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Review

Biological Testing and Interpretation of Laboratory Results Associated with Detecting Newborns with Substance Exposure

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BACKGROUND: Substance use during pregnancy is common, as is biological testing that is intended to help identify prenatal exposures. However, there is no standardized requirement for biological testing with either maternal or newborn specimens, nor is there standardization related to when testing occurs, how frequently testing occurs, what specimen(s) to test, what substances to test for, or how to perform testing.

CONTENT: We review common specimen types tested to detect maternal and newborn substance exposure with a focus on urine, meconium, and umbilical cord tissue. We also review common analytical methods used to perform testing, including immunoassay, and mass spectrometry platforms. Considerations regarding the utilization of testing relative to the purpose of testing, the drug analyte(s) of interest, the specific testing employed, and the interpretation of results are emphasized to help guide decisions about clinical utilization of testing. We also highlight specific examples of unexpected results that can be used to guide interpretation and appropriate next steps.

SUMMARY: There are strengths and limitations associated with all approaches to detecting substance exposure in pregnant persons as well as biological testing to evaluate a newborn with possible substance exposure. Standardization is needed to better inform decisions surrounding evaluation of substance exposures in pregnant people and newborns. If biological sampling is pursued, testing options and results must be reviewed in clinical context, acknowledging that false-positive and -negative results can and do occur.

Background

Substance use, whether therapeutic (prescribed or nonprescribed) or recreational (legal or illicit), is common among pregnant people. At least 70% of pregnant individuals take one or more prescription medications and more than 20% admit to use of recreational drugs including alcohol and marijuana. Illicit drug use and substance use disorder (SUD) are estimated to occur in 5%–10% of pregnant people (1–3). Detection of substance use or exposure may involve biological testing, although the appropriateness of testing specimens collected from pregnant people and newborns is a hotly debated topic without clear consensus. Universal testing of biological specimens is not currently recommended by any organization. Risk-based testing due to history of SUD, age, socioeconomic status, race and ethnicity, or late prenatal care is also not recommended (4). Results from studies evaluating the diagnostic yield of biological testing vs screening tools are conflicting and point out that there exist numerous pitfalls when interpreting a toxicology test (5–8).

It is well documented that underreporting substance use in pregnancy is common, largely because pregnant people feel stigmatized or fear the potential legal and social ramifications associated with disclosure of substance use (9). A pregnant person may not think it is important to disclose use of therapeutic or recreational substances and may be unaware of the potential for increased risk of harm to the newborn, including withdrawal symptoms. If a pregnant person reports illicit substance use, it is unlikely that the purity of the illicit substances and overall amount used is known. Illicit substances may contain unexpected compounds and often contain pharmacologically active components or adulterants such as levamisole with cocaine, and xylazine with fentanyl. Polysubstance use is also important to recognize because combinations of substances may lead to more significant hazards than

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expected. Sometimes biological testing is the only way to identify the specific substance(s) involved in an exposure.

In the context of public health, the United States Child Abuse Prevention and Treatment Act was established in 1974 to provide funding to mitigate child maltreatment, including substance exposures. There are stipulations for individual states to qualify for this funding. A 2016 amendment requires states to develop a system to notify child welfare services when a newborn is "affected" by substance use or withdrawal symptoms. However, the term "affected" is ambiguous, leading to varying interpretations and continued debate among healthcare providers (10). The Guttmacher Institute has reported that 3 states have a requirement for prenatal drug exposure testing and that 12 states consider substance use during pregnancy to be child abuse or neglect, providing grounds to potentially remove a newborn from the mothers custody. Twenty-five states prioritize and provide support for pregnant persons to receive SUD treatment (11).

Identification of substance exposure in pregnancy provides opportunity for education and intervention that could minimize risks to the newborn. Most professional organizations concur that best practice is to universally screen all pregnant individuals with validated written or verbal substance use screening tools (e.g., National Institute on Drug Abuse Quick Screen, 4/5Ps risk assessment, CRAFFT) at presentations to medical care such as entry to obstetric care, during prenatal care, and on admission to a hospital (5, 6, 12, 13). Informed consent is a critical component of screening, but does not fully mitigate concerns about biases inherent to racial and ethnic disparities. Informed consent should disclose the testing under consideration, the reasons for testing and the specific substances that can be detected by the testing. Unfortunately, informed consent is not universally adopted when biological testing of either maternal or newborn specimens is requested (14–16).

Outcomes such as preterm birth, low birth weight, overall small-for-gestational age, neonatal abstinence syndrome (NAS), and neonatal opioid withdrawal syndrome are associated with substance use during pregnancy and may be triggers for ordering biological testing. However, these adverse perinatal outcomes are multifactorial and substance testing should not occur solely based on these outcomes. Neonatal withdrawal syndromes are managed clinically in the acute setting, based on specific signs and symptoms. The American Academy of Pediatrics states that newborn toxicology testing should be considered only if it will help to inform clinical decision-making (12).

In practical terms, testing newborn specimens may not provide medically important information. If a pregnant person has received consistent prenatal care, there is no obvious need for testing a newborn specimen. A healthy newborn is likely to be discharged after birth in a hospital before biological test results are available, such that results could not be used to make medical decisions. If results are available during the birth hospitalization, the hospital care model may change. For example, the length of observation may be extended, and care plans for treatment of withdrawal symptoms may take substance exposure results into consideration. Substance exposure data may also be used to discuss risks associated with breast feeding.

Given this often-confusing landscape, the primary objective of this review is to describe specimen and analytical testing options related to biological testing, and highlight challenges associated with interpreting unexpected results. Considerations and cautions related to key aspects of the testing process are also discussed.

Biological Specimens

The most common specimen for detection of recent substance use or exposure in adults is urine, due to ease of collection and widely available testing options. Substances and associated metabolites are often present in high concentrations for hours to days after the last exposure. One concern about collecting urine is the risk of dilution, adulteration, or substitution. The best way to mitigate this risk is to observe collections, but doing so may not be practical in a clinical setting. Practice guidelines recommend specimen validity checks for temperature, appearance, and pH at the point of collection (17). In addition, measuring creatinine concentration or specific gravity in the laboratory is recommended to evaluate specimen dilution. The most common alternative to urine is venous blood, for which collections are observed. Blood is a better specimen than urine for correlating signs and symptoms of intoxication with substance exposure. Regardless of results, testing maternal blood or urine can only infer risk of exposure to the fetus; testing newborn specimens is required to detect possible fetal exposure. For newborns, urine, meconium, and umbilical cord (UC) tissue are commonly collected and tested specimens.

Collection of newborn urine is challenging. The first void after birth is likely to be of highest diagnostic yield but is easy to miss. Common collection strategies include pedibags, squeezing urine from diapers, and collecting with cotton balls, which all run the risk of contamination (18) or insufficient volume to test. Specimen validity testing, including determination of creatinine concentration, is not recommended for newborn urine that is commonly dilute in comparison with adult urine. Newborn urine will reflect only recent (hours to days) exposures and may include substances administered to the mother during the birth hospitalization. Finally, because newborns may exhibit differences in substance metabolism compared with

adults, assays developed for adult urine may not perform well for newborn urine (19).

