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Blood Lead Testing

Although Blood Lead Levels Have Declined, Testing Retains a Critical Role

By He Sarina Yang, PhD

Lead is a naturally occurring heavy metal that exists in both organic and inorganic forms. Although recent data have shown a decline in the prevalence of high blood lead levels (BLL) in developed countries, lead poisoning remains a major public health concern globally because of its long-lasting adverse health and behavioral effects.

U.S. public health services and health professionals have dedicated more than 40 years of effort to screening children, especially those at high risk, for lead exposure and to identifying and reducing sources of exposure. Policies adopted to cut exposure include phasing out leaded gasoline, eliminating lead in paints, and testing homes for lead-based paint.

The 2007–10 National Health and Nutrition Examination Survey (NHANES) estimated the mean blood lead level in the U.S. to be 1.3 µg/dL, which was a 90% decrease compared with the 1976–80 NHANES estimate of 12.8 µg/dL (1). The 2009–15 NHANES survey, which included 3.8 million venous blood samples from children 6 years old or younger, revealed that only 3% exhibited blood lead levels ≥ 5 µg/dL (2).

Risk factors associated with higher BLLs include living in an area with a high proportion of pre-1950 housing, low income, race, and ethnicity, with the non-Hispanic black population at greatest risk (3).

The Centers for Disease Control and Prevention (CDC) has designated the 97.5 percentile from NHANES—5 µg /dL—as the threshold for an elevated BLL in children and adults (2). The National Institute for Occupational Safety and Health also uses 5 µg/dL in venous blood samples as the reference level for adults.

The U.S. Occupational Safety and Health Administration requires workers to be removed from lead exposure when BLLs equal or exceed 50 µg/dL in the construction industry or 60 µg/dL in general industry. Workers are allowed to return to work when their BLL drops below 40 µg/dL.

Sources of exposure

Common sources of lead exposure include lead-based paint in old houses, household dust containing lead, drinking water delivered through lead-soldered pipes, soil contaminated by lead, food stored in lead-soldered cans or painted ceramic containers, and folk remedies (3). Young children are at higher risk than adults because they engage in hand-to-mouth activities and sometimes swallow nonfood items. The primary source of excessive exposure for young children is chronic ingestion and inhalation of lead-contaminated dust (4). In addition, cases of lead poisoning in children of pottery artisan families have been reported recently (5,6).

Adults who work with lead in their job or as a hobby risk exposure. High-risk occupations include battery manufacturing and recycling, metal smelting, welding, printing, pottery making, glass making, and stained-glass work (7). Workers at shooting ranges have also been found to have elevated BLLs (8,9).

Clinical presentation

Signs and symptoms of lead exposure vary greatly. Early symptoms are often nonspecific. Most lead-exposed children are asymptomatic at the time of screening. Initial symptoms may include gastrointestinal problems such as anorexia, vomiting, abdominal pain, or constipation. For example, a nine-year-old child who was later diagnosed with lead poisoning presented to the hospital with strong ab-

Continued on page 2

Inside...

Blood Lead Testing

Continued from page 1

dominal pain and vomiting (10). Even low levels of lead can affect the nervous system, causing behavioral changes such as hyperactivity, attention deficit disorder, memory loss, hyperirritability, ataxia, and hallucinations (11). Lead-induced anemia can result from impaired heme biosynthesis and decreased hemoglobin production.

High lead levels can impair renal function, with symptoms including aminoaciduria, glycosuria, and hyperphosphaturia (3). Numerous studies have shown that lead interferes with normal brain development and can result in delayed development, intellectual deficit, reduced IQ, increased adolescent impulsivity, anxiety, depression, and poor academic achievement (6,12). Because these changes are usually irreversible, clinicians must take lead poisoning into consideration when examining a child with gastrointestinal and/or neurological symptoms.

Pathophysiology

Although adults absorb only 5–10% of ingested lead, children can absorb as much as 50% because they have higher rates of absorption for the divalent cations iron and calcium (4). Because absorbed lead is initially found in the blood and primarily bound to red cells, the whole blood lead level is the most sensitive biomarker of recent exposure and has the strongest correlation with toxicity. Serum and plasma are not appropriate specimens because lead concentrations are too low in these matrices.

Lead redistributes from the blood to the body's soft and mineralizing tissues. Lead in soft tissues binds covalently with cysteine thiol groups of proteins, resulting in changes in protein tertiary structure and function. Cells of the nervous system are particularly susceptible to this effect (13). Renal tubular cells are also susceptible to lead.

In addition, lead inhibits the enzyme δ-aminolevulinic acid dehydratase (δ-ALAD) and blocks the ferrochelatase insertion of iron into protoporphyrin IX. These blockades increase circulating δ-ALAD and free erythrocyte protoporphyrin and can result in anemia (12).

Lead is ultimately distributed among all tissues with the greater body burden deposited as the divalent ion in trabecular bones. Lipid-dense tissues, such as the central nervous system, are particularly sensitive to organic forms of lead. The elimination half-life of lead from blood is 30–50 days in adults, which likely reflects both elimination from the body and redistribution to bone (4).

Testing Methodologies

A number of laboratory methods are available to determine BLL. Currently, the best-known technologies are flame atomic absorption spectrometry (FAA), electrothermal atomic absorption spectrometry (also called graphite furnace atomic absorption spectrometry, GFAA), and inductively coupled plasma-mass spectrometry (ICP-MS).

