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Drug Exposure in Newborns

Many Specimen Types Have Been Used In Drug Testing, But No Type Is Ideal

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Substance abuse during pregnancy is a growing problem. In the U.S., an estimated 10–11% of births are affected by prenatal alcohol or illicit drug exposure (1). The incidence of neonatal abstinence syndrome (NAS) increased by 300% from 1999 to 2013 in the 28 states surveyed, and more than 75% of newborns exposed to drugs in utero have major medical problems (2,3). In utero drug exposure can lead to chronic behavioral, cognitive, and developmental problems. Antenatal care and interventions can improve maternal and neonatal outcomes and decrease the need for long-term care. For that reason, accurate and timely detection of in utero exposure to drugs is critical to making informed social and medical management decisions for the newborn, mother, and caregivers (3,4).

Testing of biological specimens to identify drug-exposed newborns is important because maternal self-reporting is often unreliable and inadequate (3,4). In addition to the problem of mothers intentionally denying or underreporting their drug use, the length of pregnancy makes it difficult for anyone to recall an accurate history of the specific drugs used, amounts of each drug, frequency and duration of use, and other details.

To overcome these problems, healthcare providers use screening tools such as questionnaires to determine the risk of substance abuse. Maternal risk factors that have been correlated with illicit drug use include a history of high-risk behaviors such as drug abuse or prostitution, regular use of nicotine, limited prenatal care, and unexplained obstetric events such as placental abruption or premature labor. Newborn risk factors include low birth weight and size, symptoms of NAS, and unexplained neurological complications (5). A standardized and objective protocol can help avoid bias, prevent discrimination in testing, and minimize the risk of drug-exposed newborns being missed (4). The most common specimens used for detection of drug exposure in newborns include urine, meconium, and umbilical cord tissue, but several other matrices have been used. Table 1 summarizes the characteristics of each of them.

Urine vs. Meconium

Urine is the most widely used specimen for drug-testing in adults because sample collection is noninvasive and standardized assays are available. However, urine has had limited success in neonates (4). A neonate's urine is not easy to collect; the first void is often missed because the newborn may void during or shortly after the delivery. Later urine voids are not as reflective of in utero drug exposure. In any case, urine tests generally detect maternal drug use only in the days just prior to delivery.

The use of a single, easy-to-collect specimen to detect all drugs used during pregnancy would be ideal, but is not currently possible. Maternal specimens that test positive for drugs may not definitively demonstrate that a fetus was exposed because of variation among individuals in the transfer of drugs through the placenta. For these reasons, meconium—the stool passed by the newborn in the first days after birth—is preferred over urine for neonatal testing. The best-characterized of neonatal specimens, it theoretically reflects drug exposures during the latter half of pregnancy (6).

Meconium starts to form around the 12th week of gestation, when the fetal swallowing reflex develops. Drugs and drug metabolites enter the fetal circulation directly through placental transfer and

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through swallowing of amniotic fluid. Drug analytes are subsequently deposited in meconium. However, the production of meconium is nonlinear, with more than two-thirds forming during the last eight weeks of gestation. For a full-term birth, third-trimester exposures are more readily detected than second-trimester exposures. Regarded as the most sensitive of neonatal specimens, meconium is better able to detect frequent than sporadic drug use.

A major disadvantage of meconium is that the collection process is laborious and somewhat unpredictable. Because meconium is heterogeneous and passage often occurs over multiple days, the collections should be combined and mixed thoroughly prior to submission to the laboratory for testing. Meconium is typically collected from a newborn's diaper, but insufficient sample size is common, particularly with premature births and neonates who are constipated due to exposure to opioids in utero. Meconium can also be lost if it is expelled during birth or in utero, if diapers are disposed of prior to specimen collection, or if the neonate was transferred from an outside facility (6).

