

# Toxicology News

June 2008

*An AACC/CAP Educational Newsletter for Toxicology Laboratories*

## **CLSI Publishes Guideline on Toxicology and Drug Testing**

*By David Armbruster*

**T**he Clinical and Laboratory Standards Institute (CLSI) provides guidelines for the international clinical laboratory community ([www.clsi.org](http://www.clsi.org)). Most, if not all, clinical labs are involved in toxicology testing to some extent. Therapeutic drug monitoring (TDM) is routine and testing for drugs of abuse (DOA) is also common to support the emergency room.

In large medical centers, a separate toxicology department may perform TDM and DOA testing, but in smaller facilities, the general clinical chemistry lab provides this service. Toxicology testing also takes place in specialized reference labs, in physicians' office labs, and even in workplace settings with no connection to medical facilities. These activities, which bring together clinical chemistry and toxicology, require a balancing act between the two disciplines. The waters can be muddied when it comes to abused drugs because the testing may be for clinical (medical) purposes, for forensic (legal) purposes, or in a gray zone between the two.

Published in 2007, "C52: Toxicology and Drug Testing in the Clinical Laboratory Guideline," is intended to apply to both routine and specialized laboratories and to both clinical and forensic toxicology procedures (1).

C52 was originally published in 1999 as T/DM8. The "T/DM" designation was for therapeutic drug monitoring, but the guideline was actually very specific for forensic testing of drugs of abuse. T/DM8 was subject to periodic review and revision as per routine CLSI procedures. CLSI decided in 2006 that the document should be updated and that its emphasis should be shifted to routine toxicology testing for clinical purposes, while retaining information pertaining to forensic testing.

The National Academy of Clinical Biochemis-

try (NACB) published practice guidelines in 2003 for emergency toxicology (2). It was desirable to rework the CLSI guideline to incorporate the new NACB recommendations. It was also desirable to retain the guidance for forensic toxicology testing because this area is fraught with difficulty for clinical laboratories.

C52 addresses prescription drugs, drugs of abuse, and some nonprescription drugs such as ethanol, acetaminophen, and salicylates. Other classes of compounds that could have both therapeutic and abuse potential, such as steroids, are not included because they are beyond the scope of routine clinical laboratories. C52 carefully delineates clinical from forensic toxicology testing because every laboratory must clearly understand the distinction between the two and not undertake forensic testing unless fully prepared to do so. C52 is organized according to the preanalytical, analytical, and postanalytical aspects of toxicology testing, an approach that is logical for describing clinical laboratory operations for many types of testing.

### **Reasons for testing emphasized**

The guideline emphasizes at the beginning that laboratories should establish their reason for performing toxicological analyses. Clinical testing involves situations such as support of the emergency room, testing of pregnant women and newborns, and general purpose TDM. Forensic testing involves situations such as post-accident investigation for suspected driving while intoxicated, workplace drug testing of applicants for safety-related positions, and investigation of potential sexual assault (for example, date rape facilitated by drugging of the victim).

*Continued on page 7*

### **Inside...**

|  |          |
|--|----------|
| <b>Butyrylcholinesterase Activity .....</b>    | <b>2</b> |
| <b>Regional Toxicology Organizations .....</b> | <b>4</b> |

## Butyrylcholinesterase Action Is Vital, But Ill-Understood

By Gillian Johnson

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE, also known as plasmacholinesterase or pseudocholinesterase) are two closely related enzymes found in all vertebrate species. AChE plays a crucial role in the neuromuscular junction by terminating cholinergic transmission. By contrast, the physiological function of BuChE is a matter of some speculation.

Many drugs are hydrolyzed and inactivated by these esterases. Because of these enzymes' high activity and ubiquitous expression, drug metabolism was long thought to be predictable. However, hereditary and acquired conditions along with drug–drug or drug–toxin interactions may cause variability in metabolism of many drugs.