The CLIA-waived LeadCare Testing Systems, including LeadCare, LeadCare II, LeadCare Plus, and LeadCare Ultra (Magellan Diagnostics) are popular because they are portable; use electronic calibration, simple procedures, and disposable consumables; and can generate results for capillary blood specimens in a few minutes in physicians' office or small clinical laboratory settings. However, in April 2017, Magellan Diagnostics notified customers that the LeadCare Testing Systems may underestimate BLLs and give inaccurate results when used with venous blood. The Food and Drug Administration issued a Class I product warning that laboratories and healthcare professionals should not use any Magellan Diagnostics' LeadCare device for testing venous blood specimens. The product warnings do not apply to capillary specimens.

Atomic Absorption Methods

Atomic absorption (AA) methods have long been the mainstay for blood lead testing. AA uses the principle of generating ground-state free atoms in a flame or a graphite-coated furnace and measuring the amount of light absorbed from a wavelength-specific light source.

Lead is vaporized and atomized at a high temperature, and lead atoms absorb light at characteristic wavelengths. Within limits, the amount of light absorbed can be linearly correlated to the BLL. The most common application of FAA in lead testing is to analyze dried blood spots on filter paper with no preliminary sample preparation (4).

The analytical principle of GFAA is the same as FAA, except that a small GFAA-heated graphite tube replaces the flame. Most laboratories have replaced FAA with GFAA due to its improved sensitivity and lower specimen volume requirements. GFAA technology has sub-parts per billion (mg/L) sensitivity, relatively narrow dynamic range, and low throughput. It is a single-element technique that measures only one analyte at a time.

ICP-MS

ICP-MS uses a high temperature plasma discharge to generate positively charged ions that are measured for their mass-to-charge (m/z) ratio by mass spectrometry. Liquid sample is pumped through a peristaltic pump into a nebulizer where the liquid sample is broken up into a fine aerosol. A spray chamber filters out big droplets and injects the smallest droplets into an ICP plasma torch. Inside the ICP plasma torch, argon gas ionizes to argon ions and free electrons, and forms what is called ICP discharge. When tiny droplets of the sample are injected

at a high speed into the ICP plasma torch, the hot plasma dries the aerosol, dissociates the molecules, and forms singly-charged positive ions by stripping off an electron from the analyte atoms. The positively charged analyte ions are transported through the interface, which consists of two metallic cones with very small orifices. Ions are transported from the plasma at atmospheric pressure via the interface cones and propelled into the mass analyzer, which is under high vacuum. Ion optics focuses these ions into a beam for transmission into the quadrupole. The quadrupole acts as a mass filter to sort ions by m/z and allows only ions with a specific m/z to pass through and hit the detector. The detector counts individual ions passing through the quadrupole and translates the signal-to-analyte concentration (14).

ICP-MS is a rapid, ultra-trace, multi-element technique. It has parts-per-trillion (ng/L) sensitivity, a dynamic range of eight orders of magnitude, and high throughput. ICP-MS can measure multiple elements simultaneously and has a unique capability to perform isotopic analysis and speciation analysis.

Although ICP-MS is powerful, it may not be the best choice for every laboratory. It is highly complex and requires great technical expertise. In addition, ICP-MS instrumentation usually costs three times more than GFAA, and the operating and consumables costs are also higher. A thorough evaluation is needed before a laboratory purchases one.

Lead Testing Tips

In the development of an ICP-MS assay, an internal standard is used to correct for signal suppression or enhancement and improve precision. The internal standard should have a mass number close to that of the analyte element. For example, bismuth (^{209}Bi) or iridium (^{193}Ir) can serve as a standard for lead.

Lead has several isotopes, with ^{206}Pb, ^{207}Pb, and ^{208}Pb being the most stable and abundant. In an ICP-MS method, the counts of 206, 207 and 208 m/z should be summed to account for the natural isotopic variation of lead in the environment. Failure to do so will result in inaccurate results. Because lead accumulates in red blood cells, which can settle in the specimen, whole blood specimens should be thoroughly mixed prior to testing.

Contamination issues are especially challenging in element testing. The ICP-MS should be in a clean room and placed on a clean bench. Specimens should be collected in trace-metal-free tubes. All reagents, such as methanol, ethanol, nitric acid, and other cleaning solutions, should be tested for lead contamination prior to use. Lead contamination in the reagents causes background noise and negatively affects the precision of the assay.

Capillary blood specimens are often used for pediatric lead screening. False-positive results are occasionally seen in capillary specimens. For example, at the author's lab, a four-year-old child had a capillary blood lead of 125 µg/dL, but the confirmatory venous blood result was 0.5 µg/dL (data not published). Lead on the skin can contaminate specimens, so children's hands must be washed prior to capillary sample collection. Clinicians should interpret capillary results with caution and order a confirmatory venous blood test if capillary BLL is higher than 5 µg/dL.

Conclusion

Although blood lead levels have dropped significantly in developed countries, lead is a legacy poison that remains an environmental health threat globally, associated with social and economic factors. According to the CDC, there is no safe level of lead in children's blood. Several methodologies are available for blood lead testing in clinical laboratories, with a variety of sensitivity, simplicity, cost, and speed. Compared with traditional flame or graphite furnace atomic absorption spectrometry, ICP-MS is an ultra-trace, multi-element technique that provides superior sensitivity, dynamic range, and throughput. Contamination is possible from skin, the environment, and a non-certified collection device. Proper specimen collection and controlling for contamination are critical for element testing.

Learning Objectives

After reading this article, the reader will be able to discuss the signs and symptoms of lead poisoning. The reader will also be able to compare the strengths and weaknesses of current technologies in trace element testing, such as FAA, GFAA, and ICP-MS; choose an appropriate instrument that fits the needs of the clinical laboratory; and describe the basic principles and major components of an ICP-MS.

