

Toxicology News

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Immunoassays Continue to Improve, Expand, and Evolve

By Ruth E. Winecker

Developed during the 1950s, the first commercial immunoassays measured concentrations of hormones in blood. Today's immunoassays provide many different types of laboratories, from public health to environmental, with the ability to detect an abundance of analytes on a wide variety of platforms, from single-use, point-of-care devices to high throughput systems performing hundreds of tests per hour. Results are tailored to the test function: In most forensic laboratories results are strictly qualitative, while clinical laboratories use immunoassays for both qualitative and quantitative purposes. This article focuses on new developments in immunoassays used by clinical and forensic toxicology laboratories.

The basics

In immunoassays, antibodies along with labeled antigen (the analyte of interest) are combined with an aliquot of specimen. Any analyte present in the specimen competes with the labeled analyte to bind with the antibodies. Labels include radioisotopes, colloidal gold, enzymes, polymers as microparticles, and fluorescent molecules. The labeled compound provides for either direct or indirect signaling, and the signal is a function of the amount of analyte present in the specimen.

Table 1 briefly describes the mechanics of the different immunoassays and some of their strengths and weaknesses. For a more in-depth review of immunoassays, see references 1 and 2.

In general, immunoassays are divided into heterogeneous and homogeneous forms. Heterogeneous immunoassays are usually more labor-intensive and difficult to automate, as they require that bound and free antigen be separated before measurement of the labeled antigen. Thus, any sample type from post-

mortem whole blood to meconium is amenable to analysis by a heterogeneous assay with little or no sample preparation. Homogeneous immunoassays do not require separation of bound and free antigen before measurement and hence can be easily automated. However, if the sample is highly colored, pretreatment such as protein precipitation and centrifugation is needed prior to analysis (1,2).

Table 2 lists different types of immunoassay techniques, including their form, shelf life, automation capabilities, and applicable sample types. Table 3 lists some of the suppliers for radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), and point-of-care testing (POCT) devices.

New assays

Manufacturers are constantly expanding their test menus to meet the demands of the drug-testing community. Some of the assays developed in the past few years have focused on an increased need for screening for therapeutic drugs with a potential for abuse or impairment (for example, fluoxetine and methylphenidate) (3, 4). The demand for these types of assays is expected to increase as new research is conducted in these areas.

Another reason for expanding test menus beyond the "typical" drugs of abuse is an increased demand for flexibility in the screening menus offered by entities from poison control centers to substance abuse treatment and monitoring centers. New assays offered in the past two years include those directed at heroin metabolite, 6-acetylmorphine (6-AM); methylenedioxymethamphetamine (MDMA or ecstasy); fluoxetine; zolpidem; and methylphenidate. The

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Many Resources Help Labs Cope with Pesticide Exposures

By John G. Fisher III and Donald L. Frederick

The call comes in at 3 p.m. on a Friday afternoon from an area hospital's emergency department, the start of a scenario we all dread: a possible exposure to a specific pesticide.

The likely questions are: How toxic is the compound? Do diluents or "inactive ingredients" play a role in toxicity? To what class of pesticides does this chemical belong? What role does the route of exposure play in its toxicity and treatment? Do the age, sex, state of pregnancy, pre-existing medical conditions, concomitant drugs, and known allergies of the victim change the toxicity, especially given the route of exposure? The most important yet difficult question to answer is often the extent of exposure or dose of the toxin. Can we perform any laboratory test to detect and/or quantitate the exposure to evaluate any potential treatment? Are there accepted treatments?

All these questions may be confounded by the unfortunate practices of mixing poisons and storing them in food containers (empty milk jugs are the worse offenders). In addition, the labeled container may be left at home during the rush to the hospital.

Immediate resources

The first resource that can be used to evaluate a pesticide is the label from the package, if it is available. The government has put substantial resources into laws that require the manufacturer to put specific toxicity information on labels. While one needs a magnifying glass to read the information in many cases, there is helpful information on these labels.