Meconium and UC can reflect substance exposures over approximately the last trimester of a full-term pregnancy. This may seem confusing because UC begins to form in the first trimester and meconium begins to form in the second trimester. The accumulation of both meconium and UC is a nonlinear process that mirrors the growth of the fetus. As such, substance exposure in the first or second trimesters is extensively diluted by the accumulation that occurs in the third trimester. This greatly limits the ability to detect substance exposure in the first or second trimesters. Studies comparing longitudinal history of maternal substance use that document cessation in the first or second trimester were associated with negative substance testing results in newborn specimens. These data support an estimated detection window limited primarily to the third trimester (20–22). The detection window may also be analyte specific because it also depends on analyte stability over time, at physiological temperatures. Insufficient or inadequate sampling of meconium and inappropriate handling of UC can also affect the detection window.

Meconium is the combination of initial stool passages of the newborn that occur before the transition to milk stool. Meconium begins forming when the fetus starts swallowing, typically in the second trimester, and reflects substances introduced through both the placental vasculature and amniotic fluid. The sample itself is usually a green-brown or blackish color and heterogenous, with an uneven deposition of substances and other components. Meconium typically begins to pass 24–48 hours after birth, and may be passed for several days, making time of collection and the actual collection process challenging. Concerns with the collection process include involvement of multiple collectors, storage during collection, contamination of the specimen from components of diapers, contamination with newborn urine and transitional stool, collection of insufficient quantity for testing, and inadequate mixing before testing. In approximately 10% of cases, meconium is passed into amniotic fluid during birth and cannot be used for testing. Positive detection in meconium may be representative of maternal substance use, but substance exposure during the birth hospitalization, or substance administration directly to the newborn after delivery but before meconium collection, can also be detected (23, 24).

UC is an attractive complementary or alternative specimen to meconium. Substances are deposited evenly along the UC, in the Wharton jelly, a gelatinous substance that envelops the UC vessels. Substance analytes may also be present in the cells lining the vasculature and in UC blood. UC has significant logistical advantages over meconium as it is available for collection at birth and is plentiful. It is common practice to rinse the cord in saline to remove exterior blood as part of the specimen collection process. Squeezing the cord to expel blood from the UC vasculature, or storing the UC in liquid, is not recommended due to the potential risk of expelling the Wharton jelly, which could lead to false-negative results. Theoretically, very small UC specimens, such as might be collected from a stillborn or extremely premature newborn, may not have developed adequate tissue and Wharton jelly to support UC testing, but this hypothesis has not specifically been evaluated. Positive detection in UC may be representative of maternal substance use, but substances administered to the mother during labor and delivery may be present in the circulating blood supply at the time of UC collection and can also be detected (25, 26). Substances administered directly to the newborn after birth will not be detected in UC. Owing to ease of collection, some hospitals have developed processes by which a segment of UC is retained for all newborns in the event that testing is needed.

Both meconium and UC have been used to measure similar analytes, although the concentrations measured in UC are often lower than those in meconium. There are several hypotheses as to why this is, including the comparatively higher hydrophilicity of Wharton jelly preventing hydrophobic analytes from depositing, and loss of analyte due to cells sloughing off the UC. Laboratory practice could also contribute to this difference because the results and cutoff concentrations used to determine positivity of each analyte are normalized to sample weight. For UC samples, results are normalized to a specimen weight that includes structural material that has lower amounts of substance deposition than Wharton jelly. These differences in substance detection have been a hurdle for acceptance of UC as a specimen type. Urine, meconium, and UC are compared in Table 1 to summarize key differences. In addition, the agreement between these specimen types when collected and compared in published studies is summarized in Table 2, and detailed in Supplemental Table 1 (in the online Data Supplement).

As might be expected based on the chronology reflected by the specimen types, results from urine testing do not agree very well with meconium and UC whereas, in general, good agreement is observed when comparing meconium and UC. There are nuances in substance and metabolite patterns that can influence substance detection. For example, meta-OH-benzoylecgonine is an important cocaine metabolite seen in meconium but not commonly observed in other specimen types. Some studies found UC to be less sensitive for certain substances and metabolites compared with meconium, while other studies have found the opposite (30–32). More recently, it was demonstrated that adopting similar analytical approaches and cutoff concentrations is critical to increasing comparability between the 2

Table 1. Comparison of specimens used to detect substance exposure.

Specimen	Estimated detection period	Considerations	
		Collection	Analytical
Urine	Hours to days prior to collection	Adults: observed collection minimizes risk of adulteration, substitution, or dilution. Newborn: first void is often missed. Contamination possible if collection is indirect (e.g., from diaper or cotton balls)	Adults: many commercially available tests and formats. Creatinine measurement is commonly used to detect dilution. Newborn: tests designed for adults may not be appropriate. Substances administered during labor and delivery may be detected
Meconium	Starts to form in 2nd trimester but detection generally reflects exposure in 3rd trimester due to nonlinear accumulation and analyte stability	Typically passed 24–48 h postbirth but may take several movements to pass full specimen. Avoid milk stool. May be passed and lost during delivery May contain newborn urine	Commercial immunoassays have been adapted for meconium but performance of mass spectrometric testing is generally superior. Substances administered during labor and delivery may be detected May detect substances administered directly to the newborn, based on timing of substance administration relative to specimen collection
UC tissue	Starts to form in 1st trimester but detection generally reflects exposure in 3rd trimester due to nonlinear accumulation and analyte stability.	Available immediately after birth Substances are deposited evenly across the length of the UC May not be appropriate for stillbirth or premature infants if the UC is not well developed May contain maternal blood	Commercial immunoassays have been adapted for UC but performance of mass spectrometric testing is generally superior. Concentrations of substance analytes are often lower in UC than in meconium; requires lower cutoff concentrations to obtain similar detection rates Substances administered during labor and delivery may be detected

specimen types (27, 35). However, this information is often lacking in publications, making it challenging to compare studies.

There are many other specimens that could be useful for detecting substance exposure in the newborn. For example, collecting blood from a newborn heel prick with specialized collection cards is routinely performed to support detection of metabolic diseases shortly after birth. Once dried, the blood spots offer long-term analyte stability at ambient temperature and the cards can

Table 2. Agreement between specimens collected from the same birth scenario.

Study	Maternal and newborn urine	Urine and meconium	Urine and UC	Meconium and UC
Haizler-Cohen, 2023 (23)		42%		
Gersch, 2023 (14)			13%–100%	
Pandya, 2023 (27)				96%
Villarreal, 2023 (28)	66%			
Simpson, 2022 (4)	49%	Maternal urine: 72% Newborn urine: 40%		
Mark, 2021 (29)	44%			
Colby, 2019 (30)				80%–100%
Colby, 2017 (31)				76%–100%
Larabee, 2017 (32)				40%–90%
Gray, 2010 (33)		65%–98%		
Montgomery, 2008 (34)				91%–100%

be transported by mail. Dried blood spots have been evaluated as a specimen type for detection of phosphatidylethanol, a biomarker of chronic exposure to ethanol (36). Newborn oral fluid has also demonstrated promise as a specimen for detecting in utero substance exposure (37).