### Role of BuChE in drug metabolism

There has been growing awareness of the role of BuChE in drug metabolism and the factors that may affect it. BuChE is synthesized in the liver, so parenchymal damage will lead to a decrease in its synthesis. In addition, heredity, pregnancy, malignancy, malnutrition, heart disease, renal disease, burns (1), dietary factors (2), and environmental toxins (3) can cause decreased activity of this esterase.

Factors associated with an increase in BuChE activity include thyroid disease, nephritic syndrome, mental retardation, obesity (1), and anxiety (4). Interventions such as plasmapheresis, cardiopulmonary bypass surgery, and oral contraceptives also increase variation.

### Decreased BuChE effects

The group of drugs affected by decreased cholinesterase activity is best represented by succinylcholine. Anesthesiologists commonly use this drug for rapid induction of anesthesia in patients with reflux disease, in full-stomach situations, and in difficult-airway situations, when a return to full muscle strength is required minutes after administration of the drug. The rapid onset of paralysis allows the airway to be secured, and rapid metabolism of the drug means that strength can be expected to return within five minutes. If the surgery is short or there is difficulty in securing the airway, then the drug is used in the hope that it will allow a rapid return to spontaneous ventilation. A prolonged reversal of action could be fatal.

Other drugs that may be affected by decreased

cholinesterase activity include:

- Mivacurium (increased paralysis time);
- Ester local anesthetics including cocaine (higher likelihood of toxicity);
- Heroin (decreased effectiveness);
- Aspirin (increased analgesic effect, prolonged bleeding time, and decreased platelet aggregation);
- Methylprednisolone (relative resistance is suspected but not proven); and
- Bambuterol (decreased function because it is not converted to terbutaline)

### Mechanism of action

BuChE has a lower affinity for acetylcholine than AChE does and is not inhibited by high concentrations of acetylcholine. BuChE's model substrate is benzoylcholine, so the activity of plasma cholinesterase can be measured by adding plasma to benzoylcholine and using spectrophotometry after the reaction.

The tertiary structure of BuChE has recently been elucidated by Nicolet et al. (5). Similar to AChE, it has an active site containing an oxyanion hole, an anionic substrate-binding site, an acyl group binding site, and a peripheral anionic binding site. BuChE cleaves the cholinic esters such as succinylcholine.

### Measuring BuChE activity

The tests used most often for BuChE activity are dibucaine number (6), endpoint enzyme assay with spectrophotometric measurement (7), and kinetic studies (8). Newer tests include a modified electro-metric method (9, 10) and a method using dried blood spots on filter paper (11).

The BuChE knockout mouse offers a new way of studying BuChE deficiency. This model is expected to aid in identifying drugs that may be harmful to BuChE-deficient humans. The mice may also be useful in identifying the enzyme's physiological functions (12).

### Genetic polymorphism

Mutations in the BuChE gene affect its function, leading to prolonged neuromuscular blockade. The more frequent variations from the normal phenotype are the atypical (A) and Kalow (K) variants, which occur secondary to nucleotide substitutions.

Genetic investigations have provided evidence that the K variant alone causes moderate prolongation of neuromuscular blockade; however, the effect is more pronounced with patients carrying both variants. Until recently, distinction among the four genotypic combinations of A and K variants was only possible by molecular genetic methods. In 2007, Le-

vano et al. (13) established a denaturing high-performance liquid chromatography method for A and K variants in the BuChE gene. These advances are moving the practice of medicine toward individualized therapies and increased patient safety based on the known genetic differences of each patient.

### Environmental and chemical warfare agents

Organophosphates are toxic substances predominantly used as pesticides. In recent times, these substances are frequently discussed as potential nerve warfare agents. BuChE is involved in both the toxicity and detoxification of these agents. The cholinesterases therefore play a role in both the detection of organophosphates and antidotes for exposure. Measurement of changes in the activity of cholinesterases in human blood allow detection of exposure to organophosphates. The toxins are targeted against AChE; however, inhibition of BuChE is seen more rapidly and is hence a more sensitive indicator of exposure.

Because of the increased threat of chemical warfare, recent work has been done to find antidotes to (14) and protective measures against (15) nerve agents. One such investigation used recombinant human BuChE from the milk of transgenic animals.