An important clue to toxicity is the "warning word" on the label. From least to most toxic, these words are "Caution," "Warning," and "Danger: Poison." These words are typically in bolder print than other label data, and may also be enclosed in a box.

While label information about the substance(s) in the compound can be relied upon, treatment information may be outdated, or even potentially harmful. For instance, using salt water to induce vomiting is not only ineffective but has caused hypernatremia, including death in small children.

A call to a Poison Control Center may yield valuable information. The Centers for Disease Control and Prevention (CDC) has established a nationwide toll-free number for poisoning emergencies. Calling 800-222-1222 will connect the caller to the closest certified poison center. Nurses or pharmacists supervised by a clinical or medical toxicologist staff

poison centers. In addition to providing treatment information, poison centers do follow-up calls to check on poisoning victims and capture data for the Toxic Exposure Surveillance System (TESS), a nationwide database maintained in Washington, D.C. This data can be used to identify poisoning trends and can guide efforts to treat exposures and improve safety.

In 2003, there were 2,395,582 human exposures causing 1,106 deaths reported to TESS (1). Pesticides accounted for 97,677 exposures and 41 deaths. Twenty-three of the pesticide deaths were from rodenticides, with 16 due to organophosphates. Four deaths were caused by rodenticides, and most of the remainder were due to herbicides. Most of the deaths occurred after deliberate ingestion, although some occurred after accidental exposure.

Anticoagulant rodenticides deserve a special comment. One study concluded, "Normal preschool-aged children with unintentional acute exposures to superwarfarin rodenticides do not require any routine follow-up laboratory studies and do not require any medical intervention" (2). The deaths attributed to anticoagulant rodenticides only occurred following deliberate, suicidal ingestions.

Web resources

One of the first things you can try is a standard search engine to retrieve sources of information. The resultant 100,000 to 200,000 pages would take forever to go through; however, within the first page of results, the site, www.pesticideinfo.org, was located. This site contains toxicity information on more than 350,000 pesticide products, including product labels and materials safety and data sheets (MSDS). The website is sponsored by the Pesticide Action Network (PAN), an association of environmental groups. The PAN Pesticide Database contains a vast amount of information.

The site also connects to the U.S. Environmental Protection Agency's Pesticide Product Information System (PPIS) database of all U.S. current and historical pesticide information (www.epa.gov/oppmsd1/PPISdata/index.html). The organization also has a "Pesticide Poisoning Diagnostic Tool," in which the user inputs signs and symptoms and the tool lists possible pesticides that may elicit these conditions.

Various educational institutions also have websites dedicated to providing information on the safe use of pesticides to consumers. For example, Pennsylvania State College has <http://tfpg.cas.psu.edu/default.htm>, with information on the safe use of pesticides in orchards. Another example of an educational institution providing data for pesticide expo-

sure is Oklahoma State University, <http://osuextra.okstate.edu>.

The CDC has several sources of information, including cooperative work with the regional National Institute for Occupational Safety and Health (NIOSH) agricultural centers established to conduct research, education, and prevention projects to address the nation's pressing agricultural health and safety problems. This group maintains several large databases that contain information on pesticide exposure and toxicity. One database contains analytical methods to test for the presence of specific pesticides and other chemicals. Another useful source of information from NIOSH is a CD-ROM containing the complete *Pocket Guide to Chemical Hazards* and other databases. A free copy can be obtained through the NIOSH website (www.cdc.gov/niosh) or by calling 800-35-NIOSH.

Other governmental sources of information include the Columbia Environmental Research Center, which is a U.S. Geological Survey research facility in Columbia, Mo., that maintains a database on the acute toxicity of pesticides (<http://www.cerc.cr.usgs.gov>).

The National Pesticide Information Center (NPIC) is maintained at Oregon State University. In addition to online databases and fact sheets, the center operates a toll-free phone number to answer questions about pesticide exposures (800-858-7378). In cooperation with four other major universities, Oregon State also operates the Extension Toxicology Network, which contains a searchable database on most current pesticides (<http://extoxnet.orst.edu>).