If there is sufficient time between birth and sample collection, iatrogenically administered medications can accumulate in meconium and cause a positive test that reflects postpartum drug exposure that may confuse interpretation (5). Prolonged sample storage can also contribute to degradation of drugs; in particular, the heroin metabolite 6-acetylmorphine is unstable (7).

Umbilical Cord Tissue

In recent years, umbilical cord tissue has gained popularity as an alternative to meconium. The umbilical cord is formed by the fifth week of development. Drugs are thought to deposit in the cord's Wharton jelly, which consists of mucopolysaccharides and other components that pass through the placenta. Drug testing using umbilical cord tissue is argued to accurately reflect fetal exposure because it is on the fetal side of the placenta and is physically attached to the fetus.

Unlike meconium, umbilical cord is universally available in large amounts at birth, making collection easy and noninvasive. Many facilities collect cords for all births, and either send cords for drug testing right away (if drug exposure is likely) or store the cords in case symptoms of NAS emerge. Umbilical cord tissue contains no drugs administered after birth, although drugs administered during labor and delivery or otherwise present in the maternal circulation may be detected (5). For that reason, maternal pharmacy history must be considered when interpreting umbilical cord drug-test results.

Segmental analysis of umbilical cord has shown consistent drug concentrations throughout the specimen. As with meconium, tissue formation is nonlinear, with the bulk of the tissue formed in the third trimester, making detection of maternal drug use during

Table 1. Biological Specimens for Detection of Drug Exposure in Newborns

	Neonatal urine	Placenta	Amniotic fluid	Vernix caseosa	Meconium	Umbilical cord tissue	Maternal hair	Neonatal hair	Neonatal nails
Detects recent drug exposure (hours to days)	X	X	X						
Potential detection window of days to weeks	X*		X*	X					
Potential detection window of weeks to months					X	X	X	X	X
Noninvasive collection	X	X		X	X	X	X	X	X
Adulteration or external contamination is concern	X						X	X	X
Commercially available methods	X				X	X	X	X	
May detect drugs given during labor and delivery	X	X	X		X	X	X		
May detect drugs given directly to newborn	X				X			X	X
May require multiple collections	X		X		X				

* Detection window may be longer based on pharmacokinetics, dosing frequency, and unique characteristics of the specimen.

the first and second trimesters unlikely. Drug concentrations in umbilical cord tissue are typically much lower than in meconium, sometimes in the picogram per gram range, so cutoff concentrations are not equivalent for the two. Several comparison studies of cord and meconium have found mixed degrees of concordance, which may reflect differences in analytical methods, cutoff concentrations, and target analytes. Additional studies will be required to evaluate the concordance of these two specimen types, which each offer unique pros and cons.

Other Matrices: Hair

Other matrices that have been used for fetal drug exposure include maternal hair, neonatal hair, placenta, amniotic fluid, and vernix caseosa (3,8,9).

Hair, both maternal and neonatal, has historically been used to detect drug exposure during pregnancy (3). The exact mechanism of drug incorporation into hair is not known, and likely varies with specific drugs. Drug analytes are believed to be incorporated into the hair shaft through passive diffusion from systemic circulation during the formation of hair in the follicle, exposure to sweat and sebum secretions, and via external contamination. The drug analytes are trapped in the hair shaft indefinitely and can be detected several months after drug use, depending on the length of hair available for testing.

Maternal hair has been reported as the most sensitive specimen for the detection of some abused drugs during pregnancy and offers the advantage of chronological estimation of drug use (3,8). Adult hair grows at a rate of about 1 cm per month, so maternal hair collected at delivery may not reflect the most recent days to weeks of drug use, but can provide an estimated history of when drug exposure occurred. Biases in adult hair testing results are extensive, relating to the color, consistency, and cosmetic processing of hair. Hair testing may not be available if the mother cuts, shaves, or otherwise removes hair prior to collection. Therefore, the testing of maternal hair is highly controversial.