References

1. Pirkle JL, Brody DJ, Gunter EW, et al. The decline of blood lead levels in the United States: the National Health and Nutrition Examination Surveys (NHANES). JAMA 1994;272:284–91.
2. McClure LF, Niles JK, Kaufman HW. Blood lead levels in young children: U.S., 2009–2015. J Pediatr 2016;175:173–81.
3. Dapul H, Laraque D. Lead poisoning in children. Adv Pediatr 2014;61:313–33.
4. Kwong TC, Magnani BJ, Rosano TG, et al. The clinical toxicology laboratory: contemporary practice of poisoning evaluation, 2nd ed. Washington, D.C.: AACC Press, 2013.
5. Marginean CO, Melit LE, Moldovan H, et al. Lead poisoning in a 16-year-old girl: a case report and a review of the literature (CARE compliant). Medicine (Baltimore) 2016;95:e4916.
6. Estrada-Sánchez D, Ericson B, Juárez-Pėrez CA, et al. Intelligence quotient loss in Mexican pottery artisan's children. Rev Med Inst Mex Seguro Soc 2017;55:292–9.
7. Qasim SF, Baloch M. Lead toxicity in battery workers. J Coll Physicians Surg Pak. 2014;24 Suppl 3:S284–6.
8. Laidlaw MA, Filippelli G, Mielke H, et al. Lead exposure at

firing ranges—a review. Environ Health 2017;16:34.
9. Mathee A, De Jager P, Naidoo S, et al. Lead poisoning in shooting-range workers in Gauteng Province, South Africa: two cases studies. S Afr Med J 2017;107:302–3.
10. Mottiera DM, Cargnel E. Abdominal pain as a presentation by lead poisoning. Case report. Arch Argent Pediatr 2017;11592:e96–8.
11. Lyer S, Sengupta C, Velumani A. Lead toxicity: an overview of prevalence in Indians. Clin Chim Acta 2015;451:161–4.
12. Winter AS, Sampson RJ. From lead exposure in early childhood to adolescent health: a Chicago birth cohort. Am J Public Health 2017;107:1496–1501.
13. Burtis CA, Bruns DE. Tietz fundamentals of clinical chemistry and molecular diagnostics, 7th ed. St. Louis: Elsevier/Saunders, 2015.
14. Thomas R. Practical guide to ICP-MS: a tutorial for beginners, 3rd ed. Boca Raton, Fla.: CRC Press, 2013.

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AACC Publishes Guide on Testing To Monitor Pain Management

The AACC Academy, formerly the National Academy of Clinical Biochemistry, has developed a laboratory medicine practice guideline for using laboratory tests to monitor drug therapy in pain management patients. The guideline provides evidence-based recommendations for using laboratory and point-of-care urine drug tests for over-the-counter medications, prescribed and non-prescribed drugs, and illicit substances in pain management patients. Opioids are the mainstay therapy for moderate and severe pain, but they pose the risk of addiction and abuse, so monitoring of patients for compliance is commonplace.

The guideline writers reviewed the literature regarding the clinical utility and use of urine and alternative specimen types, assorted assay formats, different assay types (screening vs. confirmatory), and the inclusion of specimen validity testing and pharmacogenomics testing, as well as the reporting, communication, and interpretation of test results back to clinicians. The target audience is the laboratorians who perform pain management testing and the clinicians who order, use, and interpret these tests.

The guideline is available at: https://www.aacc.org/science-and-practice/practice-guidelines/using-clinical-laboratory-tests-to-monitor-drug-therapy-in-pain-management-patients.

Challenge of Synthetic Opioids *Analytical Detection Needs to Keep up with Emerging Trends*

By Kara L. Lynch, PhD

According to the U.S. Centers for Disease Control and Prevention (CDC), overdose deaths from synthetic opioids (excluding methadone) increased tenfold from 2006 to 2016. These drugs are now the leading cause of overdose deaths annually in the U.S. Many of the deaths can be attributed to a proliferation of adulterated heroin and counterfeit opioid pills in the illicit drug market. A smaller percentage has been attributed to the increased availability of synthetic opioids on the internet and darknet.

Synthetic opioids—such as fentanyl, counterfeit fentanyl pills (known as nonpharmaceutical fentanyl or NPF), fentanyl analogs, and novel compounds such as U-47700 and MT-45—are an emerging public health threat. These compounds have been detected in heroin and illicit pills across the U.S. Laboratory professionals should be aware of the adulteration trends and analytical methods that can be used to aid in understanding the ongoing epidemic.

Epidemiology

The rise in counterfeit fentanyl pills and NPF-laced heroin and cocaine started in the mid-2010s. From 2012 to 2014, the number of NPF-related deaths reported in the U.S. more than doubled (1). NPF proliferation has created a significant public health crisis because those exposed are unaware that they consumed an adulterated product, but rather assume they are using standard heroin, cocaine, or prescription-strength opioid pills. Street-purchased counterfeit Xanax and Norco pills containing fentanyl were responsible for two overdose outbreaks in northern California from late 2015–16 (2–4) and subsequent outbreaks across the U.S. Thirteen fentanyl overdoses were reported in June 2016 in Connecticut related to fentanyl-adulterated cocaine (5).

The rise in the production of counterfeit pills and NPF-laced heroin and cocaine is expected to continue due to the ease of manufacturing and ready availability precursors.