### New drug could bring changes

A new drug about to launch will likely change the landscape of succinylcholine use and thus the relevance of BuChE measurement. Sugammex is a selective relaxant binding drug that antagonizes or reverses steroidal non-depolarizing neuromuscular blockers in one tenth the time of conventional drugs. The advantages of this drug over conventional cholinesterase inhibitors include its speed of action, completeness of reversal, and decreased likelihood of dry mouth after surgery (16).

This drug could obviate the need for succinylcholine in many cases, which would make measurement of a patient's BuChE activity less relevant in anesthesiology. However, with the rapidly expanding pharmacological armamentarium and the constant threat of nerve agents, the physiological role of BuChE will likely remain an important consideration for some time to come.

### References

1. Davis L, Britten J, Morgan M. Cholinesterase. Its significance in anaesthetic practice. *Anaesthesia* 1997;52:244–60.
2. McGehee DS, Krasowski MD, Fung DL, Wilson B, Gronert GA, Moss J. Cholinesterase inhibition by potato glycoalkaloids slows mivacurium metabolism. *Anesthesiology* 2000;93:510–9.
3. Mone J, Mathie W. Qualitative and quantitative defects of pseudocholinesterase activity. *Anesthesia* 1967;22:55–68.
4. Ledowski T, Bein B, Hanss R, Tonner P, Roller N, Scholz J. Pseudocholinesterase activity increases and heart rate variability decreases with preoperative anxiety. *Eur J Anaesthesiol* 2005;22:289–92.
5. Nicolet Y, Lockridge O, Masson P, Fontecilla-Camps J, Nachon G. Crystal structure of human butyrylcholinesterase and of its complexes with substrate and products. *J Biol Chem* 2005;278:41141–7.
6. Kalow W, Genest K. A method for the detection of atypical forms of human serum cholinesterase; determination of dibucaine numbers. *Can J Biochem Physiol* 1957;35:339–46.
7. Garry P, Routh J. A micro method for serum cholinesterase. *Clin Chem* 1965;11:91–6.
8. Ellman G, Courtney, Andres K, Featherstone R. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961;7:88–95.
9. Al-Jobory MM, Mohammad FK. Validation of an electrometric blood cholinesterase measurement in goats. *J Vet Sci* 2005;6:299–303.
10. Mohammad F, Alias A, Ahmed O. Electrometric measurement of plasma, erythrocyte, and whole blood cholinesterase activities in healthy human volunteers. *J Med Toxicol* 2007;3:25–30.
11. Trudeau S, Mineau P, Cartier G, Fitzgerald G, Wilson L, Wheler C, Knopper L. Using dried blood spots stored on filter paper to measure cholinesterase activity in wild avian species. *Biomarkers* 2007;12:145–54.
12. Li B, Duysen EG, Carlson M, Lockridge O. The butyrylcholinesterase knockout mouse as a model for human butyrylcholinesterase deficiency. *J Pharmacol Exp Ther* 2008;324:1146–54.
13. Levano S, Keller D, Schobinger E, Urwyler A, Girard T. Rapid and accurate detection of atypical and Kalow variants in the butyrylcholinesterase gene using denaturing high performance liquid chromatography. *Anesth Analg* 2008;106:147–51.
14. Reiner E, Radic Z, Simeon-Rudolf V. Mechanisms of organophosphate toxicity and detoxication with emphasis on studies in Croatia. *Arh Hig Rada Toksikol* 2007;58:329–38.
15. Huang YJ, Huang Y, Baldassarre H, et al. Recombinant human butyrylcholinesterase from milk of transgenic animals to protect against organophosphate poisoning. *Proc Natl Acad Sci USA*. 2007;104:13603–8.
16. Sacan O, White PF, Tufanogullari B, Klein K.

Sugammadex reversal of rocuronium-induced neuromuscular blockade: a comparison with neostigmine-glycopyrrolate and edrophonium-atropine. *Anesth Analg* 2007;104:569–74.