Analytical Approaches

The 2 primary analytical technologies used for drug testing are immunoassays and chromatography coupled to mass spectrometry (38, 39). See Table 3 for a comparative overview of immunoassays, chromatography, and common mass spectrometry analyzers. The traditional approach to urine drug testing, which became widespread in the United States after Executive Order 12564 in 1986, is a two-step approach in which the initial test is based on a panel of immunoassays. Any positive result generated by the initial test is confirmed by a second, more specific method, typically gas or liquid chromatography coupled to a single stage or tandem mass analyzer. This "screen with reflex to confirmation" testing approach is very effective at minimizing the risk of false-positive results. However, false-negative results can occur as well, with any technology, when an expected analyte is not detected (17).

The major advantages of immunoassays are wide availability, low cost, and rapid time to result. There are many commercially available configurations for urine testing that can be performed at the point of collection and are "waived" of regulatory oversight, making them easy to implement in nearly any setting. A major concern with immunoassays is poor specificity. The reason for poor specificity is that immunoassays are designed to detect substances based on antibody-antigen reactions. As part of assay design, monoclonal or polyclonal antibodies are characterized for cross-reactivity to a specific substance (calibrator). Based on chemical similarity to the calibrator, substances in a biological specimen will bind to the testing antibodies with variable affinity, thus yielding cross-reactivity profiles for a range of chemically similar substances. Consequently, an immunoassay may detect a single substance or a combination of substances. Immunoassay-based results, whether positive or negative, are considered presumptive because the specific analytes detected or missed cannot be discriminated (38).

Mass spectrometry methods that are coupled to gas or liquid chromatography are referred to as hyphenated techniques. Common examples are gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). These methods generally require hours to days to perform, in part due to preanalytical handling and processing of a biological specimen required to prepare a sample for testing. Typical steps associated with sample preparation are shown in Fig. 1. Dilution and hydrolysis are commonly employed for urine tests. Derivatization is common for tests that involve gas chromatography or for which chiral separations are required; digestions and homogenization may be required to convert a solid specimen (e.g., meconium and UC) to a liquid specimen. Extraction methods are designed to isolate and concentrate the analyte(s) of interest to maximize detection. Detection technologies are aligned with the chemistry of the targeted analytes and desirable range of concentrations measured (38, 40–42).

A specimen that is prepared for analysis may undergo chromatography to separate components in the sample by size and chemistry. A chromatogram is used to

Table 3. Overview of analytical methods used for drug testing.

Characteristics	Immunoassay	Chromatography	Single stage mass analyzer	Tandem mass analyzers	Accurate mass analyzers
Principle	Antibodies bind the drug analytes of interest in traditional antibody-antigen reaction; competitive assay design common between labeled and unlabeled analytes	Liquid or gas flows through a solid phase to which analytes can be retained, based on chemical and physical characteristics, before traveling to a detector	Analytes are ionized, sorted, and detected based on mass-to-charge (*m/z*); variable electric and magnetic fields in a single quadrupole (Q)	Analytes are ionized, sorted, and detected based on *m/z*; variable electric and magnetic fields in a triple quadrupole configuration wherein Q1 measures precursor ions; Q2 is a collision cell; Q3 measures product ions	Analytes are ionized, sorted, and detected based on *m/z*; multiple instrument designs differentiate ions with mass accuracy to several decimal places; often includes isotope analyses
Data elements captured	Signal generated by chemical reaction and measured by detector	Signal measured by detector, retention time, and relative retention time; peak shapes	Ion abundance per *m/z*	Ion abundance per *m/z* and unique transitions to product ions	Ion abundance per *m/z*, may be summed with isotopes
Common types	Lateral flow, enzyme immunoassay	Liquid, gas	Mass spectrometry	MS/MS	Orbitrap, TOF
Data generated	Absorbance units	Chromatogram; requires a detector such as a mass analyzer	Mass spectra; total ion, extracted chromatogram; for select or multiple ions; nominal resolution	Mass spectra; total ion, extracted chromatogram; for select or multiple ion transitions; nominal resolution	Mass spectra and related chromatograms for select or multiple ions and isotopes; high resolution
Results generated	Presumptive positive or negative	Assay design dictates analytical measurement range, quality of results, and whether quantitative values are reported. Assay parameters vary widely between laboratories and specific test designs			
Time to result	Minutes	Hours to days			

evaluate the retention of the analytes over time. Retention occurs through interactions between a mobile phase (gas or liquid), the flow rate of the mobile phase, and the solid phase (an analytical column). Each analyte exhibits a unique retention time that is compared to traceable standards, and quality of response is monitored

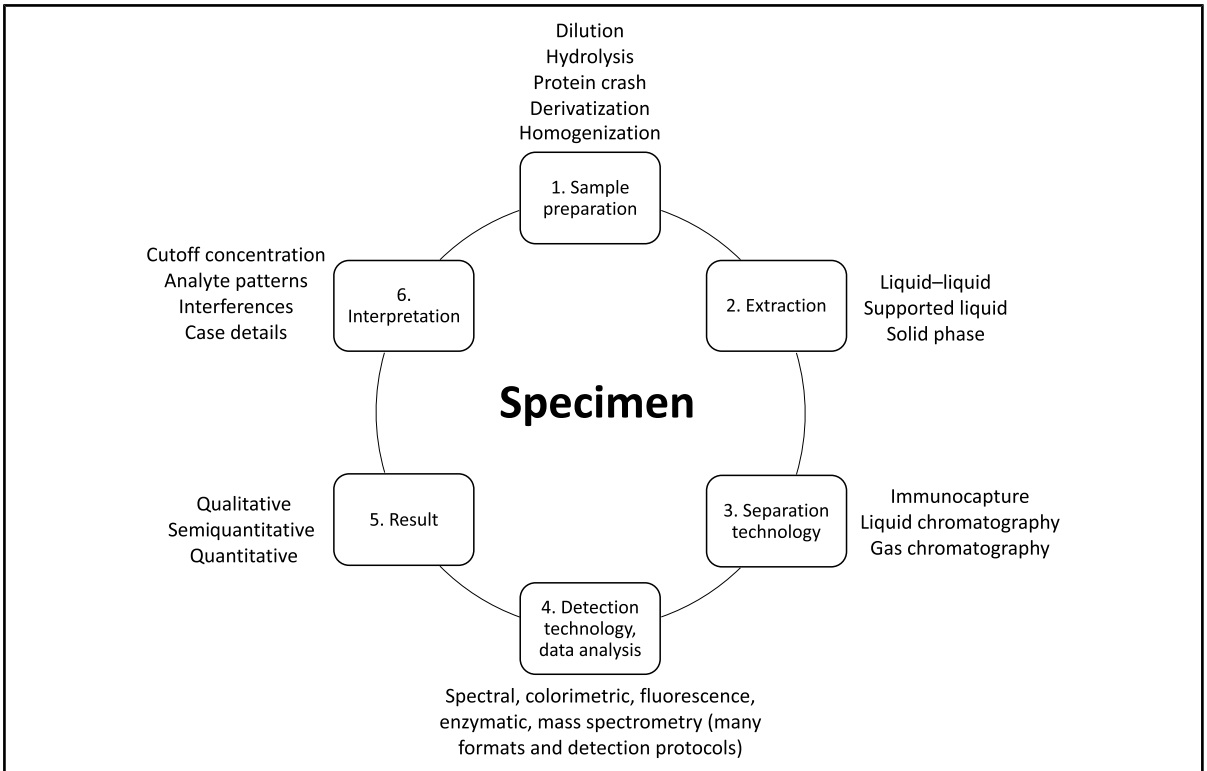

Fig. 1. Common variables of laboratory methods that could affect detection of substances in biological specimens, organized based on major steps in the testing process.

throughout analysis. Retention times of internal standards are also measured relative to the analyte(s) of interest. Visualizing a chromatogram requires a detector, which is often a mass analyzer.