Fentanyl Analogs and Novel Opioids

The issue is bigger than just NPF and extends to potent fentanyl analogs and novel synthetic opioids. In 2013, acetylfentanyl emerged as a fentanyl analog responsible for numerous fatalities in Rhode Island, Pennsylvania, and North Carolina (6,7). The magnitude of this outbreak may have been underestimated because clinical and forensic toxicology laboratories did not routinely monitor for acetylfentanyl. The CDC published a public health advisory in 2015 rec-

ommending more extensive toxicological analysis when sudden increases in opioid overdoses occur.

In 2016, the Drug Enforcement Administration (DEA) declared that butyryl fentanyl and beta-hydroxythiofentanyl were associated with numerous fatalities in 2015. At least 40 confirmed overdose deaths involving butyryl fentanyl abuse were reported in Maryland, New York, and Oregon, and at least seven confirmed overdose fatalities involving beta-hydroxythiofentanyl were reported in Florida (8).

In 2016, carfentanil, which is used legally as a general anesthetic for large animals, made its way into the U.S. heroin supply. The first outbreak occurred in the Midwest and Appalachian regions in August and September 2016. The DEA estimated there were 300 carfentanil overdoses during this time. Since then, carfentanil has swept across the country, causing numerous fatalities. Recently there have also been reports of overdoses due to acrylfentanyl, octfentanil, and furanyl fentanyl, to name a few.

In addition to fentanyl analogs, the novel synthetic opioids AH-7921, U-47700, and MT-45 have become available primarily via the internet or hidden services on the darknet. In 2013, AH-7921 was discovered as an active ingredient in synthetic cannabis products in Japan (9). It has since been identified in numerous overdose cases across Europe and the U.S. (10–14). Similarly, the DEA received reports of at least 46 confirmed fatalities in 2015 and 2016 from the use of U-47700. MT-45 was first reported as a novel psychoactive substance through the early warning system of the European Monitoring Centre for Drugs and Drug Addiction in December 2013. Since then, there have been reports of abuse and overdose in Europe and the U.S. (14–17).

Analytical Detection

The true extent of the synthetic opioid epidemic is unknown due to the lack of routine diagnostic monitoring. The opiate immunoassays approved by the Food and Drug Administration (FDA) included in routine urine toxicology testing do not cross-react with the synthetic opioids because the drugs have little structural homology to morphine. Definitive detection requires the use of more complex methods, such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS). In the early 2010s, the need for fentanyl compliance monitoring in chronic pain management patients led to the development of automated fentanyl immunoassays; however, not all clinical laboratories offer fentanyl testing in real time.

The first automated homogeneous enzyme immunoassay for the detection of fentanyl in urine was developed in 2011 (Immunalysis Corporation) (18). This assay cross-reacts with two of the major fentanyl metabolites, hydroxyfentanyl and depropionylfentanyl, but does not detect norfentanyl. An independent laboratory evaluation of this assay found it was rapid and accurate (99%) for fentanyl detection in monitoring fentanyl compliance and abuse (19). The manufacturer claims that the assay cross-reacts (>10% cross-reactivity) with 4-metylbutyrl fentanyl, acetyl fentanyl, butyryl fentanyl, carfentanil, furanyl fentanyl, isobutyryl fentanyl, trans-methylfentanyl, and valeryl fentanyl; however, this claim has not been independently confirmed.

A similar automated homogeneous enzyme immunoassay (DRI Fentanyl Assay, Thermo Scientific) was proven to cross-react with acetylfentanyl, risperidone, and 9-hydroxyrisperidone, but not with norfentanyl (20). Fentanyl, acetylfentanyl, risperidone, and 9-hydroxyrisperidone all share an intramolecular alkylated piperidine (3-methyl-5-piperidino-2-pentene) that is not present in norfentanyl. This is likely recognized in part by the antibody for the immunoassay. This moiety could potentially be used to predict cross-reactivity with other fentanyl analogs, but the assay has not been evaluated for the detection of other analogs. These fentanyl immunoassays are generally for "forensic use only."

In July 2017, the FDA approved the first fentanyl immunoassay for clinical diagnostics (Fentanyl Urine SEFRIA, Immunalysis).

Specific enzyme-linked immunosorbent assays (ELISAs) are also available for fentanyl, MT-45, AH-7921, and U-47700 (Randox Toxicology), but involve more manual processes compared with automated immunoassays. Randox Toxicology also offers an automated designer fentanyl and opioids biochip array that detects fentanyl and 12 additional synthetic opioids. However, this assay has not been independently evaluated in the literature or approved by the FDA.

GC-MS and LC-MS/MS

The fentanyl immunoassays and ELISAs can be used to screen for fentanyl, select fentanyl analogs, and novel synthetics. Mass spectrometry is required for definitive detection, but is not routinely available in hospital laboratories for real-time testing. Numerous methods have been published dating back to the early 1980s.

GC-MS has the advantage of offering untargeted data acquisition with library searching of acquired mass spectra for compounds detected in biological specimens. Large GC-MS mass spectral libraries are commercially available and transferrable across different vendors' instruments. In recent years, considerable focus has been devoted to adding spectra for designer drugs and novel psychoactive substances to commercial libraries. However, GC-MS methods cannot directly analyze drugs that are nonvolatile, polar, or thermally labile. Thus, the methods require lengthy sample preparation, so are not amenable to rapid testing.

LC-MS/MS methods do not require lengthy sam-

ple preparation, but they are commonly targeted such that they detect only the synthetic opioids for which they were designed. Targeted LC-MS/MS methods designed to simultaneously detect fentanyl, fentanyl analogs, and other synthetic opioids were first published in 2009 and variations are still used in many laboratories (21,22).