*Gillian Johnson, MD, is a resident with the department of anesthesiology at the University of Colorado Health Sciences Center in Denver.*

## Regional Organizations Offer Access to Education Programs

*By Donald L. Frederick*

Toxicology is a diverse field with clinical, forensic, and environmental branches, each having its own professional organizations. Professional associations can be international, national, or regional, but these boundaries are blurred by international organizations having national chapters or sponsoring national organizations (see Tables 1, 2, and 3). National associations also have regional or state chapters or affiliations with state or regional organizations.

Within these broad disciplines, groups are organized around special practice disciplines. For example, within clinical toxicology there are organizations of laboratory toxicologists, physicians that are board-certified in toxicology, and professionals associated with poison control centers. Forensic toxicology and environmental toxicology both have international, national, regional, and even local organizations.

Practicing toxicologists may find all of these professional organizations helpful with their practice, and many cases cross these arbitrary boundaries. For example, lead toxicology crosses all toxicology disciplines, and professionals in other associations may provide help in specific cases. A clinical toxicologist may find the information from a local environmental toxicologist helpful at times. Likewise, an environmental toxicologist may need information on the number of cases of a particular poison found in clinical or forensic cases. The goal of this article is to give a short description of some of the regional toxicology groups that readers may find helpful.

### Regional organizations

Regional toxicology professional organizations may be divisions or subsidiaries of national organizations, such as the regional sections of the American Association for Clinical Chemistry, or they may be independent with little or no association with na-

tional organizations. There are three regional associations focused on clinical and forensic toxicology that are independent from national groups. One of the first regional toxicology associations in the United States originated in the northeast but rapidly became a major national association, the Society of Forensic Toxicologists (SOFT).

### California Association of Toxicologists

One of the oldest regional groups is the California Association of Toxicologists (CAT), founded in 1967. The first meeting had 11 attendees, but the organization has grown to more than 350 members. One of the original goals for CAT was to bring together toxicologists who normally would not meet together, such as environmental, clinical, and medical examiner toxicologists, to discuss common problems. For the first six years, CAT met quarterly throughout the state. To minimize time away from work, the meetings were generally held on Saturdays. The goal was to keep the discussion at the practical level of day-to-day problems. The organization was not formally structured until 1973, when P.C. Reynolds became president.

Goals over the years have been primarily to exchange ideas and provide educational opportunities. Activities have included quarterly meetings, listservs, book exchanges, one-day workshops, and online educational formats, including newsletters, drug monographs, and publication of meeting activities. CAT usually has three meetings a year and publishes a newsletter restricted to members. The next CAT meeting is Oct. 26, immediately preceding the SOFT meeting in Phoenix.

### Southwestern Association of Toxicologists

The Southwestern Association of Toxicologists (SAT) began life as a regional meeting in Dallas in 1975 and was given a more formal structure the following year. Modeled after the CAT, SAT was officially incorporated in 1984. SAT continues an early goal to provide a regional exchange of information with inclusion of bench-level toxicologists in presentations at the biannual meetings. The meetings are held mostly in Texas, Oklahoma, Louisiana, Arkansas, Kansas, and New Mexico, with occasional joint meetings with other organizations in Arizona and Las Vegas. SAT's next meeting is in the fall in Austin, Texas.

### Midwest Association

One of the newest regional associations is the Midwest Association for Toxicology and Therapeutic Drug Monitoring (MATT). MATT originated in 1995 and covers the states of Minnesota, Wisconsin,