Some of the most common first-line information sources in the hospital or at the poison control center are the databases supplied by Micromedex (www.micromedex.com), including POISINDEX. Although these subscriptions are expensive, they provide the first line of inquiry for many toxicology cases.

Several additional private environmental groups also provide information on pesticides, including the Natural Resources Defense Council (www.nrdc.org). Medical groups have also entered the online world with sites such as <http://emedicine.com>, which has clinical information on pesticide exposures seen in emergency departments. Reference laboratories that offer bio-monitoring have sites providing information on pesticide exposure, such as Pacific Toxicology Laboratories (www.pactox.com/library/article.php?articleID=15).

Any of the above sources of information may cite specific literature references that may be needed for more extensive evaluation. The National Library of Medicine (www.ncbi.nlm.nih.gov) is a main source to search for literature, whether regarding a specific

poison or a method of treatment. The search goes across multiple databases including Medline/PubMed and Toxline.

Laboratory resources

Depending on the specific pesticide, the laboratory can provide crucial data.

For anticoagulant rodenticide exposures, prothrombin time is the laboratory test of choice. Laboratorians should remember that after an acute exposure, abnormal prothrombin time results may not develop for 48–72 hours. In general, specific analysis for individual anticoagulants is not warranted for clinical care, although specific agent analysis may be warranted for other reasons.

Organophosphate and carbamate insecticides are acetylcholinesterase inhibitors. The definitive measure for organophosphate exposure is a red blood cell cholinesterase assay. This test is rare in clinical laboratories because demand is so low. A cholinesterase measurement much more widely available is the plasma or pseudo-cholinesterase analysis. This is often used as a screening test by anesthesiologists who are considering the use of succinylcholine in a patient. Another typical finding in the organophosphate- or carbamate-exposure victim is serum glucose, which is commonly elevated following exposure to these agents.

Other laboratory data may provide important clues to pesticide exposure. In cyanide exposure, the normal gap between arterial pO₂ and venous pO₂ narrows dramatically, and patients have a severe ion-gap acidosis. Hemolytic anemia may result from exposure to naphthylene mothballs. Heavy metals, through rarely used in modern pesticides, may cause their own constellations of symptoms, which often include abnormal renal function or changes in red cell appearance.

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Oleander Poisoning Creates Challenges for Laboratories

By Amitava Dasgupta

Oleanders are evergreen shrubs with various colors of flowers that belong to the dogbane family. There are two common types. *Nerium oleander*, also known as pink oleander, is an ornamental shrub that grows in the southern United States, Australia, India, Sri Lanka, China, and other parts of the world. *Thevetia peruviana*, commonly called yellow oleander, is a common plant in many tropical countries. It was named in honor of the French monk Andre Thevet, who traveled extensively in South America, where this species is common. Yellow oleander is also found in Sri Lanka and India. The trees can attain heights of 20 to 30 feet.

Human exposure and toxicity

All parts of the oleander plant are poisonous due to the presence of cardiac glycosides.

Human exposure to oleander can occur via accidental exposure, ingestion by children, purposeful administration in food or drink, medicinal herbal products, and criminal poisoning (1–4). In Australia, 27% of plant poisonings involve oleander (5).

Despite its toxicity, oleander is used in folk medicines (6). The fatality rate from oleander toxicity is around 10% in Sri Lanka, with approximately 40% of patients requiring specialized management in a tertiary care hospital. Deliberate ingestion of oleander seeds is a popular method of suicide in Sri Lanka (7). A small amount of the plant can have toxic effects. Boiling or drying the plant does not inactivate the toxins. Death from drinking herbal tea containing oleander has been reported (8).

Oleander toxicity was studied from 1983 to 1998 in a Tunisian toxicology intensive care unit in connection with plant poisonings and use of herbal medicines. The authors reported that 7% of all herbal medicine poisonings were due to oleander (9).