Neonatal hair forms at about 20 weeks gestation and reaches the scalp surface about three weeks later. Neonatal hair retains drugs present in the fetal circulation and amniotic fluid and reflects exposure during the last trimester of a full-term birth (8). A major advantage of neonatal hair is that it does not contain drugs administered during labor and delivery. However, neonates often have so little hair that collection of an adequate specimen is impossible. In addition, cutting or shaving neonatal hair may be culturally undesirable, making its use controversial and thus not a preferred specimen (3,8).

Placenta

The placenta forms at approximately four weeks of gestation to connect the fetus to the mother. The development of the maternal blood supply to the placenta is complete by the end of the first trimester (10–12 weeks). The interface between maternal and fetal blood, the placenta is responsible for maternal–fetal exchange of oxygen, nutrients, and waste products. It protects the fetus by serving as a barrier to entry that not all substances can cross (9). Most drug analytes cross the placenta via passive diffusion, but the extent of transfer to fetal blood circulation is determined by the physicochemical properties of the drugs and metabolites, as well as their affinity for placental drug transporters. The detection window of drugs in placenta has not been well-defined, but many studies suggest it is only hours to days. Due to the relatively large size of the placenta at birth (~500 g), storage and handling pose logistical challenges. That said, most studies suggest that distribution of drug analytes is relatively homogenous, so there is no need to collect and process the entire placenta.

Amniotic Fluid

Amniotic fluid is the liquid that surrounds the embryo/fetus in the amniotic sac. The composition of amniotic fluid changes as the fetus develops. In early pregnancy, the amniotic fluid consists of a filtrate of maternal blood and fetal cells. At 10–20 gestational weeks, the fluid is similar to fetal plasma. In the second half of pregnancy, amniotic fluid is composed of fetal secretions, including lung fluid and urine (3,8).

Drug tests of amniotic fluid provide information about fetal exposure based on the time of collection. Amniotic fluid is collected using an amniocentesis procedure in which a long needle is inserted through the maternal abdomen and uterine wall to sample the fluid in the amniotic sac. This invasive procedure can be dangerous for the fetus (3,9).

Vernix Caseosa

Vernix caseosa is the white, creamy substance covering the skin of the fetus during the last trimester of pregnancy and is usually present on neonatal skin at birth (10). It is held on the skin by the baby's body hair and can be easily removed after delivery by swabbing the skin with gauze or other wipe, although the amount of sample available for testing varies. The window of detection of maternal drug use is estimated to be the last 24 weeks of gestation, but few analytical methods using this specimen have been described. Its potential utility and routine analytical methods remain to be defined (3).

Nails

Nails begin to develop at about 10 weeks of gestation and typically grow to the tips of the fingers and toes by the ninth month. Neonatal nails collected at birth theoretically reflect exposure during the second and third trimester, but a sufficient sample size can be difficult to collect, and testing is not widely available (8). Maternal nail testing reflects chronic exposure from months to weeks, based on the length of the nail available. Adult nails grow at about 0.1 mm/day for fingernails and 0.03 mm/day for toenails. The growth

rate varies among individuals and depends on age, sex, health status, weather, diet, time of year, environment, and exercise. As with hair, adult nails are subject to contamination from the environment, the body (sweat and sebum), and cosmetic treatment. Sample preparation typically involves wash steps; an acid, base, or enzymatic hydrolysis; and hours of incubation. False-negative results are likely if the recovery of drugs incorporated in the nails is poor and if drugs degrade intrinsically over time or as a consequence of the pre-analytical processing.

Combining Results

Aligning results from multiple biological matrices could theoretically improve confidence in detection of drug exposure during pregnancy and be used to establish the chronology of exposure. In practice, however, the discrepancies in testing results from different specimens can be confusing. Confounding factors include the stability and pharmacokinetics of individual drugs and drug metabolites and deposition of drugs and metabolites in each specimen type. Detection windows also depend on the specific drugs, patterns of use, quality and quantity of the specimen, and performance characteristics of the tests.