The initial step for mass spectrometric analysis is to produce ions from a neutral atom or molecule in an ion source. The most common ion sources add one or more protons to basic sites on the molecule, and thereby function in positive ion mode; some substances are better detected based on generating negatively charged ions (e.g., loss of a proton). Ions formed in the ion source are sorted and filtered based on mass-to-charge ratio values (*m/z*). Depending on the ionization technique and configuration of the mass analyzer, substances may undergo fragmentation. The fragments produced are characteristic of the precursor molecule and corresponding *m/z* measured. Traditionally confirmation testing of substances was based on GC–MS, which is a nominal mass analyzer that generates mono-isotopic *m/z* data, generally with a resolution of 1 Da.

Today, the most common configuration for confirmation testing is LC–MS/MS. In this configuration an intact precursor molecule is measured in the first quadrupole (Q1), fragmented in the second quadrupole (Q2), and the fragments are measured in the third quadrupole (Q3). As such, the instrument is often referred to as a triple quadrupole analyzer. The monitoring of precursor and characteristic product ions, typically called multiple reaction monitoring adds specificity to monitoring the precursor ion alone. Performance criteria such as retention time of the substance of interest, relative retention time of the associated internal standard, ion abundances of both the precursor and product ions, and ratios of ion abundances representing the transitions from precursor to product ions are routinely monitored to identify and discriminate analytes. Like GC–MS, the typical LC–MS/MS generates nominal mass, mono-isotopic *m/z* data, with a resolution of 1 Da. One concern about nominal mass analysis is that some substances have very similar *m/z* values (isobars), and cannot be discriminated. For example, the nominal *m/z* for the unique heroin metabolite 6-acetylmorphine cannot be distinguished from the *m/z* for the opioid antagonist naloxone by a nominal mass analyzer. Chromatographic separation of analytes that share the same *m/z* is a common way to manage isobaric interferences.

High-resolution mass spectrometry (HRMS) can resolve isobaric interferences without chromatographic separation, by detecting substances based on the sum of the natural isotopes for a *m/z*. Examples of common HRMS platforms include the Orbitrap and time of

flight (TOF) mass analyzers. These instruments may be coupled to chromatographic separation to increase the number of analytes that can be detected. An interesting approach is to apply HRMS in an untargeted test design in which a method can detect a wide range of candidate analytes that are not known before testing, such as fentanyl analogs or other novel psychoactive substances (NPS). Detection is typically based on identification of physical and chemical characteristics that are subsequently compared to a library or database of characteristics and patterns established previously with known analytes. Because untargeted methods are not optimized for detection of specific analytes, detection limits and performance are often not known and may not compare well with targeted methods (1, 27, 43–45). However, when a candidate analyte is identified, the sample could be confirmed by another method and/or search for metabolites (44). A similar approach has also been used to search for and identify adulterating substances and NPS in newborn specimens (45, 46).

The specific drugs included in a test is not standardized for newborn specimens, and varies widely. Some laboratories use the workplace drug testing scheme as a guide, and others focus on controlled substances only. Standardization in analyte content for testing newborn specimens would help improve consistency of testing between laboratories, but may not meet clinical needs. With the exception of commercial immunoassays waived or approved by the Food and Drug Administration, most clinical testing that is performed to detect substance use or exposure occurs with laboratory-developed tests as defined by the Clinical Laboratory Improvement Amendments or forensic standards. As such, the design and performance characteristics of an analytical approach is determined and verified by the laboratory that designed and performs the test. Testing between laboratories is monitored over time by participation in external proficiency testing program, but nuances in assay design and associated detection limits are to be expected. Unfortunately, proficiency testing is not currently available for substance testing of newborn specimens.

Challenges with Interpretation of Meconium and UC Test Results

A common question regarding interpretation of meconium and UC results relates to timing and extent of substance exposure(s). As discussed before, the specific time window reflected by each specimen is approximately the last trimester of a full-term pregnancy, but may vary relative to each substance involved in an exposure, specimen quality and handling, and analytical variables. The fact that substance exposure during the birth hospitalization can be detected is also problematic. In addition, detection of multiple analytes may imply polysubstance exposure, but meconium and UC test results cannot determine whether multiple substances were used simultaneously or at asynchronous time points. Matrix-specific analyte targets, fetal metabolic pathways, mechanisms for analyte deposition, and elimination kinetics represent substrate-specific challenges and can contribute to discrepancies in results between specimens. The discussion in this section, along with Table 4, summarizes some of the common interpretive concerns and likely explanations.

Unexpected results, disputed results, and results that do not correlate with clinical presentation require investigation of the case details, including input from the laboratory that performed the testing (50, 67). Unexpected or false negatives can occur when analysis is negative for substances and/or substance metabolites despite known or suspected exposure. This can theoretically happen with any substance, especially if maternal exposure is limited, or if the test performed is not designed to detect the substance analytes of interest. For example, NPS are not detected by most toxicology tests. There is also a growing literature on the potential effects of antidepressants and other prescription medications during pregnancy that may contribute to clinical symptoms consistent with NAS, yet will not be detected by most laboratory tests (68). Nonmedical use during pregnancy of prescription substances such as gabapentin (65, 69) or over-the-counter compounds such as kratom (43, 70) may affect the neonate, and may not be included in routine meconium and UC testing. Unexpected or disputed positives can also occur with meconium and UC testing. The common use of mass spectrometry-based techniques for analysis of meconium and UC reduces the potential for false positives compared with immunoassay-based techniques that are limited by cross-reactivity of analytes with assay antibodies (34, 47, 71, 72). A thorough review of maternal inpatient and outpatient prescriptions along with history of other therapeutic and recreational substances is critical for interpretation of toxicology testing.

Newborn substance testing using meconium or UC may be performed in multiples (e.g., twins, triplets) (61–63). Discordance in results between multiples can potentially create confusion for the clinical team. Fortunately, results between multiples are frequently concordant or with minor differences that do not impact overall interpretation of results (e.g., oxycodone found in UC of both twins but a metabolite such as noroxycodone or oxymorphone not detected in one twin). Occasionally, analysis may result in more discordant findings, including even for identical twins, although discrepancies are overall more common in dizygotic twins (64). In both UC and meconium, the most common reason for discrepancies is when one or more analytes are not detected in one of the newborns due to insufficient analytical quality or a concentration slightly below the cutoff for positive reporting. In meconium samples, there is the additional variable that

Table 4. Examples of interpretive challenges with meconium and UC results.