**Table 1. International Toxicology Organizations**

| Organization  | Primary Focus   | Founded |
|---|---|---------|
| Asia Pacific Association of Medical Toxicology                                    | Clinical toxicology   | 1989    |
| Association of Inhalation Toxicologists   | Research in inhalation toxicology   | 1981    |
| European Association of Poison Centres and Clinical Toxicologists                 | Diagnosis and treatment of poisons  | 1964    |
| European Workplace Drug Testing Society   | Workplace drug testing  | 1998    |
| Federation of European Toxicologists and European Societies of Toxicology         | All areas of toxicology, mostly research oriented   | 1962    |
| International Association for Chemical Testing                                    | Tests on drivers  | 1988    |
| International Association for Therapeutic Drug Monitoring and Clinical Toxicology | Forensic and clinical toxicology  |         |
| International Council on Alcohol, Drugs and Traffic Safety                        | Traffic safety: alcohol and drugs   | 1950    |
| Society of Environmental Toxicology and Chemistry                                 | Environmental toxicology  | 1979    |
| Society of Hair Testing   | Clinical and forensic testing in hair samples   | 1995    |
| Society of Toxicology of Canada   | Research in toxicology  | 1964    |
| Society of Toxicologic Pathology  | Pathologists from 22 countries  |         |
| The British Toxicology Society  | All areas of toxicology in United Kingdom   | 1971    |
| The International Association of Forensic Toxicologists                           | Forensic toxicology   | 1963    |
| The International Union of Toxicology   | Federation of national and international organizations, including 53 national organizations | 1980    |

**Table 2. National Toxicology Professional Organizations**

| Organization                                      | Primary Focus  | Founded |
|---|--|---------|
| American Academy of Clinical Toxicology           | Unite scientists and clinicians whose research, clinical, and academic experience focuses on clinical toxicology | 1968    |
| American Academy of Forensic Sciences             | All 10 branches of forensic science, including toxicology  | 1957    |
| American Association of Poison Control Centers    | Operation of poison control centers to disseminate emergency toxicology information                              |         |
| American College of Emergency Physicians          | All emergency medicine   | 1968    |
| American College of Medical Toxicology            | Physicians board-certified in medical toxicology   | 1993    |
| American College of Toxicology                    | Basic toxicology research and safety assessment  |         |
| Canadian Society of Forensic Science              | Forensic sciences  | 1953    |
| Society of Environmental Toxicology and Chemistry | International organization of environmental scientists with 18 North American chapters                           | 1979    |
| Society of Forensic Toxicologists                 | Forensic toxicology and drugs of abuse testing   | 1970    |
| Society of Toxicologic Pathology                  | Pathology study of toxins  | 1978    |
| Society of Toxicology                             | Research scientists and academics with 18 regional chapters  | 1961    |
| Society of Toxicology Canada                      | Fundamental and basic to clinical and applied toxicology   | 1964    |

**Table 3. Toxicology Meeting Dates**

| Date         | Location                  | Organization  |
|--------------|---------------------------|---|
| <b>2008</b>  |                           |   |
| June 22–26   | San Francisco, California | Society of Toxicologic Pathology  |
| July 26–31   | Washington, D.C.          | American Association for Clinical Chemistry                                       |
| August 3–7   | Sydney, Australia         | SETAC 5 <sup>th</sup> World Congress  |
| Sept. 6–14   | Bodensee, Germany         | International Union of Toxicology Risk Assessment Summer School XIII              |
| Sept. 8–9    | Liverpool, U.K.           | British Toxicology Society Autumn Meeting   |
| Sept. 11–16  | Toronto, Canada           | American Academy of Clinical Toxicology   |
| Sept. 15–18  | Halifax, Canada           | Canadian Society of Forensic Sciences   |
| Oct. 5–8     | Rhodes, Greece            | 45th Congress of the European Societies of Toxicology                             |
| Oct. 8–10    | Dublin, Ireland           | Association of Inhalation Toxicologists   |
| Oct. 27–31   | Phoenix, Arizona          | Society of Forensic Toxicologists   |
| Nov. 9–12    | Tucson, Arizona           | American College of Toxicology  |
| Nov. 16–20   | Tampa, Florida            | SETAC North America 29 <sup>th</sup> Annual Meeting                               |
| Dec. 1–2     | Montreal, Canada          | Society of Toxicology of Canada   |
| Dec. 7–10    | Chandigarh, India         | Asia Pacific Association of Medical Toxicology                                    |
| <b>2009</b>  |                           |   |
| Date unknown | Copenhagen, Denmark       | European Workplace Drug Testing Society   |
| Feb. 16–21   | Denver, Colorado          | American Academy of Forensic Sciences   |
| March 15–19  | Baltimore, Maryland       | Society of Toxicology   |
| March 22–25  | Warwick, Coventry, U.K.   | British Toxicology Society Annual Congress  |
| July 19–23   | Chicago, Illinois         | American Association for Clinical Chemistry                                       |
| August 16–21 | Montreal, Canada          | International Association for Therapeutic Drug Monitoring and Clinical Toxicology |
| Sept. 6–10   | Sun City, South Africa    | 7 <sup>th</sup> Congress of Toxicology in Developing Countries                    |
| Sept. 21–26  | San Antonio, Texas        | American Academy of Clinical Toxicology   |
| Oct. 1       | Dresden, Germany          | 46th Congress of the European Societies of Toxicology                             |
| Oct. 19–23   | Oklahoma City, Oklahoma   | Society of Forensic Toxicologists   |
| Nov. 19–23   | New Orleans, Louisiana    | SETAC North America 30 <sup>th</sup> Annual Meeting                               |
| <b>2010</b>  |                           |   |
| Feb. 22–27   | Seattle, Washington       | American Academy of Forensic Sciences   |
| March 7–11   | Salt Lake City, Utah      | Society of Toxicology   |
| July 11–15   | Barcelona, Spain          | The XII International Congress of Toxicology                                      |
| August 22–26 | Oslo, Norway              | International Council on Alcohol, Drugs and Traffic Safety                        |
| Oct. 18–22   | Richmond, Virginia        | Society of Forensic Toxicologists   |