There are many analytical methods for drug testing; the most widely used are immunoassay-based screens followed by confirmation of positive results using chromatographic separation and mass spectrometric detection (4). This two-step approach essentially eliminates the likelihood of reporting false results. Because immunoassay-based screening is prone to both false-positive and false-negative results, some laboratories skip the immunoassays and go directly to a mass spectrometric method targeted for specific drug analytes in the context of neonatal testing. Such methods can deliver fast results with qualitative or quantitative results.

The time to the results is an important consideration in neonatal drug testing. A long turnaround time may delay the identification of prenatal exposure. While most births are associated with two to three days in the hospital, NAS treatment may require weeks (3). If a newborn is released from the hospital before a positive result is available and before symptoms of NAS appear, the newborn may be lost to follow-up. Both mother and newborn may not receive the needed care.

The specific drug analytes included in a testing program vary among laboratories, geographic regions, and specimens. The predominant drug analytes present vary according to the time interval between drug administration and specimen collection, and may vary from specimen to specimen. For example, metabolites found in meconium but not in urine include para-hydroxymethamphetamine for methamphetamine, meta-hydroxybenzoylecgonine for cocaine, and the 11-hydroxy metabolite of THC (11). Umbilical cord is suitable for detection of heroin use because it contains the heroin metabolite 6-acetylmorphine and meconin, a metabolite of noscapine that is an impurity in illicit heroin (12).

Summary

Protection of newborns and mothers requires detection of drug use and associated exposures. Laboratory testing of newborn biological specimens can provide evidence of maternal drug use and in utero exposure. Each specimen type has its own pros and cons and reflects exposure during a specific time period. Because the availability and quality of specimens, specimen handling, analytical method performance, and properties of specific drugs influence detection rates, one specimen is not always preferred over another. Additional studies are required to optimize and standardize testing, with needs for a better understanding of the predominant drug analytes in each specimen type and other variables such as clinically relevant cutoff concentrations and the value of quantitative results.

Learning Objectives

After reading this article, the reader will be able to list the most commonly encountered specimens in neonatal drug testing and summarize the advantages and disadvantages of each.

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Sports Drug Testing Update

Laboratories Continue to Develop Sensitive Assays to Identify Doping

By Brian N. Kelly, PhD

In response to the doping program of the former German Democratic Republic (1) as well as widespread doping at the 1998 Tour de France, the World Anti-Doping Agency (WADA) was formed in 1999 to assist sporting authorities in curbing the use of performance-enhancing substances by athletes. In the ensuing years, WADA emerged as the leading international sports drug-testing body. However, doping continues to plague sports despite these efforts.

Recent efforts by WADA include investigating government-supported doping programs, such as the sample-swapping scheme of Russia during the 2014 Winter Olympic Games in Sochi (2). There is a continuing need to improve detection of prohibited substances in biological samples obtained from athletes.

To increase the likelihood of catching athletes who cheat in sports, anti-doping laboratories must be aware of new and re-emerging compounds, develop robust assays for these compounds, and keep abreast of new methods of doping.

Meeting this challenge requires improving limits of detection and using alternative matrices for detection of the prohibited substances and methods. Improved analytical technology helps drive the ever-decreasing limits of detection as well as the ability to detect previously undetectable substances and doping methods. For example, the minimum required reporting limit for clenbuterol in urine is 0.2 ng/mL. To put that in perspective, a four-gram sugar cube dissolved into an Olympic-size swimming pool (2,500,000 L) would be at a concentration of 1.6 ng/mL.

Very few clinical assays can regularly quantify to these concentrations, but the anti-doping laboratories are required to have robust and sensitive assays to meet these low minimum required reporting limits. To do so, anti-doping laboratories have moved from traditional gas chromatography-mass spectrometry (GC-MS) to gas chromatography-tandem mass spectrometry (GC-MS/MS) assays for many anabolic steroid compounds.