Issue examples	Comments	References
Unexpected or false negatives		
Buprenorphine	• May be difficult to detect due to variable dosing and pharmacokinetics during pregnancy, poor placental transfer of buprenorphine, and analytical limitations of testing	(47–49)
Heroin	• Difficult to detect due to rapid metabolism of heroin and thermal instability of 6-acetylmorphine • Morphine may be primary metabolite detected	(24, 50–52)
Unexpected or disputed positives		
Fentanyl	• Perinatal administration (e.g., epidural) may explain detection in any specimen type, including UC tissue or meconium	(25, 26)
Methamphetamine	• Result based on immunoassay only is limited by assay cross-reactivity and potential for false positives • Regular use of over-the-counter formulation that contains l-methamphetamine, or use of a prescription substance such as selegiline that metabolizes to l-methamphetamine may be interpreted as positive	(53–56)
Morphine	• May be difficult to interpret source due to multiple possibilities including poppy seeds, administration of morphine, heroin, and codeine • Common medication to newborn in hospital setting	(24, 52)
Cannabis	• Use of CBD or hemp products that contain small amounts of delta-9 THC may be detected in UC tissue or meconium • Delta-8 and delta-10 THC may be analytically confused with delta-9 THC in many assays, including mass spectrometry-based • Passive exposure could explain unexpected positives (theoretically possible with some other substances as well)	(57–60),
Discrepant results		
Multiples (e.g., twins, triplets)	• Often explained by analyte concentrations slightly below reporting cutoff for one newborn specimen but not the other or different medication(s) administered after, birth but before specimen collection (urine, meconium)	(61–64)
Newborn symptoms despite negative toxicology testing	• Maternal use of substances not detected by analysis (e.g., designer stimulants, fentanyl analogs, xylazine, synthetic cannabinoids, gabapentin, antidepressants, mitragynine) • Concentrations of analyte(s) of suspected substance below reporting cutoff	(50, 65, 66)

medications prescribed to the newborn prior to meconium passage may be detected. This type of discrepancy would involve medications commonly prescribed to newborns in the hospital setting (e.g., lorazepam, morphine, phenobarbital) that are administered in one twin or triplet and not the others (61–63, 73). This phenomenon is more common in premature newborns that have delayed passage of meconium, providing a greater time window for newborn medications to distribute into meconium before specimen collection.

Although existing data cannot definitively guide interpretation of unexpected results, specific issues related to amphetamines, opioids, and cannabinoids are common.

Amphetamines remain common substances of use in the United States, with amphetamine and methamphetamine being the most common (74). The main metabolite of methamphetamine is amphetamine; consequently, maternal exposure to methamphetamine often results in the detection of both methamphetamine and amphetamine in meconium or UC. False-positive amphetamine or methamphetamine screening results generated by immunoassay are known to occur because of use or exposure to many other substances or substance metabolites (53, 54, 75). This may affect urine drug screening of mother or newborn. For example, the antihypertensive substance labetalol is used frequently in pregnancy and has been associated with false-positive immunoassay results that are resolved by mass spectrometric methods (55). The detection of amphetamine in isolation (i.e., without methamphetamine) in UC and meconium would likely imply exposure to amphetamine alone, including prescription medications used for treatment of attention-deficit/hyperactivity disorder.

The detection of methamphetamine by mass spectrometric assays in meconium or UC typically implies nonmedical use. However, for the last few decades, certain over-the-counter products have contained l-methamphetamine, an isomer exempt from Controlled Substance Scheduling in the United States. Although manufacturers have since reformulated some of these products without l-methamphetamine (76), use of these over-the-counter products can potentially result in positive results for methamphetamine. Many MS-based methods do not distinguish between the 2 methamphetamine stereoisomers, with specialized chiral analysis required for this differentiation (77, 78).

Opioids and their metabolites are substances commonly detected in newborns. Several factors can affect interpretation of opioid testing in meconium and UC. For example, possible maternal sources of morphine include dietary poppy seeds, administration of morphine to the mother (including perinatally), and as a metabolite of other substances (codeine, heroin). Heroin has traditionally been difficult to detect in meconium and UC due to the rapid metabolism of the parent substance and thermal instability of the diagnostic metabolite 6-acetylmorphine, which degrades significantly at body temperature and additionally after specimen collection (51). Targeting analytes that are suggestive of heroin use such as meconin (a metabolite of the heroin contaminant noscapine) and the common impurity codeine may help improve detection of heroin exposure (52). However, heroin use by the pregnant person may be only evident by the presence of morphine, and perhaps further downstream metabolites such as hydromorphone (24, 52). Administration of morphine, fentanyl and other opioids during the birth hospitalization further complicates interpretation. The potential for fentanyl administration as part of neuraxial anesthesia (e.g., epidural injection) to be detectable in UC has been illustrated by a series of published reports (25, 26, 79). Although cutoff concentrations and use of HRMS can help discriminate the likelihood of false-positive detection of recreational opioids, interpretation of meconium and UC results should consider the perinatal administration of opioids during the birth hospitalization.

Buprenorphine has increasingly been used for medication for opioid use disorder (MOUD) in pregnant people and thus buprenorphine and/or metabolites may be expected in meconium and UC. However, buprenorphine pharmacokinetics change during pregnancy, and placental transfer of buprenorphine is relatively low (48, 49). Analytical challenges in relation to the detection of buprenorphine and its metabolites in meconium and UC may lead to analyte concentrations below limits of reporting (47). This can potentially result in a false negative for buprenorphine and its metabolites in UC that may be interpreted by the clinical team as maternal nonadherence to MOUD treatment. Assessing maternal adherence to testing, if desired, should be achieved by testing of maternal specimens such as urine.

Interpretation of testing for cannabis use and exposure is quite complex (57). Some of the factors impacting interpretation include varying laws between states related to recreational and medical use of cannabis products, increased sales of cannabidiol (CBD) products, and provisions of the 2018 United States Agricultural Improvement Act (Farm Bill) regarding hemp-derived compounds. In states that have legalized recreational cannabis, healthcare providers and clinical laboratories may decide to discontinue or test less often for tetrahydrocannabinol (THC) and its metabolites (80, 81).

The increasing popularity of CBD and hemp products complicates interpretation of results. While federally regulated CBD and hemp formulations are intended to contain <0.3% delta-9-THC by dry weight, many over-the-counter CBD products exceed this threshold (58, 59). Further, THC metabolites may accumulate and be detected for several days to weeks after last use. Therefore, pregnant people may be using CBD products without realizing that there could be sufficient THC to cause positivity in newborn specimens such as meconium and UC, especially given the wide time window for detection. Another complicating factor is the fact that many assays for delta-9 THC, including mass spectrometric tests, cannot distinguish between delta-9 THC and isomers such as delta-8 THC, delta-10 THC, and their metabolites (60). Passive exposure to cannabis theoretically has the potential to lead to positive maternal and/or neonate substance testing results as well.

Future Needs and Directions

Biological testing is a frequently used tool for detecting and documenting substance exposure among pregnant

Table 5. Summary of considerations and cautions relative to biological testing for detection of drug use or exposure.