Given the continued emergence of novel synthetics, the major disadvantage of GC-MS and LC-MS/MS is that they are targeted in nature or limited by the availability of pre-established mass spectral libraries.

LC-HRMS

Liquid chromatography-high resolution mass spectrometry (LC-HRMS) using quadrupole time-of-flight or orbitrap technology offers potential advantages in the detection of emerging synthetic opioids. This method can provide tentative identification of unknowns without a reference standard or a library spectrum. It acquires data in an untargeted manner and can be retrospectively analyzed for new and emerging synthetics.

In recent years, LC-HRMS has been used to detect synthetic opioids such as butyrfentanyl, 4-fluorobutyrfentanyl, acetylfentanyl, 4-methoxybutyrfentanyl, furanylfentanyl, acrylfentanyl, 4-chloroisobutyrfentanyl (4Cl-iBF), 4-fluoroisobutyrfentanyl (4F-iBF), tetrahydrofuranfentanyl (THF-F), cyclopentylfentanyl, AH-7921, MT-45, and U-47700 in a variety of contexts, including individual cases, case series, outbreaks, and epidemiological surveillance efforts (3,17,23–34). It has also been used for the elucidation of the metabolic pathways of emerging synthetic opioids, such as AH-7921, butyrfentanyl, carfentanil, U-47700, furanyl fentanyl, acetylfentanyl, acrylfentanyl, and 4-fluoroisobutyrylfentanyl (24,28,30,31,33–35).

LC-HRMS is clearly the predominant method for the detection of synthetic opioids, but it is not currently available in most clinical and forensic laboratories.

Conclusion

The opioid epidemic calls for increased vigilance across all sectors of public health. Clinical and forensic laboratories are often on the frontlines of determining the causative agent in outbreaks and individual cases presenting to hospitals across the country. Although most cases are identified as an opioid overdose and treated as such immediately by first responders and emergency room clinicians, definitive detection of the causative agent provides a more complete picture of the epidemic. It also provides an understanding of the clinical effects of specific synthetic opioids. More research is needed to understand the extent of cross-reactivity of fentanyl analogs with fentanyl immunoassays. At the very least, clinical laboratories should offer immunoassay screening for fentanyl. The identification of emerging synthetic opioids will continue to require untargeted mass spectrometry approaches, such as LC-HRMS.

Learning Objectives

After reading this article, the reader will be able to list fentanyl analogs and synthetic opioids that have been identified in the U.S. over the past five years. The reader will also be able to describe different analytical approaches that can be used to identify fentanyl, fentanyl analogs, and synthetic opioids in the clinical laboratory.

References

1. Gladden RM, Martinez P, Seth P. Fentanyl law enforcement submissions and increases in synthetic opioid-involved overdose deaths—27 states, 2013–2014. MMWR Morb Mortal Wkly Rep 2016;65:837–843.
2. Arens AM, van Wijk XM, Vo KT, et al. Adverse effects from counterfeit alprazolam tablets. JAMA Intern Med 2016;176;1554–1555.
3. Vo KT, van Wijk XM, Lynch KL, et al. Counterfeit norco poisoning outbreak—San Francisco Bay Area, California, March 25–April 5, 2016. MMWR Morb Mortal Wkly Rep 2016;65:420–423.
4. Sutter ME, Gerona R, Davis MT, et al. Fatal fentanyl: one pill can kill. Acad Emerg Med 2016;24:106–113.
5. Tomassoni AJ, Hawk KF, Jubanyik K, et al. Multiple fentanyl overdoses—New Haven, Connecticut, June 23, 2016. MMWR Morb Mortal Wkly Rep 2017;66:107–11.
6. Lozier MJ, Boyd M, Stanley C, et al. Acetyl fentanyl, a novel fentanyl analog, causes 14 overdose deaths in Rhode Island, March–May 2013. J Med Toxicol 2015;11:208–217.
7. Rogers JS, Rehrer SJ, Hoot, NR. Acetylfentanyl: an emerging drug of abuse. J Emerg Med 2015;50:433–6.
8. U.S. Department of Justice, Drug Enforcement Administration. Rules – 2016. Notice of intent: temporary placement of butyryl fentanyl and beta-hydroxythiofentanyl into Schedule I. https://www.deadiversion.usdoj.gov/fed_regs/rules/2016/fr0323_16.htm (Accessed February 2018).
9. Uchiyama N, Matsuda S, Kawamura M, et al. Two new-type cannabimimetic quinolinyl carboxylates, QUPIC and QUCHIC, two new cannabimimetic carboxamide derivatives, ADB-FUBINACA and ADBICA, and five synthetic cannabinoids detected with a thiophene derivative α-PVT and an opioid receptor agonist AH-7921 identified in illegal products. Forensic Toxicol 2013;31:223–240.
10. Vorce SP, Knittel JL, Holler JM, et al. A fatality involving AH-7921. J Anal Toxicol 2014;38:226–230.
11. Karinen R, Tuv SS, Rogde S, et al. Lethal poisonings with AH-7921 in combination with other substances. Forensic Sci Int 2014;244:21–24.
12. Coppola M, Mondola R. AH-7921: a new synthetic opioid of abuse. Drug Alcohol Rev 2015;34:109–10.
13. Katselou M, Papoutsis I, Nikolaou P, et al. AH-7921: the list of new psychoactive opioids is expanded. Forensic Toxicol 2015:33:195–201.
14. Fels H, Krueger J, Sachs H, et al. Two fatalities associated with synthetic opioids: AH-7921 and MT-45. Forensic Sci Int 2017;S0379-0738:30140–8.
15. Helander A, Bäckberg M, Beck O. MT-45, a new psychoactive substance associated with hearing loss and uncon-