New Approaches to Testing

Faced with expanding test menus and requirements for fast turnaround times, anti-doping laboratories have developed multi-analyte methods. Recent improvements to these assays include the use of supercritical fluid (SFC) liquid chromatography (LC) interfaced via positive electrospray ionization (ESI) to a triple-quadrupole MS system and LC-silver ion coordination ion spray (Ag^+CIS) MS. An assay using SFC-LC is reported to have a limit of detection of 0.1–10 ng/mL for most of the 53 anabolic agents and metabolites included in the panel, along with a run time of eight minutes, allowing a quick turnaround time. An Ag^+CIS MS technique can detect 86% of relevant compounds at or below 2 ng/mL, which makes it the sensitivity leader in sports drug-testing MS assays (3).

Other developments include quantification of transcriptional signatures in biological matrices. One study demonstrated an increase of 32 transcripts in whole blood among volunteers administered 50 IU/kg of erythropoietin every two days over a period of four weeks. The increase in transcripts was observed for up to four weeks post-administration.

Growth hormone causes a similar change in the concentration profile of two mRNA transcripts, FN1 and RAB31, for seven days post-administration. These studies indicate that transcriptional profiling may be a useful addition to anti-doping testing (3).

Emerging Compounds

To track emerging compounds, WADA has a program that determines the prevalence of certain substances in anti-doping samples. In 2017, the monitoring program list contains substances prohibited in-competition, including the stimulants buproprion, caffeine, nicotine, phenylephrine, phenylpropanolamine, pipradrol, and synephrine as well as the narcotics codeine, mitragynine, and tramadol. The monitoring program also includes both the in-competition and out-of-competition use of glucocorticoids, beta-2-

agonists, and telmisartan (a peroxisome proliferator-activated receptor agonist).

A substance recently moved from the monitoring program to the prohibited list is meldonium, the drug that caused Maria Sharapova to fail a drug test in 2016. Developed in 1970, meldonium is an anti-ischemic drug that has limited use outside of Latvia, where it is produced.

Sample collection and shipping are among the largest costs of anti-doping testing. This has led to research into alternative sample collection strategies and test matrices. In a proof-of-principle study that may allow unsupervised urine collection, athletes ingested a polyethylene glycol marker 30 minutes prior to submitting urine samples for testing (3). Other studies have examined using exhaled breath as a matrix for anti-doping testing (4).

Although many of the general methods are similar between a clinical laboratory and an anti-doping laboratory, there are some important differences, including the limits of detection required of anti-doping laboratories. Except when there is a positive test of a well-known athlete, the work of anti-doping laboratories often goes unnoticed by the general public, but their efforts are an important part of maintaining fair athletic competitions.

Learning Objectives

After reading this article, the reader will be able to summarize the latest techniques used in testing for doping in sports and list some of the emerging compounds added to the prohibited testing list.

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Revisions to Federal Testing Guidelines Take Effect October 1

By Jennifer Collins, PhD

The U.S. Department of Health and Human Services (HHS) Substance Abuse and Mental Health Services Administration (SAMHSA) published a final rule revising the Mandatory Guidelines for Federal Workplace Drug Testing Programs on Jan. 23. The new rules will take effect on Oct. 1 (1). The document finalizes the changes published for public comment on May 15, 2015, and includes revisions to initial and confirmatory drug test analytes, approval of new testing methods, and a revision in the cutoffs for reporting a urine specimen as adulterated based on low pH.

The changes to the guidelines affect drug tests performed for federal employees and job applicants, as well as for other public and private sector employees working in industries regulated by the guidelines. The U.S. Department of Transportation (DOT) also published proposed revisions to 49 CFR Part 40, its workplace drug-testing regulations, on Jan. 23 to harmonize them with the revised HHS guidelines (2). The final DOT rule has not yet been published, however, so the HHS and DOT implementation timelines will not coincide. This is the first time that a major change in the federal guidelines will not be consistent across the two entities.