Symptoms of oleander toxicity vary from contact dermatitis from touching freshly cut leaves (10) to life-threatening conditions due to ingestion. Oleander ingestion can cause irritation of contacted membranes, nausea, vomiting, abdominal pain, increased salivation, diarrhea, headache, altered mental status, mydriasis, peripheral neuritis, and cardiovascular complications (11).

Animal toxicity

Dogs, cats, goats, and monkeys are very sensitive to oleander poisoning, but rodents and avian

species are less sensitive or insensitive (12). Severe toxicity has been reported in donkeys, sheep, horses, and cattle.

Laboratory role in poisoning detection

Oleandrin, the toxic glycoside in oleander, can be detected in blood by high performance liquid chromatography and mass spectrometry (HPLC/MS) (13). However, this technique is too complex to be used routinely in small hospitals. Early reports indicated that cardiac glycosides in oleander cross-react with digoxin radioimmunoassays (14). Cheung et al. reported detection of poisoning by plant origin (including oleander) using the digoxin immunoassay on the TDx analyzer (15). Jortani et al. reported rapid detection of oleandrin and oleandrogenin using fluorescence polarization immunoassay (FPIA), fluorometric enzyme assay on the Stratus analyzer, radioimmunoassay, ACS:180, and On-Line digoxin assays (16).

Osterloh et al. reported an apparent digoxin level of 5.8 ng/mL after suicidal ingestion of oleander tea in a patient with no history of taking any cardioactive drug. The person eventually died from oleander toxicity (17). Eddleston et al. reported a mean apparent serum digoxin concentration of 1.5 nmol/L (1.2 ng/mL) in patients who were poisoned with oleander but eventually discharged from the hospital. Severe oleander toxicity correlated with a mean apparent serum digoxin concentration of 2.8 nmol/L (2.2 ng/mL) as measured by the digoxin FPIA (18).

In our experience, the digoxin FPIA has the highest cross-reactivity with oleander extract as well as oleandrin, an active component of oleander. The chemiluminescent assay of Bayer Diagnostics is free from oleander interference and thus cannot be used to detect oleander poisoning.

Patient management

Supportive therapy and activated charcoal, along with expensive digibind therapy and cardiac pacing, are used to manage patients with oleander poisoning. De Silva et al. recently reported that multiple doses of activated charcoal are effective in reducing deaths and life-threatening cardiac arrhythmias after yellow oleander poisoning and should be considered in all patients (19).

Digibind therapy has been proposed for treating patients poisoned with oleander. Shumanik et al. reported a case in which a 37-year-old man poisoned with oleander leaves was successfully treated with a single dose of five vials (200 mg) of digibind (20). Zylber-Katz et al. reported a case in which treatment with digoxin-specific fab fragment was successful in treating a 24-year-old man who ingested oleander

leaf extract (21). Safadi et al. demonstrated effectiveness of digibind in treating a patient who ingested oleander leaf (22). Clark et al. reported that digibind therapy is effective in treating experimentally induced oleander poisoning in a canine model (23). A recent article indicated that in the absence of digibind therapy, fatalities from oleander poisoning increased threefold in Sri Lanka (24).

Conclusion

Oleander poisoning is a problem in the southern United States and several Asian countries, in part because oleander tea is used as an herbal supplement. Toxicity from oleander is due to its cardiac glycosides, and its manifestation resembles digoxin toxicity. Digibind therapy along with life-support measures may be useful for managing oleander poisoning.

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Oleander Case Studies Show Dangers of Eating Wild Things

By Dan Anderson and Michelle Sandberg

In the past five years, the Los Angeles County coroner's department has encountered several deaths in which oleandrin was detected. Our laboratory's procedure for screening oleandrin is to conduct a "poor man's" screening test using a digoxin immunoassay. A positive screen demonstrates that oleander could have been ingested. Positive specimens are sent to the University of California, Davis, Veterinary Diagnostic Laboratory System for liquid chromatography/mass spectrometry/mass spectrometry confirmation and quantitation.