Practice topics	Considerations	Cautions
Choice to pursue biological testing	Decide how results will be used before testing	Harm and disparities can occur; consenting is critical
Specimen to test	Align specimen(s) with clinical workflow, purpose of testing, timing of possible exposure	Detection window is dependent on the substance, the timing of substance use or exposure relative specimen collection, laboratory method, and many other factors
Preferred maternal specimens to test	Urine or blood collected at clinical presentation represent recent substance use or exposure	Results between specimens may not agree
Preferred newborn specimens to test	UC tissue is preferred due to ease of collection; positivity rate may be higher in meconium	Drugs administered in the hospital prior to specimen collection may be detected
Testing in multiples (e.g., twins or triplets)	Each newborn is unique and should be treated as such	Results may not agree
Analytical method	Assay design and analytical approach defines sensitivity and specificity of results	Avoid immunoassays for newborn specimens
Cutoff concentrations	Lack of standardization among laboratories contributes to variation in detection	Lower cutoffs may detect passive exposures; higher cutoffs may yield false-negative results
Proficiency testing	Interlaboratory comparisons of results not available	Intralaboratory comparisons demonstrate consistency rather than appropriateness of testing
Specific analytes to detect	Panels often focus on controlled substances, similar to established urine panels	No test can detect all possible analytes or exposures; detection of multiple metabolites and/or adulterants may add value
Interpretation of unexpected results	Investigate relative to possible sources of exposure, timing of exposure, specimen quality, limitations of testing performed	Testing alternate specimen types or analytical approaches may add value
Interpretation of quantitative results	Reserve interpretation of quantitative results for evaluating metabolite and unusual result patterns	Correlation between exposure patterns with concentrations of analytes is not well established for most analytes
Assessment of maternal compliance with MOUD	Apply tools for evaluating maternal behavior and test maternal specimens	Testing newborn specimens is not appropriate for evaluating maternal compliance

people and their newborns. The results of testing can be difficult to interpret and may not be consistent when multiple specimens are collected and compared, or when specimens are tested by >1 laboratory method. There may also be inconsistency between results of testing on or during maternal admissions and pretest expectations. These differences can often be explained with investigation and education but the need for better understanding of test and specimen strengths and limitations, standardization of testing content and cutoff concentrations, as well as a reduced time to result would improve the value of biological testing toward informing both medical and social support decisions. These and other important knowledge gaps for the field of

biological testing for substance-exposed newborns are summarized in Table 5, along with considerations and cautions, with the hope that related stakeholders will commit to better understand and guide development of common approaches, and ultimately draft clinical practice guidelines. In the meantime, we suggest the following specific clinical practice recommendations that should occur prior to ordering any biological testing: (*a*) laboratories and clinicians should discuss testing options prior to biological testing to ensure that strengths and limitations of available options are understood; (*b*) develop protocols for standardized collection and handling of biological specimens; (*c*) obtain informed consent; and (*d*) define and communicate actions that will be taken based on biological testing results.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: SUD, substance use disorder; NAS, neonatal abstinence syndrome; UC, umbilical cord; HRMS, high-resolution mass spectrometry; TOF, time of flight; NPS, novel psychoactive substances; MOUD, medication for opioid use disorder; CBD, cannabidiol; THC, tetrahydrocannabinol.

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References

1. Hudson RE, Metz TD, Ward RM, McKnite AM, Enioutina EY, Sherwin CM, et al. Drug exposure during pregnancy: current understanding and approaches to measure maternal-fetal drug exposure. Front Pharmacol 2023;14:1111601.
2. ELNahas G, Thibaut F. Perinatal psychoactive substances use: a rising perinatal mental health concern. J Clin Med 2023;12:2175.
3. Board A, D'Angelo DV, Salvesen von Essen B, Denny CH, Miele K, Dunkley J, et al. Polysubstance use during pregnancy: the importance of screening, patient education, and integrating a harm reduction perspective. Drug Alcohol Depend 2023;247:109872.
4. Simpson EA, Skoglund DA, Stone SE, Sherman AK. A prediction model for positive infant meconium and urine drug tests. Am J Perinatol 2022;39:1104–11.
5. Committee opinion No. 711 summary: opioid use and opioid use disorder in pregnancy. Obstet Gynecol 2017;130:488–9.
6. Ecker J, Abuhamad A, Hill W, Bailit J, Bateman BT, Berghella V, et al. Substance use disorders in pregnancy: clinical, ethical, and research imperatives of the opioid epidemic: a report of a joint workshop of the Society for Maternal-Fetal Medicine, American College of Obstetricians and Gynecologists, and American Society of Addiction Medicine. Am J Obstet Gynecol 2019;221:B5–B28.
7. Gonzalez-Colmenero E, Concheiro-Guisan A, Lorenzo-Martinez M, Concheiro M, Lendoiro E, de-Castro-Rios A, et al. Drug testing in biological samples vs. Maternal surveys for the detection of substance use during whole pregnancy. J Addict Dis 2021;39:175–82.
8. Smith BL, Hall ES, McAllister JM, Marcotte MP, Setchell KDR, Megaraj V, et al. Rates of substance and polysubstance use through universal maternal testing at the time of delivery. J Perinatol 2022;42:1026–31.
9. Garg M, Garrison L, Leeman L, Hamidovic A, Borrego M, Rayburn WF, Bakhireva L. Validity of self-reported drug use information among pregnant women. Matern Child Health J 2016;20:41–7.
10. Child Welfare Information Gateway. About CAPTA: a legislative history. Washington, DC: U.S. Department of Health and Human Services, Children's Bureau; 2019. https://www.childwelfare.gov/resources/about-capta-legislative-history/ (Accessed February 2024).
11. Guttmacher Institute. Substance use during pregnancy. https://www.guttmacher.org/