sciousness. Clin Toxicol 2014;52:901–4.
16. Papsun D, Krywanczyk A, Vose JC, et al. Analysis of MT-45, a novel synthetic opioid, in human whole blood by LC-MS-MS and its identification in a drug-related death. J Anal Toxicol 2016;40:313–7.
17. Helander A, Bradley M, Hasselblad A, et al. Acute skin and hair symptoms followed by severe, delayed eye complications in subjects using the synthetic opioid MT-45. Br J Dermatol 2017;176:1021–7.
18. Wang G, Huynh K, Barhate R, et al. Development of a homogeneous immunoassay for the detection of fentanyl in urine. Forensic Sci Int 2011;206:127–31.
19. Snyder ML, Jarolim P, Melanson SE. A new automated urine fentanyl immunoassay: technical performance and clinical utility for monitoring fentanyl compliance. Clin Chim Acta 2011;412:946–51.
20. Wang BT, Colby JM, Wu AH, et al. Cross-reactivity of acetylfentanyl and risperidone with a fentanyl immunoassay. J Anal Toxicol 2014;38:672–5.
21. Lurie IS, Iio R. Use of multiple-reaction monitoring ratios for identifying incompletely resolved fentanyl homologs and analogs via ultra-high-pressure liquid chromatography-tandem mass spectrometry. J Chromatogr A 2009;1216:1515–9.
22. Gergov M, Nokua P, Vuori E, et al. Simultaneous screening and quantification of 25 opioid drugs in post-mortem blood and urine by liquid chromatography-tandem mass spectrometry. Forensic Sci Int 2009;186:36–43.
23. Backberg M, Beck O, Jönsson KH, et al. Opioid intoxications involving butyrfentanyl, 4-fluorobutyrfentanyl, and fentanyl from the Swedish STRIDA project. Clin Toxicol 2015;53:609–17.
24. Wohlfarth A, Scheidweiler KB, Pang S, et al. Metabolic characterization of AH-7921, a synthetic opioid designer drug: in vitro metabolic stability assessment and metabolite identification, evaluation of in silico prediction, and in vivo confirmation. Drug Test Anal 2016;8:779–91.
25. Helander A, Bäckberg M, Beck O. Intoxications involving the fentanyl analogs acetylfentanyl, 4-methoxybutyrfentanyl and furanylfentanyl: results from the Swedish STRIDA project. Clin Toxicol 2016;54:324–32.
26. Schneir A, Metushi IG, Sloane C, et al. Near death from a novel synthetic opioid labeled U-47700: emergence of a new opioid class. Clin Toxicol (Phila) 2017;55:51–4.
27. Armenian P, Olson A, Anaya A, et al. Fentanyl and a novel synthetic opioid U-47700 masquerading as street "Norco" in Central California: a case report. Ann Emerg Med 2017;69:87–90.
28. Jones MJ, Hernandez BS, Janis GC, et al. A case of U-47700 overdose with laboratory confirmation and metabolite identification. Clin Toxicol 2017;55:55–9.
29. Breindahl T, Kimergård A, Andreasen MF, et al. Identification of a new psychoactive substance in seized material: the synthetic opioid N-phenyl-N-[1-(2-phenethyl)-piperidin-4-yl]prop-2-enamide (acrylfentanyl). Drug Test Anal 2017.;9:415–22.
30. Steuer AE, Williner E, Staeheli SN, et al. Studies on the metabolism of the fentanyl-derived designer drug butyrfentanyl in human in vitro liver preparations and authentic human samples using liquid chromatography-high resolution mass spectrometry (LC-HRMS). Drug Test Anal 2017;9:1085–92.
31. Fleming SW, Cooley JC, Johnson L, et al. Analysis of U-47700, a novel synthetic opioid, in human urine by LC-MS-MS and LC-QToF. J Anal Toxicol 2017;41:173–80.
32. Guerrieri D, Rapp E, Roman M, et al. Postmortem and toxicological findings in a series of furanylfentanyl-related deaths. J Anal Toxicol 2017;41:242–9.
33. Goggin MM, Nguyen A, Janis GC. Identification of unique metabolites of the designer opioid furanyl fentanyl. J Anal Toxicol 2017;41:367–75.
34. Watanabe S, Vikingsson S, Roman M, et al. In vitro and in vivo metabolite identification studies for the new synthetic opioids acetylfentanyl, acrylfentanyl, furanylfentanyl, and 4-fluoro-isobutyrylfentanyl. AAPS J 2017;19:1102–22.
35. Feasel MG, Wohlfarth A, Nilles JM, et al. Metabolism of carfentanil, an ultra-potent opioid, in human liver microsomes and human hepatocytes by high-resolution mass spectrometry. AAPS J 2016;18:1489–99.

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The author has nothing to disclose.

Revisions to DOT Drug-Testing Program Took Effect January 1

By Jennifer A. Collins, PhD

In the September 2017 issue, *Clinical & Forensic Toxicology News* reported on revisions to the Department of Health and Human Services' Substance Abuse and Mental Health Services Administration (HHS/SAMHSA) Mandatory Guidelines for Federal Workplace Drug Testing Programs effective Oct. 1. At that time, the proposed revisions to the Department of Transportation (DOT) drug-testing rule, 49 CFR Part 40, had not been finalized. On Nov. 13, DOT published the final rule in the *Federal Register*, for implementation on Jan. 1, 2018 (1).