Case 1

An 81-year-old female's business ventures had failed and she had become depressed over her financial situation, which included being 18 months behind on her rent. On the day her eviction was to take place, the landlord found her unresponsive body with a suicide note near a large potted oleander plant. During the autopsy, the pathologist recovered green leaf-like material from the esophagus, stomach, and duodenum. Her toxicology results for diphenhydramine were: heart blood, 5.5 µg/mL; femoral blood, 0.71 µg/mL; liver, 58 µg/g; gastric, 360 mg.

Oleandrin was present but it was not necessary to quantitate it because of the lethal level of diphenhydramine. The manner and cause of death were suicide by diphenhydramine and oleander intoxication.

Case 2

A family with two healthy children adopted two three-year-old Russian boys. They entered the United States malnourished and with symptoms of pica. Over the course of three days, the mother observed the two boys spitting out plant material and stating, "Yucky." They were also seen grabbing for the oleander plants in the neighborhood. During this period, the boys were lethargic, vomiting, and had no appetite. On the third day, the boys appeared to be regaining their strength, ate a healthy dinner, and went to bed for the night. In the morning, the parents found the boys cold and unresponsive in their cribs. Toxicology tests found lethal levels of oleandrin, detected in the urine and intestinal tract of each boy.

Case 3

A 41-year-old teacher married a woman after an eight-week courtship. After three months, this apparently healthy male fell ill during a field trip with his students and was diagnosed with a severe case of

food poisoning. He was found unresponsive by his wife four days later. The autopsy revealed no significant findings and initial toxicology results indicated medications consistent with therapeutic use.

His widow could not collect life insurance until the cause of death was determined, so she became impatient and called the laboratory frequently. She eventually left hints guiding the toxicologist to test for oleander and antifreeze. Oleandrin was found to be: heart blood, ~9 ng/mL; urine, ~1 ng/mL; liver, >5 ng/g; gastric, 0.055 mg. Ethylene glycol was: heart blood, 2,994 µg/mL; femoral blood, 2,436 µg/mL; vitreous, 3,075 µg/mL; gastric, 1.7 mg.

The manner and cause of death were determined to be homicide by oleander and ethylene glycol toxicity. The wife was convicted of murder. This marriage was her fourth. Seven years before, her baby had died due to a pacifier lodged in the throat. She had collected a \$710,000 settlement from the manufacturer, and that case is being re-investigated.

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AACC Annual Meeting to Feature TDM and Toxicology

The upcoming AACC Annual Meeting, to be held July 24–28 in Orlando, Florida, will feature a large number of sessions on toxicology, pharmacogenomics, and therapeutic drug monitoring (TDM).

EduTrak sessions include "Immunosuppressive Drug Monitoring: The Rationale and Application Following Transplantation" (3409).

Workshops include "Eliminating False Positive and False Negative Results in the Toxicology Laboratory" (2202) and "A New Generation of Antiepileptic Drugs: Application and Analysis" (2207).

Roundtables include "Drug Interferences—The Unsolved Problem" (4213/5213), "New Approaches for Therapeutic Monitoring of Immunosuppressive Drugs" (4221/5221), "Unlocking the Mystery of Pain Management with TDM and Pharmacogenetics" (4309/5309), "How People Try to Beat Drug Tests and Defend Positive Results" (4416/5416), "LC/MS/MS for TDM, Toxicology, and Pharmacogenomics" (4418/5418), "What A Pain: Drug Testing for Pain Management Programs" (4426/5426), and "Incorporating Music, History, and Famous People into Teaching Toxicology and TDM" (5429).

The AACC website lists many sessions on pharmacogenomics as well. You can register for the meeting at www.aacc.org/2005AM/default.stm.