Illinois, Indiana, Michigan, and Ohio. Meeting locations vary between the states, with an occasional meeting in Kansas. The goal is to provide meetings within driving distance of most members.

The American Academy of Forensic Sciences encompasses all 11 forensic science disciplines. It has associated organizations at both the regional and international levels. Some of the more active of these associations are the California Association of Criminalistics, Northwest Association of Forensic Scientists, and Southern Association of Forensic Scientists (SAFS). SAFS was formed in 1966 with 47 members and has expanded to more than 500. Although these regional meetings include related forensic science information, there are very few toxicology presentations.

### Functions of regional organizations

Regional toxicology associations provide several important functions. They meet within driving distance for most members, which helps members who may not have the opportunity to travel to national or international meetings. In addition to educational presentations, these meetings provide the opportunity to informally exchange ideas and socialize with colleagues in the profession. Regional meetings afford younger, less experienced members the opportunity to begin their professional careers with presentations in smaller, less formal arenas.

On occasion, these groups have joint meetings with other regional or national associations. For example, CAT and SAT have had joint meetings; CAT is having a joint meeting with SOFT this fall; and MATT has had a joint meeting with an international group, the Society of Hair Testing.

Most regional associations publish a high-quality newsletter for additional information exchange. These associations also provide opportunities for younger members to gain experience in areas such as meeting planning and development or committee assignments for specific projects. Several provide additional benefits such as lending libraries, online education, and a listserv program for members to exchange ideas.

*Donald L. Frederick, PhD, DABFT, is with the Peoria Tazewell Pathology Group in Peoria, Illinois.*

## CLSI Guideline

*Continued from page 1*

An easy rule of thumb to distinguish between the two situations is that forensic testing requires a chain of custody to document the collection and handling of the specimen, whereas clinical testing does not. In forensic situations, the specimen is legal evidence and must be treated as such. Failure to control the specimen and document its handling invalidates the findings of the analytical procedure.

There can be gray areas between clinical and forensic testing. For example, a positive result for an abused drug on a specimen from a pregnant woman may have legal as well as medical implications. The same applies for an ethanol test requested on a patient being treated in the emergency room after a vehicular accident. All potential situations cannot be foreseen, but it's important that laboratories have a basic understanding of clinical vs. forensic testing and set up their procedures accordingly.