Similar to the revised HHS guidelines, the DOT rule adds four semisynthetic opioids to the testing panel and removes methylenedioxyethylamphetamine (MDEA). DOT is required by law to harmonize with HHS guidelines on scientific and technical issues such as included drugs and cutoffs (2). The new cutoffs are shown in Table 1. However, DOT has discretion in many aspects of testing regulations governing transportation industries, and the new rule diverges from the revised HHS guidelines in several areas.

The DOT published its original drug-testing rule on Dec. 1, 1989, based on the first HHS guidelines. The effective date was Jan. 1, 1990, coincident with the implementation of the HHS guidelines. DOT revised its rules in 1994, 2000, and 2010 to harmonize with HHS changes. The most recent revision is the first time a major change has been introduced without simultaneous implementation by these federal programs.

Table 1. New Cutoffs for DOT and HHS Drug-Testing Programs (1)

Initial test analyte	Initial test cutoff[1]	Confirmatory test analyte	Confirmatory test cutoff
Marijuana metabolites (THCA)[2]	50 ng/mL[3]	THCA	15 ng/mL
Cocaine metabolite (Benzoylecgonine)	150 ng/mL[3]	Benzoylecgonine	100 ng/mL
Codeine/ Morphine	2000 ng/mL	Codeine Morphine	2000 ng/mL 2000 ng/mL
Hydrocodone/ Hydromorphone	300 ng/mL	Hydrocodone Hydromorphone	100 ng/mL 100 ng/mL
Oxycodone/ Oxymorphone	100 ng/mL	Oxycodone Oxymorphone	100 ng/mL 100 ng/mL
6-Acetylmorphine	10 ng/mL	6-Acetylmorphine	10 ng/mL
Phencyclidine	25 ng/mL	Phencyclidine	25 ng/mL.
Amphetamine/ Methamphetamine	500 ng/mL	Amphetamine Methamphetamine	250 ng/mL 250 ng/mL
MDMA[4]/MDA[5]	500 ng/mL	MDMA MDA	250 ng/mL 250 ng/mL

[1] For grouped analytes (that is, two or more analytes that are in the same drug class and have the same initial test cutoff):
Immunoassay: The test must be calibrated with one analyte from the group identified as the target analyte. The cross-reactivity of the immunoassay to the other analyte(s) within the group must be 80 percent or greater; if not, separate immunoassays must be used for the analytes within the group.
Alternate technology: Either one analyte or all analytes from the group must be used for calibration, depending on the technology. At least one analyte within the group must have a concentration equal to or greater than the initial test cutoff or, alternatively, the sum of the analytes present (that is, equal to or greater than the laboratory's validated limit of quantification) must be equal to or greater than the initial test cutoff.
[2] An immunoassay must be calibrated with the target analyte, Δ-9-tetrahydrocannabinol-9-carboxylic acid (THCA).
[3] Alternate technology (THCA and benzoylecgonine): The confirmatory test cutoff must be used for an alternate technology initial test that is specific for the target analyte (that is, 15 ng/mL for THCA, 100 ng/mL for benzoylecgonine).
[4] Methylenedioxymethamphetamine (MDMA).
[5] Methylenedioxyamphetamine (MDA).

Major Changes to 49 CFR Part 40

The primary policy changes include:

1. The addition of four semisynthetic opioids to the panel—hydrocodone, hydromorphone, oxycodone, and oxymorphone—and removal of MDEA. These changes harmonize 49 CFR Part 40 with the HHS guidelines and are required by federal statute.

2. The elimination of the requirement for employers and service agents to submit blind specimens to laboratories as a component of drug-testing programs. The requirement to submit blind specimens to laboratories has been part of the DOT testing program since 1990. These blind specimens were intended as a quality monitor. DOT has indicated that the blind program can be eliminated to reduce costs for employers because the extensive experience with the program, quarterly proficiency-testing requirements, and laboratory inspections provide sufficient quality monitors. In contrast, the HHS guidelines continue to require federal agencies to submit at least 3 percent blind samples along with test subject specimens each year (3).

3. Several changes to the medical review officer (MRO) review process. First, the term "prescription" has been clarified to mean "a legally valid prescription consistent with the Controlled Substances Act" (CSA). This language reinforces the intent to follow the CSA regarding what an MRO may consider a legitimate medical explanation when reviewing positive results. It also clarifies that a prescription for medical marijuana, although legal in some states, cannot be considered legally valid because marijuana is listed on the CSA as a Schedule I substance with no medical use. This is consistent with DOT's position on medical and recreational marijuana posted on its website (4,5).

The new rule formalizes the ability of the MRO to request testing for the d,l-methamphetamine isomer to verify methamphetamine use and to request tetrahydrocannabivarin (THC-V) testing to distinguish use of marijuana from Marinol, a Schedule III substance.

The revised rule provides for a five-day waiting period before an MRO communicates a safety concern to a third party (such as an employer or healthcare provider) to permit the test subject to facilitate contact between the MRO and a prescribing physician. At times, the test subject has a legal prescription for a controlled substance consistent with the positive laboratory result, but the MRO determines the medication presents a safety concern. The MRO reports the drug-test result as negative, but also communicates the safety concern. Under the previous rule, the MRO would report the medical information at the same time the test results were reported. In the revision, the MRO provides the test subject five business days after receiving the verified negative result to have the prescribing physician contact the MRO regarding the safety concern. The MRO reports the information to third parties only after the waiting period or contact with the physician to determine whether the safety concern is valid.

This change was based on public comments to the proposed rule that warned of potential adverse impacts to employees with the addition of the synthetic opioids to the test menu given their prevalence as prescribed medications (1). The change has led to some concern within the MRO community regarding

safety and MRO liability should an accident occur within the waiting period (6).