Immunoassays

Continued from page 1

6-AM and ecstasy assays were long-awaited by many laboratories. Table 4 provides details of immunoassay types, cutoffs, and manufacturer information for some assays developed in the past two years.

Alternative matrices

The use of alternative matrices, such as oral fluid, sweat, and hair, for the detection of drugs of

abuse is not a recent phenomenon. However, the development of immunoassay kits and POCT devices designed for use with alternative matrices has gained prominence in the past few years. The Substance Abuse and Mental Health Services Administration (SAMHSA) proposed cutoff concentrations for screening of alternative matrices in June 2000 (5). After several revisions, SAMHSA then published these proposed rule changes in the *Federal Register* of April 13, 2004 (6). The comment period ended July 12, 2004, and final rules are expected in 2005.

Table 1. Mechanics, Advantages, and Disadvantages of Various Immunoassay Techniques (1, 2)

Radioimmunoassay

Mechanics: Isotopically labeled drug (^3H , ^{14}C , or ^{125}I) competes with free drug for antibodies coated in a tube. Sample is decanted to separate free and bound drug. Signal is read by scintillation or gamma counter. Drug concentration is inversely related to signal.

Pros and cons: High sensitivity, free from many matrix effects, relatively free from effects related to sample adulteration, disposal of radioactive waste is expensive and difficult, short shelf life for reagents, automation is limited.

EMIT (enzyme-multiplied immunoassay technique)

Mechanics: Drug labeled with glucose-6-phosphate dehydrogenase competes with free drug for antibodies in solution. Binding with antibodies inhibits enzyme activity. Signal increases as drug concentration increases.

Pros and cons: Large test menu available, easily adaptable to a wide range of commercially available chemistry analyzers, interfering substances can cause false negatives.

Fluorescence polarization immunoassay (FPIA)

Mechanics: Fluorescein-labeled drug competes with free drug for antibodies in solution. Binding to the antibodies slows the fluorescein-labeled drug's rotation, causing light to be emitted in the same plane as the polarizing filter. Drug concentration is inversely related to signal.

Pros and cons: Potential for lower limits of detection than enzyme assays, wide range of analytes available, more expensive than other immunoassays, requires Abbott instrument.

CEDIA (cloned enzyme donor immunoassay)

Mechanics: Similar to EMIT but employs genetically engineered fragments of beta-galactosidase, one of which is bound to drug. The fragments will not associate and produce a signal if the labeled fragment is bound to antibody. Signal is directly related to drug concentration.

Pros and cons: Linear over a wide range of concentrations, large test menu available, easily adaptable to a wide range of commercially available chemistry analyzers, interfering substances can cause false negatives.

KIMS (kinetic interaction of microparticles in solution)

Mechanics: Multiple drug molecules conjugated to a polymer compete with free drug for antibodies that are covalently coated to microparticles in solution. Free drug molecules in the specimen prevent the formation of aggregates of drug/polymer and antibody/microparticles. The amount of agglutination is inversely proportional to the amount of drug in the sample.

Pros and cons: More stable than enzyme conjugates, less susceptible to interfering substances than enzyme immunoassays, interfering substances can cause false positives, analyzers require more maintenance due to coating of tubing with microparticles, narrower linear range than traditional immunoassays.

Enzyme-linked immunosorbent assay (ELISA)

Mechanics: Antibodies are coated on the surface of wells, usually in a 96-well microtiter plate format. Enzyme-labeled drug competes with free drug for binding to the antibodies. Separation of bound and free ligand is by automated or manual washing of the plates. Drug concentration is inversely related to signal.

Pros and cons: High sensitivity, free from many matrix effects, requires specialized analyzers for automation.

Point-of-care testing (POCT) device

Mechanics: These devices generally use lateral-flow technology to float an antibody labeled with colloidal gold or colored latex toward immobilized drug, causing the formation of a line. The specimen provides the wetting needed for the lateral flow process. When drug is present in the specimen it binds the antibody and no line is formed.