statepolicy/explore/substance-use-during-pregnancy (Accessed December 2023).
12. Patrick SW, Barfield WD, Poindexter BB. Neonatal opioid withdrawal syndrome. Pediatrics 2020;146:e2020029074.
13. Ondersma SJ, Chang G, Blake-Lamb T, Gilstad-Hayden K, Orav J, Beatty JR, et al. Accuracy of five self-report screening instruments for substance use in pregnancy. Addiction 2019;114:1683–93.
14. Gersch H, Shah D, Chroust A, Bailey B. Can umbilical cord testing add to maternal urine drug screen for evaluation of infants at risk of neonatal opioid withdrawal syndrome? J Matern Fetal Neonatal Med 2023;36:2211706.
15. Koenigs KJ, Chou JH, Cohen S, Nolan M, Liu G, Terplan M, et al. Informed consent is poorly documented when obtaining toxicology testing at delivery in a Massachusetts cohort. Am J Obstet Gynecol MFM 2022;4:100621.
16. Jones J. Toxicology as a diagnostic tool to identify the misuse of drugs in the perinatal period. Front Pediatr 2022;10:1071564.
17. AACC Academy. Using clinical laboratory tests to monitor drug therapy in pain management patients. 2018. https://www.myadlm.org/science-and-research/practice-guidelines/using-clinical-laboratorytests-to-monitor-drug-therapy-in-pain-management-patients (Accessed December 2023).
18. Cotten SW, Duncan DL, Burch EA, Seashore CJ, Hammett-Stabler CA. Unexpected interference of baby wash products with a cannabinoid (THC) immunoassay. Clin Biochem 2012;45:605–9.
19. Barakauskas VE, Davis R, Krasowski MD, McMillin GA. Unresolved discrepancies between cannabinoid test results for infant urine. Clin Chem 2012;58:1364–7.
20. Metz TD, McMillin GA, Silver RM, Allshouse AA, Heard K, Jensen TL, et al. Quantification of prenatal marijuana use: evaluation of the correlation between self-report, serum, urine and umbilical cord assays among women delivering at two urban Colorado hospitals. Addiction 2022;117:172–81.
21. Marin SJ, Christensen RD, Baer VL, Clark CJ, McMillin GA. Nicotine and metabolites in paired umbilical cord tissue and meconium specimens. Ther Drug Monit 2011;33:80–5.
22. Gray TR, LaGasse LL, Smith LM, Derauf C, Grant P, Shah R, et al. Identification of prenatal amphetamines exposure by maternal interview and meconium toxicology in the Infant Development, Environment and Lifestyle (IDEAL) study. Ther Drug Monit 2009;31:769–75.
23. Haizler-Cohen L, Collins A, Kaplan DM, Giri P, Davidov A, Blau J, Fruhman G. Universal urine drug screening with rapid confirmation upon admission to labor and delivery. [Epub] Am J Perinatol August 4, 2023, as doi: 10.1055/a-2118-2841.
24. McMillin GA, Wood KE, Strathmann FG, Krasowski MD. Patterns of drugs and drug metabolites observed in meconium: what do they mean? Ther Drug Monit 2015;37:568–80.
25. Jones J, Coy D, Mitacek R, Thompson S, Maxwell S. Neonatal cord tox panel and maternal perinatal fentanyl exposure: a retrospective chart review. Am J Anal Chem 2021;12:324–31.
26. Siegel MR, Mahowald GK, Uljon SN, James K, Leffert L, Sullivan MW, et al. Fentanyl in the labor epidural impacts the results of intrapartum and postpartum maternal and neonatal toxicology tests. Am J Obstet Gynecol 2023;228:741.e1–e7.
27. Pandya V, Wilker C, McMillin GA. Can umbilical cord and meconium results be directly compared? Analytical approach matters. J Anal Toxicol 2023;47:96–105.
28. Villarreal M, Belmonte V, Re S, Garcia-Algar O. Detection of illicit psychoactive substances in the urine of mothers and newborn infants at a public hospital. Comparison between the 2009–2013 and 2014–2018 five-year periods. Arch Argent Pediatr 2023;121:e202202900.
29. Mark K, Pace L, Temkin SM, Crimmins S, Terplan M. Concordance and discordance between maternal and newborn drug test results. Am J Obstet Gynecol MFM 2021;3:100366.
30. Colby JM, Adams BC, Morad A, Presley LD, Patrick SW. Umbilical cord tissue and meconium may not be equivalent for confirming in utero substance exposure. J Pediatr 2019;205:277–80.
31. Colby JM. Comparison of umbilical cord tissue and meconium for the confirmation of in utero drug exposure. Clin Biochem 2017;50:784–90.
32. Labardee RM, Swartzwelder JR, Gebhardt KE, Pardi JA, Dawsey AC, Brent Dixon R, Cotten SW. Method performance and clinical workflow outcomes associated with meconium and umbilical cord toxicology testing. Clin Biochem 2017;50:1093–7.
33. Gray TR, Choo RE, Concheiro M, Williams E, Elko A, Jansson LM, et al. Prenatal methadone exposure, meconium biomarker concentrations and neonatal abstinence syndrome. Addiction 2010;105:2151–9.
34. Montgomery DP, Plate CA, Jones M, Jones J, Rios R, Lambert DK, et al. Using umbilical cord tissue to detect fetal exposure to illicit drugs: a multicentered study in Utah and New Jersey. J Perinatol 2008;28:750–3.
35. Negrusz A, Jones J. Testing umbilical cord tissue for drugs: lower analytical limits, better concordance with meconium results. J Pediatr 2019;209:258–9.
36. Henderson EMA, Tappin D, Young D, Favretto D, Mactier H. Assessing maternal alcohol consumption in pregnancy: does phosphatidylethanol measured from day 5 newborn blood spot cards have any value? An observational, population-based study. Arch Dis Child 2023;108:36–41.
37. Gesseck AM, Poklis JL, Wolf CE, Xu J, Bashir A, Hendricks-Munoz KD, Peace MR. A case study evaluating the efficacy of an ad hoc hospital collection device for fentanyl in infant oral fluid. J Anal Toxicol 2020;44:741–6.
38. McMillin GA, Slawson MH, Marin SJ, Johnson-Davis KL. Demystifying analytical approaches for urine drug testing to evaluate medication adherence in chronic pain management. J Pain Palliat Care Pharmacother 2013;27:322–39.
39. Peters FT, Wissenbach D. Current state-of-the-art approaches for mass spectrometry in clinical toxicology: an overview. Expert Opin Drug Metab Toxicol 2023;19:487–500.
40. Wabuyele SL, Colby JM, McMillin GA. Detection of drug-exposed newborns. Ther Drug Monit 2018;40:166–85.
41. Silveira GO, Pego AMF, Pereira ESJ, Yonamine M. Green sample preparations for the bioanalysis of drugs of abuse in complex matrices. Bioanalysis 2019;11:295–312.
42. de Campos EG, da Costa BRB, Dos Santos FS, Monedeiro F, Alves MNR, Santos Junior WJR, De Martinis BS. Alternative matrices in forensic toxicology: a critical review. Forensic Toxicol 2022;40:1–18.
43. Hughs M, Kish-Trier E, O'Brien A, McMillin GA. Analysis of mitragynine and speciociliatine in umbilical cord by LC-MS-MS for detecting prenatal exposure to kratom. J Anal Toxicol 2023;46:957–64.
44. Mamillapalli SS, Smith-Joyner A, Forbes L, McIntyre K, Poppenfuse S, Rushing B, et al. Screening for opioid and stimulant exposure in utero through targeted and untargeted metabolomics analysis of umbilical cords. Ther Drug Monit 2020;42:787–94.
45. Lopez-Rabunal A, Di Corcia D, Amante E, Massano M, Cruz-Landeira A, de-Castro-Rios A, Salomone A. Simultaneous determination of 137 drugs of abuse, new psychoactive substances, and novel synthetic opioids in meconium by UHPLC-QTOF. Anal Bioanal Chem 2021;413:5493–507.
46. Nelson BN, Strathmann FG, Browne T, Cervantes A, Logan BK. Qualitative LC-Q-TOF analysis of umbilical cord tissue via data-dependent acquisition as an indicator of in utero exposure to toxic adulterating substances. J Anal Toxicol 2022;46:619–24.
47. Shan X, Cao C, Yang B. Analytical approaches for the determination of buprenorphine, methadone and their metabolites in biological matrices. Molecules 2022;27:5211.
48. Nanovskaya T, Deshmukh S, Brooks M, Ahmed MS. Transplacental transfer and metabolism of buprenorphine. J Pharmacol Exp Ther 2002;300:26–33.
49. Suarez EA, Huybrechts KF, Straub L, Hernandez-Diaz S, Jones HE, Connery HS, et al. Buprenorphine versus methadone for opioid use disorder in pregnancy. N Engl J Med 2022;387:2033–44.
50. Midthun KM, Nelson BN, Strathmann FG, Browne T, Logan BK. Analysis of umbilical cord tissue as an indicator of in utero exposure to toxic adulterating substances. Front Pediatr 2023;11:1127020.
51. Wu F, Marin SJ, McMillin GA. Stability of 21 cocaine, opioid and benzodiazepine drug analytes in spiked meconium at three temperatures. J Anal Toxicol 2017;41:196–204.