### Typical drugs

C52 notes the typical drugs for which clinical labs test: volatiles (alcohols), acetaminophen, salicylate, the common therapeutic drugs (such as theophylline, valproic acid, and digoxin), and the common drugs of abuse (such as amphetamines, cannabinoids, cocaine, opiates, and PCP). Although clinical testing is confined to specimens from patients for medical reasons, some of the specimens may be from patients who are comatose or under the influence of ethanol or drugs of abuse. Serum is the most common matrix for therapeutic and non-prescription drugs such as acetaminophen and salicylates, but urine is routine for DOA testing.

Specific immunoassays are commonly used for the routine TDMs and DOAs, but C52 describes various other screening methods, such as thin-layer chromatography and point-of-care-testing (POCT) devices. POCT devices for toxicology were not as prevalent when the first version of this guideline (T/DM8) was written, but are now very common. Laboratorians must understand the limitations of POCT devices and the need to ensure that testing conducted using them meets the same standards expected of testing in traditional central labs. This requirement is particularly critical if forensic testing is performed.

### Need for confirmation

Quantitative TDM assays are standard in many clinical laboratories and do not require much elaboration in C52. DOA assays, which are also readily available and in common use, receive more specific

attention because DOA testing is typically performed in two phases: screening and confirmation.

The screening immunoassays identify negative specimens and "presumptive positives." In some cases, a positive DOA immunoassay result may be adequate when coupled with other patient information; for example, in the case of initial work up of a comatose or incoherent emergency room patient or to confirm that a patient in a heroin detoxification program is taking methadone. But when performing procedures with legal consequences, confirmation testing requires using a more specific, physicochemical method, preferably a chromatographic technique such as high-performance liquid chromatography, gas chromatography (GC), or GC/mass spectrometry. Because confirmation is critical for DOA testing in forensic applications, C52 points to another CLSI guideline, C43, for detailed guidance (3).

DOA testing in the clinical laboratory is further confounded by the concept of cutoff concentrations. Qualitative screening immunoassays are designed to yield a positive result if the drug concentration exceeds a pre-set value. Laboratorians need to understand that a negative immunoassay result doesn't necessarily mean that a patient has not taken a drug. To further complicate the picture, confirmatory tests often use different cutoffs than the immunoassays.

C52 provides basic information about DOA testing, but there are specialized considerations for the DOA assays that can cause confusion for clinical laboratorians if they equate DOA tests to TDM assays. C52 also describes a variety of other topics, such as how samples should be stored; cross-reactivity considerations for immunoassays; reporting of TDM, DOA, and other toxicology tests; retention of records; and the need for confidentiality of test results.

### Forensic testing

C52 concludes with a section devoted to forensic testing. Although the revised guideline emphasizes clinical toxicology, it is exactly because most routine clinical laboratories perform little, if any, forensic toxicology testing that this testing receives detailed consideration. Laboratories must understand the requirements of non-medical testing for those atypical situations in which they engage in it or before deciding to perform these procedures on a routine basis.

The guideline reviews several applications of forensic testing, including pre-employment testing, for-cause testing, random drug testing of employees in safety-related occupations, return-to-work examinations, monitoring of drug users undergoing detoxification, criminal justice testing of prisoners and parolees, athletic testing, and driving-while-intoxicated/driving-under-the-influence testing.

The guideline devotes considerable attention to the appropriate method for collecting urine specimens. Because the subjects providing urine specimens for forensic testing are not patients but individuals whose positive tests may result in adverse consequences, such as denial of employment or incarceration, some of them will tamper with their specimens. Possible manipulation includes dilution of a specimen to drop the concentration of a drug below the screening cutoff, substitution of one's own specimen with a "negative" sample, and adulteration by the addition of substances designed to mask a drug or interfere with an analytical method. Although direct observation of specimen collection is a sure means of precluding tampering with specimens, it is considered to be unduly intrusive and is allowed only under certain circumstances.

### Chain of custody

C52 notes early on that the chain of custody is the hallmark of forensic testing and that its use clearly separates forensic from clinical testing. The final section of the guideline lays out the many requirements for proper completion of the chain of custody form, which is a legal document subject to intense scrutiny in a court of law. Careless or incomplete completion of this documentation can invalidate the most careful and scientifically rigorous testing. C52 includes an example of acceptable chain of custody documentation as an appendix.