4. The addition of three fatal flaws that result in cancellation of a test:

a) Specimen submitted with no Federal Drug Testing Custody and Control Form (CCF).

b) CCF submitted with no specimen.

c) Two separate collections performed using one CCF. This addition formalizes what has long been standard practice among laboratories.

Additional changes include a requirement for key personnel in the testing process to subscribe to the DOT Office of Drug and Alcohol Policy and Compliance listserver, changes to the "shy bladder" process at collection sites, and the removal of outdated compliance dates and links. The rule can be reviewed in its entirety at https://www.ecfr.gov/cgi-bin/text-idx?SID=44edbc0e557a4cc5ff03365810ee5b1c&mc=true&node=pt49.1.40&rgn=div5 or on the DOT website: https://www.transportation.gov/odapc/part40.

Custody and Control Form

A separate component relates to the CCF required in these testing programs (7). The CCF has been revised to accommodate the addition of semisynthetic opioids to the testing panel. As with previous changes, in which laboratories were permitted to deplete inventories of forms stored at collection sites, the old CCF may be accepted through June 30 without a "memorandum for the record" (MFR) explaining its use. After June 30, use of the revised CCF is required and any "old" forms must be accompanied by an MFR from the collector.

Summary

In summary, the proposed changes to the DOT Procedures for Workplace Drug and Alcohol Testing Programs put forth in January 2017 were published in final form on Nov. 13, to be implemented on Jan. 1. The most significant component, the addition of semisynthetic opioids to the testing panel, harmonizes with changes to the HHS Mandatory Guidelines for Workplace Drug Testing Programs implemented Oct. 1. Laboratories certified by SAMHSA and authorized to provide testing for these regulated programs were expected to implement the changes on Jan. 1. Although the most significant changes are consistent with those implemented by HHS, the DOT rule contains some policy and procedural differences specific to the transportation industry.

Learning Objectives

After reading this article, the reader will be able to summarize the most important changes to the Department of Transportation drug-testing rule, 49 CFR Part 40, which became effective on Jan. 1.

References

1. U.S. Department of Transportation. Procedures for transportation workplace drug and alcohol testing programs: addition of certain Schedule II drugs to the Department of Transportation's drug-testing panel and certain minor amendments. Fed Reg Nov. 3, 2017, Vol. 82, No. 217. https://www.gpo.gov/fdsys/pkg/FR-2017-11-13/pdf/2017-24397.pdf (Accessed February 2018).
2. Omnibus Transportation Employee Testing Act of 1991. Public Law 102–143, Title V, 105 Stat. 952. https://www.transportation.gov/sites/dot.gov/files/docs/199111028_Omnibus_Act.pdf (Accessed February 2018).
3. U.S. Department of Health and Human Services. Substance Abuse and Mental Health Services Administration. Mandatory guidelines for federal workplace drug testing programs. Fed Reg. https://www.federalregister.gov/documents/2017/01/23/2017-00979/mandatory-guidelines-for-federal-workplace-drug-testing-programs (Accessed February 2018).
4. U.S. Department of Transportation DOT Office of Drug and Alcohol Policy compliance notice. https://cms.dot.gov/sites/dot.gov/files/images/ODAPC%20Medical%20Marijuana%20Notice.pdf (Accessed February 2018).
5. U.S. Department of Transportation DOT Office of Drug and Alcohol Policy compliance notice. https://www.transportation.gov/sites/dot.gov/files/docs/odapc-notice-recreational-mj.pdf (Accessed February 2018).
6. MRO Alert, Nov/Dec 2017, Volume XXVIII, No. 10. American Association of Medical Review Officers, Chicago.
7. U.S. Department of Transportation. Notice: Federal Drug Testing Custody and Control Form, https://www.transportation.gov/odapc/Notice_CCF_December_2017 (Accessed February 2018).

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Loperamide Abuse Concerns FDA

Concerned that some individuals are taking high doses of the over-the counter (OTC) anti-diarrhea drug loperamide to treat symptoms of opioid withdrawal, the Food and Drug Administration (FDA) is working with manufacturers to use blister packs or other single-dose packaging and to limit the number of doses in a package.

The FDA continues to receive reports of serious heart problems and deaths from people taking doses much higher than recommended, primarily among people who are intentionally misusing the product. Loperamide acts on opioid receptors in the gut to slow the movement in the intestines and decrease the number of bowel movements.

More information is available at https://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm594403.htm.

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Learning Objectives

Learning objectives vary by article, but in general, after reading *CFTN*, the reader will be able to:

- Describe emerging and changing drug-abuse trends.
- Identify potential analytes of clinical significance.
- Evaluate methodologies' utility and limitations.
- Discuss relevant regulations.
- Explain analytical and regulatory issues.
- Describe the medical implications of drug abuse.

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FDA Finds Opioid Evidence in Kratom

The Food and Drug Administration (FDA) recently conducted an analysis using a novel computational model that provided strong new evidence that the plant kratom contains compounds with opioid properties. Using this model, FDA scientists analyzed the chemical structures of the 25 most prevalent compounds in kratom and concluded that they all share structural similarities with opioid analgesics, such as morphine derivatives.

The agency continues to receive reports of deaths involving kratom use and has identified additional adverse events related to it.

Drug users are employing kratom to try to treat opioid withdrawal symptoms, despite the fact that no reliable evidence exists for this use.

"Based on the scientific information in the literature and further supported by our computational modeling and the reports of its adverse effects in humans, we feel confident in calling compounds found in kratom, opioids," said FDA commissioner Scott Gottlieb, MD.

Information is available at https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm595622.htm

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