Pros and cons: Allows multiple drug analysis simultaneously, laboratory and expensive equipment not necessary, the addition of control lines to assure that sufficient specimen has been added to provide adequate wetting has decreased the coefficient of error associated with these devices, cost typically higher per test than traditional immunoassays.

Table 2. Commercial Immunoassay Techniques (1, 2)

Assay Type	Manufacturer	Form	Shelf Life	Automation	Samples
RIA	Various	He	< 60 days	None/semi	Whole blood, tissue homogenates, hair, oral fluid, meconium, urine
EMIT	Dade-Behring DRI (Microgenics)	Ho	> 1 year	Full	Urine
FPIA	Abbott	Ho	> 1 year	Semi/full	Urine, serum, plasma
CEDIA	Microgenics	Ho	> 1 year	Full	Urine, serum, plasma
KIMS	Roche	Ho	> 1 year	Full	Urine
ELISA	Various	He	> 1 year	Semi/full	Whole blood, tissue homogenates, hair, oral fluid, meconium, sweat, urine
POCT	Various	Ho	> 1 year	None	Urine, oral fluid

He=Heterogeneous, Ho=Homogeneous

Table 3. Suppliers of RIA, ELISA, and POCT Assays**RIA**

Diagnostic Products Corp., Immulysis

ELISA

Cozart Bioscience, Immulysis, International Diagnostic Systems Corp., Neogen Corp., Orasure Technologies, Venture Labs

POCT

American Bio Medica Corp., Ansys Technologies, Avitar, Bio-Rad, Biosite Diagnostics, Branam Medical Corp., Cozart Bioscience, Dade-Behring, Orasure Technologies, Princeton Biomedical Corp., Roche Diagnostic Systems, Securetec

Table 5 summarizes the proposed and current screening cutoffs for the federal workplace drug-testing program. The proposed changes would lower the urine cutoff concentrations for cocaine metabolite and amphetamines in order to increase the deterrent effect of the federal workplace drug testing program. The lower cutoffs are expected to identify 10–20% more urine cocaine metabolite positives and 5–24% more amphetamines positives (6).

For some drug classes, the proposed cutoffs for the alternate matrices are substantially lower than the urine cutoffs. This is due in part to the nature of these specimens and how drugs are secreted or incorporated into them. Urine tends to concentrate drug levels from blood, while hair, sweat, and oral fluid do not. In addition, the amount or volume of alternative specimen collected is much less than the volume of urine typically collected for a drug screen. The ramifications for immunoassay testing of these matrices are that laboratories will have to implement more sensitive screening techniques such as ELISA or current homogeneous techniques will have to be modified by decreasing antibody titer to increase sensitivity (7).

Manufacturers of POCT devices designed for

testing oral fluid have reason to hope that these devices will gain approval for use in the federal workplace program. SAMSHA has designated testing of oral fluid by POCT devices to be most suited for reasonable suspicion, cause, and post-accident testing (6). Should such approval be forthcoming, then these manufacturers could expect the market to expand to include the widespread use of these devices during roadside examination of impaired drivers. These devices could then be used much like the passive alcohol sensor or other such devices to screen drivers for probable cause and the further administration of an evidentiary test. A prime advantage of this type of testing is that oral fluid concentrations are related to blood or plasma drug concentrations, while urine drug concentrations are not. In addition, specimen collection is non-invasive and can be directly observed (8). However, improvements need to be made for these devices to perform as well as standard ELISA for oral fluid or POCT devices designed for testing urine.

In a recent study comparing a number of these POCT devices for use on oral fluid, the authors reported that most devices performed well for detecting methamphetamine and opiates, the devices varied greatly in their ability to detect cocaine and amphetamines, and all performed poorly at detecting cannabinoids (9).