As with any CLSI guideline, C52 is intended to apply to clinical and forensic testing as conducted

around the world. As such, it provides adequate detail for laboratorians without being overly prescriptive.

A recurrent theme of the document is that it is the responsibility of each laboratory to consciously decide on the type and extent of drug testing that it should undertake, guided by the needs of its clients, the medical staff, and patients. The guideline underwent a dramatic evolution from T/DM8 to the current C52 to reflect current conditions. C52 is expected to undergo future revisions to reflect new directions and developments in the field.

### References

1. Clinical and Laboratory Standards Institute. CLSI C52: Toxicology and drug testing in the clinical laboratory; approved guideline, 2nd ed. Wayne, Pennsylvania: CLSI, 2007.
2. Wu AHB, McKay C, Broussard L, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines: recommendations for the use of laboratory tests to support poisoned patients who present to the emergency department. *Clin Chem* 2003;49:357-79.
3. Clinical and Laboratory Standards Institute. CLSI C43: Gas chromatography/mass spectrometry (GC/MS) confirmation of drugs; approved guideline. Wayne, Pennsylvania: CLSI, 2002.

*David Armbruster, PhD, DABCC, FACB, is a scientific affairs manager with Abbott Diagnostics in Abbott Park, Illinois.*

The purpose of *Clinical & Forensic Toxicology News* is to provide practical and timely information about the clinical, forensic, technical, and regulatory issues faced by toxicology laboratories.

*Clinical & Forensic Toxicology News* is an educational service of the forensic urine drug testing (FUDT) program. The FUDT program, cosponsored by the American Association for Clinical Chemistry and the College of American Pathologists, includes three components: FUDT accreditation, the FUDT proficiency testing survey, and this newsletter. The accreditation program is the responsibility of the CAP. The surveys are sponsored jointly by AACC and CAP. The newsletter is published quarterly by the American Association for Clinical Chemistry, Inc., 1850 K St., N.W., Suite 625, Washington, DC 20006, (800) 892-1400 or (202) 857-0717, [cftnews@aacc.org](mailto:cftnews@aacc.org).

*Clinical & Forensic Toxicology News* does not accept advertising and is supported solely by its readers. Annual subscription prices are \$98 (U.S.) and \$109 (international). Subscribers are encouraged to reproduce copy with appropriate acknowledgment of source.

Opinions expressed are those of the authors and do not represent the position of the AACC or CAP.

### Editorial Advisory Board:

Chair: Loralie Langman, PhD, Mayo Clinic, Rochester, MN, [langman.loralie@mayo.edu](mailto:langman.loralie@mayo.edu)

Jennifer Collins, PhD, MEDTOX Laboratories, Saint Paul, MN, [jcollins@medtox.com](mailto:jcollins@medtox.com)

Amitava Dasgupta, PhD, University of Texas-Houston Medical School, Houston, TX, [amitava.dasgupta@uth.tmc.edu](mailto:amitava.dasgupta@uth.tmc.edu)

Donald L. Frederick, PhD, Peoria Tazewell Pathology Group, Peoria, IL, [dfrederick@ptpg.com](mailto:dfrederick@ptpg.com)

Nikolas P. Lemos, PhD, Office of the Chief Medical Examiner, San Francisco, CA, [nlemos@yahoo.com](mailto:nlemos@yahoo.com)

John M. Wilson, PhD, William Beaumont Hospital, Royal Oak, MI, [jwilson@beaumont.edu](mailto:jwilson@beaumont.edu)

Readers are invited to submit questions they would like answered by an expert. An e-mailable PDF copy of this newsletter is available: [cftnews@aacc.org](mailto:cftnews@aacc.org).

Editorial Consultant: Eric Seaborg

**AACC**

Advancing  
Clinical Laboratory  
Science Worldwide

© 2008 American Association for Clinical Chemistry, Inc.

Visit the AACC home page: [www.aacc.org](http://www.aacc.org)



Advancing Excellence