52. Jones JT, Jones M, Jones B, Sulaiman K, Plate C, Lewis D. Detection of codeine, morphine, 6–monoacetylmorphine, and meconin in human umbilical cord tissue: method validation and evidence of in utero heroin exposure. Ther Drug Monit 2015;37:45–52.
53. Hughey JJ, Colby JM. Discovering cross-reactivity in urine drug screening immunoassays through large-scale analysis of electronic health records. Clin Chem 2019; 65:1522–31.
54. Pope JD, Drummer OH, Schneider HG. False-positive amphetamines in urine drug screens: a 6-year review. J Anal Toxicol 2023;47:263–70.
55. Bithi N, Merrigan SD, McMillin GA. Does labetalol trigger false positive drug testing results? J Addict Med 2023;17: e209–10.
56. Miklya I. The significance of selegiline/(-)-deprenyl after 50 years in research and therapy (1965–2015). Mol Psychiatry 2016; 21:1499–503.
57. Concheiro M, Gutierrez FM, Ocampo A, Lendoiro E, Gonzalez-Colmenero E, Concheiro-Guisan A, et al. Assessment of biological matrices for the detection of in utero cannabis exposure. Drug Test Anal 2021;13:1371–82.
58. Miller OS, Elder EJ, Jones KJ, Gidal BE. Analysis of cannabidiol (CBD) and THC in nonprescription consumer products: implications for patients and practitioners. Epilepsy Behav 2022;127:108514.
59. Spindle TR, Sholler DJ, Cone EJ, Murphy TP, ElSohly M, Winecker RE, et al. Cannabinoid content and label accuracy of hemp-derived topical products available online and at national retail stores. JAMA Netw Open 2022;5:e2223019.
60. La Maida N, Di Giorgi A, Pichini S, Busardo FP, Huestis MA. Recent challenges and trends in forensic analysis: delta9-THC isomers pharmacology, toxicology and analysis. J Pharm Biomed Anal 2022;220: 114987.
61. Lewis D, Moore C, Leikin JB, Kechavarz L. Multiple birth concordance of street drug assays of meconium analysis. Vet Hum Toxicol 1995;37:318–9.
62. Nelson HA, Wood KE, McMillin GA, Krasowski MD. Concordance of umbilical cord drug screening in multiple births: experience from a reference laboratory and academic medical center. J Anal Toxicol 2022;46:611–8.
63. Wood KE, Krasowski MD, Strathmann FG, McMillin GA. Meconium drug testing in multiple births in the USA. J Anal Toxicol 2014;38:397–403.
64. Alexander A, Abbas L, Jones M, Jones J, Lewis D, Negrusz A. Discordant umbilical cord drug testing results in monozygotic twins. J Anal Toxicol 2018;42: e47–9.
65. Loudin S, Murray S, Prunty L, Davies T, Evans J, Werthammer J. An atypical withdrawal syndrome in neonates prenatally exposed to gabapentin and opioids. J Pediatr 2017;181:286–8.
66. Nellhaus EM, Murray S, Hansen Z, Loudin S, Davies TH. Novel withdrawal symptoms of a neonate prenatally exposed to a fentanyl analog. J Pediatr Health Care 2019; 33:102–6.
67. Concheiro M, Lendoiro E, de Castro A, Gonzalez-Colmenero E, Concheiro-Guisan A, Penas-Silva P, et al. Bioanalysis for cocaine, opiates, methadone, and amphetamines exposure detection during pregnancy. Drug Test Anal 2017;9: 898–904.
68. Wang J, Cosci F. Neonatal withdrawal syndrome following late in utero exposure to selective serotonin reuptake inhibitors: a systematic review and meta-analysis of observational studies. Psychother Psychosom 2021;90:299–307.
69. Okoye NC, McMillin GA. Patterns of neonatal co-exposure to gabapentin and commonly abused drugs observed in umbilical cord tissue. J Anal Toxicol 2021;45:506–12.
70. Smid MC, Charles JE, Gordon AJ, Wright TE. Use of kratom, an opioid-like traditional herb, in pregnancy. Obstet Gynecol 2018;132:926–8.
71. Marin SJ, McMillin GA. LC-MS/MS analysis of 13 benzodiazepines and metabolites in urine, serum, plasma, and meconium. Methods Mol Biol 2010;603:89–105.
72. Ristimaa J, Gergov M, Pelander A, Halmesmaki E, Ojanpera I. Broad-spectrum drug screening of meconium by liquid chromatography with tandem mass spectrometry and time-of-flight mass spectrometry. Anal Bioanal Chem 2010; 398:925–35.
73. Wang P, Molina CP, Maldonado JE, Bernard DW. In utero drugs of abuse exposure testing for newborn twins. J Clin Pathol 2010;63:259–61.
74. Toske SG, McKibben TD. Monitoring methamphetamine in the United States: a two-decade review as seen by the DEA methamphetamine profiling program. Drug Test Anal 2022;14:416–26.
75. Saitman A, Park HD, Fitzgerald RL. False-positive interferences of common urine drug screen immunoassays: a review. J Anal Toxicol 2014;38:387–96.
76. Barkholtz HM, Hadzima R, Miles A. Pharmacology of R-(-)-methamphetamine in humans: a systematic review of the literature. ACS Pharmacol Transl Sci 2023;6: 914–24.
77. Paul BD, Jemionek J, Lesser D, Jacobs A, Searles DA. Enantiomeric separation and quantitation of (+/-)-amphetamine, (+/-)-methamphetamine, (+/-)-MDA, (+/-)-MDMA, and (+/-)-MDEA in urine specimens by GC-EI-MS after derivatization with (R)-(-)- or (S)-(+)-alpha-methoxy-alpha-(trifluoromethy)phenylacetyl chloride (MTPA). J Anal Toxicol 2004;28:449–55.
78. Shin I, Choi H, Kang S, Kim J, Park Y, Yang W. Detection of l-methamphetamine and l-amphetamine as selegiline metabolites. J Anal Toxicol 2021;45:99–104.
79. Fleet JA, Belan I, Gordon AL, Cyna AM. Fentanyl concentration in maternal and umbilical cord plasma following intranasal or subcutaneous administration in labour. Int J Obstet Anesth 2020;42:34–8.
80. Schoneich S, Plegue M, Waidley V, McCabe K, Wu J, Chandanabhumma PP, et al. Incidence of newborn drug testing and variations by birthing parent race and ethnicity before and after recreational Cannabis legalization. JAMA Netw Open 2023;6:e232058.
81. Skelton KR, Hecht AA, Benjamin-Neelon SE. Association of recreational cannabis legalization with maternal cannabis use in the preconception, prenatal, and postpartum periods. JAMA Netw Open 2021; 4:e210138.