An overview of the most significant changes is provided below. SAMHSA has been developing procedures and supporting documents—including the federal custody and control form, National Laboratory Certification Program (NLCP) Laboratory Checklist and Manual, and Medical Review Officer (MRO) Manual—to reflect the revisions. The new custody and control form was approved on August 8 for use in HHS testing programs. The expiration date of the current form has been extended until June 30, 2018, and it should be used for the DOT testing program until the program's final version is published.

Interested parties should monitor the SAMHSA and DOT websites and the *Federal Register* for up-to-date information on revised policies and documents (3,4,5).

Compounds Added to Test Panel

In accordance with a recommendation from the Drug Testing Advisory Board in July 2011, additional Schedule II drugs have been added to the test panel. Specifically, hydrocodone, hydromorphone, oxycodone, and oxymorphone will now be included in federal workplace programs. The inclusion of these drugs is supported by a wealth of data indicating that the abuse of prescription pain relievers in the U.S. poses a significant public health problem (6,7).

At the same time, methylenedioxyethylampheta-

mine (MDEA) will be removed from the panel, based on program data showing extremely low prevalence of this drug.

Table 1 shows the revised panel and cutoffs.

Revised Testing Requirements

The revised guidelines provide more flexibility in testing methods. For an initial drug test, a laboratory may use an immunoassay *or* an alternate technology such as mass spectrometry, whereas in the current version, immunoassay is the only approved method. This change is based on advances in the alternate technologies that enable their use as efficient and cost-effective screening methods that provide the required sensitivity, specificity, and accuracy. The guidelines and NLCP manual define specific validation requirements for alternate methods.

For immunoassays used to detect multiple initial test analytes such as opioids, the guidelines specify cross-reactivity requirements for each component. For example, an opiate assay optimized to morphine can be used to detect codeine, morphine, hydrocodone, and hydromorphone as long as the cross-reactivity to each component is at least 80%. Otherwise, separate, more specific assays must be used. Quality control requirements have also been modified to ensure that testing for each of the new analytes is appropriately monitored.

An additional significant change to the testing protocol is a revised threshold for reporting a urine sample as "adulterated" based on low pH. The current threshold is a pH of less than 3.0; that threshold is being raised to 4.0. This change is based on data supporting a minimum physiologically achievable urine pH of about 4.5, with no known medical conditions or medications that produce urine pH below that value (8). The upper pH limit of 11.0 remains unchanged. Specimen validity testing and identification of altered or substituted urine samples continues to be a challenge and focus for the testing program. Although the changes to the drug panel will not change for the DOT program on Oct. 1, NLCP laboratories have received guidance to implement the revised pH threshold for both HHS and DOT programs at that time.

Table 1. Revised Screening and Confirmation Cutoff Concentrations (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	2,000 ng/mL	Codeine Morphine	2,000 ng/mL 2,000 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).

Specimen Collection

A significant change to the specimen collection procedure is that a federal agency may authorize collection of an alternate specimen type (for example, oral fluid) when a donor is unable to provide an adequate urine sample. This option is predicated on publication of the final version of the HHS guidelines specific to oral fluid testing. This proposed change was published for public comment in May 2015, but a timeline for publication and implementation of the final oral fluid mandatory guideline has not yet been provided.

Revised Standards for MROs

With the addition of more Schedule II prescription medications to the guidelines, SAMHSA has included additional training requirements for MROs. While MROs are currently required to be certified by a recognized entity, the revised guidelines require that an MRO complete requalification training and examination at least every five years from initial certification. Initial and requalification training must include regulatory issues and procedures for interpretation, review, and reporting of results. Certified MROs must also complete training on any revisions to the guidelines prior to the effective date.

There are other changes that affect the overall program, but those described above will have the greatest impact on laboratories. Since the original publication of the guidelines in the *Federal Register* in 1988, this is only the sixth revision. The implementation of these changes, with the expansion of opioid testing, will likely increase the positive rate and better reflect concerns related to opioid use in transportation and other federally regulated industries.

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