The future of immunoassays

The need for improvements in limits of detection, especially for the analysis of alternate matrices, is expected to increase research and development in the area of new detection technologies. One area enjoying renewed interest is the development of chemiluminescence detection systems, due in part to an expected order-of-magnitude increase in sensitivity over RIA (2). The development of more sensitive confirmatory techniques such as liquid chromatography/mass spectrometry/mass spectrometry will continue to drive this trend. In addition, implementation

Table 4. Recently Developed Assays

Drug	Assay Type	Manufacturer	Cutoff (ng/mL)	Specimen Type	Comments
6-Acetylmorphine (10)	CEDIA	Microgenics	10	U	16.7% cross reactivity to heroin, <0.1% to other opioids
Buprenorphine (11)	CEDIA	Microgenics	5	U	
Carisoprodol (12)	ELISA	Immunalysis	5000	B,T,U	
Carisoprodol (13)	ELISA	International Diagnostic Systems Corp.	np	B,T,U	
Ecstasy* (14)	EMIT	DRI (Microgenics)	500	U	< 0.8% cross-reactivity to pseudoephedrine, phentermine, ephedrine, amphetamine, and methamphetamine
Fentanyl (15)	ELISA	Orasure Technologies	1	B,T,U	
Fluoxetine (12)	ELISA	Immunalysis	200	B,T,U	
Hydrocodone (11)	ELISA	International Diagnostic Systems Corp.	np	B,T,U	
Ketamine (11)	ELISA	International Diagnostic Systems Corp.	np	B,T,U	
Methadone (12)	ELISA	Immunalysis	300	B,T,U	
Methylphenidate (12)	ELISA	Immunalysis	100	B,T,U	
Naltrexone (11)	ELISA	International Diagnostic Systems Corp.	np	B,T,U	
Propoxyphene (11)	ELISA	International Diagnostic Systems Corp.	np	B,T,U	
Propoxyphene (15)	ELISA	Orasure Technologies	25/50/300	B,T,U	
Sertraline (12)	ELISA	Immunalysis	200	B,T,U	
Zolpidem (12)	ELISA	Immunalysis	25	B,T,U	
Zolpidem (11)	ELISA	International Diagnostic Systems Corp.	np	B,T,U	

*3,4-Methylenedioxymethamphetamine (MDMA), 3,4-Methylenedioxyamphetamine (MDA), and 3,4-Methylenedioxyethylamphetamine (MDEA)

B=Whole blood/serum, T=Tissue, U=Urine, np=not provided

Table 5. Federal workplace drug-testing program urine screening cutoffs and proposed alternative matrices cutoffs (16)

Drug/Drug Class	Current Urine (ng/mL)	Proposed Urine (ng/mL)	Proposed Hair (pg/mg)	Proposed Sweat (ng/patch)	Proposed Oral Fluid (ng/mL)
Amphetamines	1000	500*	500*	25*	50*
Cocaine metabolites	300	150	500	25	20
Marijuana metabolites	50	50	1	4	4
Opiates	2000	2000	200**	25***	40****
Phencyclidine	25	25	300	20	10

*Screening technique must use d-methamphetamine as the target compound and must exhibit 50–150% cross-reactivity with MDMA, MDA, and MDEA..

** Due to limited specimen amounts, laboratories are permitted to initially test all specimens for 6-AM using a 300 pg/mg cutoff.

*** Due to limited specimen amounts, laboratories are permitted to initially test all specimens for 6-AM using a 25 ng/patch cutoff.

**** Due to limited specimen amounts, laboratories are permitted to initially test all specimens for 6-AM using a 4 ng/mL cutoff.

of biochemical terrorism readiness programs in medical examiner and clinical laboratories could potentially bring commercially available ELISAs for agents like botulin toxin out of the public health arena and into the toxicology laboratory. Finally, continued expansion of test menus to meet market demand will most likely lead to manufacturers intro-

ducing a long-awaited assay for gamma-hydroxybutyrate in the next year.

Conclusion

A wealth of immunoassay techniques and assays are available to the modern-day toxicologist. As always, implementation of new assays and tech-

niques will depend on cost considerations, sensitivity needs, sample type, and drug-abuse trends.

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