Newborn Toxicology Testing: Comparison of Umbilical Cord, Meconium and Urine

Clinical Monograph

Introduction

Drug Exposure During Pregnancy

Fetal drug exposure is not uncommon, and each year an estimated 15% of infants are affected by prenatal alcohol or illicit drug exposure. Drug exposure in utero can have both short-term effects on the newborn and long-term effects on the developing child. With the ongoing opioid epidemic, opioid withdrawal is now one of the most commonly seen disorders in newborn infants and is referred to as Neonatal Abstinence Syndrome (NAS), a term also used to describe withdrawal from other drugs. Opioid withdrawal in a newborn can occur when a pregnant women uses heroin or other opioids that have been obtained illegally, or when the drugs are prescribed for conditions such as chronic pain.

Substance abuse during pregnancy also has important legal ramifications, with approximately half of the states in the United States considering substance abuse during pregnancy child abuse under civil child-welfare statutes. In addition, 25 states require healthcare professionals to report suspected prenatal drug use, 8 require testing for prenatal drug exposure if drug use is suspected, and a number of states mandate reporting of NAS for public surveillance.

Neonatal Abstinence Syndrome

NAS is caused when a fetus is exposed to drugs during pregnancy. The most common cause of NAS is maternal use of illicit drugs of abuse during pregnancy (Table 1). However, it is important to know that withdrawal symptoms and NAS can also be caused by the use of prescribed drugs, such as barbiturates, benzodiazepines, and antidepressants.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ASSOCIATED COMPLICATIONS</th>
</tr>
</thead>
</table>
| Heroin and other opioids, including methadone | • Serious withdrawal in the infant  
• Some symptoms can last 4 to 6 months  
• Seizures may also occur |
| Amphetamines                       | • Low birth weight  
• Premature birth                                                      |
| Cocaine                            | • Poor fetal growth  
• Low birth weight  
• Increased risk of complications such as placental abruption |
| Marijuana                          | • Low birth weight  
• Learning and behavior problems                                      |
| Alcohol                            | • Low birth weight  
• Failure to thrive  
• Anatomical defects of the head, face, and heart  
• Learning and psychological problems later in life |
| Tobacco (cigarette smoking)        | • Low birth weight  
• Increased risk of premature birth and stillbirth                      |
NAS rates have been increasing and have significantly impacted healthcare systems.

- From 2004 to 2013 the rate of neonatal intensive care unit (NICU) admissions for NAS in the United States increased from 7 cases per 1,000 admissions to 27 cases per 1,000 admissions.4
- The average hospital stay for an infant with NAS is 3.5 times longer than for an unaffected infant.11
- Overall, additional hospital charges associated with NAS were approximately $1.5 billion in 2012.12

Physical manifestations of NAS withdrawal in the newborn include irritability, excessive crying and/or a high-pitched cry, seizures, twitching, increased muscle tone (hyperactive reflexes) and tremors, rapid breathing, poor feeding and gastrointestinal disturbances (vomiting, loose stools), and sleep disturbances. Skin excoriations and mottling may occur, as well as manifestations of autonomic dysfunction such as excessive sweating. Fever of unknown origin, nasal stuffiness, and excessive sneezing may also be present. Infants with NAS may require long-term support, and can experience developmental problems.9,10 Children who have been exposed to drugs during pregnancy, even if they do not develop NAS after birth, may have developmental problems.9,10 Drug use during pregnancy is also associated with intrauterine growth retardation, premature birth, and birth defects.9,10

Determining Intrauterine Drug Exposure

Testing newborns to determine drug exposure and drug type is necessary when drug abuse is suspected based on maternal history or neonatal symptoms in order to provide appropriate treatment for the newborn and maximize good outcomes.2,4,5

Traditionally, determining if an infant has been exposed to a drug in utero has been done by testing the infant’s urine or meconium. Over the past decade, methods to test for in utero drug exposure using umbilical cord have been developed. This monograph discusses these 3 methods for testing if an infant has been exposed to drugs in utero and the advantages of testing. It also discusses NMS Labs’ product offering details for umbilical cord and meconium toxicology testing.

Testing Urine and Meconium for In Utero Drug Exposure

Testing urine and meconium are the most common methods of determining fetal drug exposure during pregnancy. Both have certain advantages and limitations, and the limitations are especially important when identification of drug use is critically important. For example, premature birth and low birth weight are common among infants with NAS; however, these conditions make the collection of urine or meconium difficult.14

The use of urine or meconium for toxicology testing may miss identifying maternal drug use during pregnancy if a “risk-based” protocol for testing is used. Risk factors are often not identified until days after delivery and the meconium or urine sample has been discarded.15 The prevalence of drug use during pregnancy can also vary with the population being served.16 Thus, some authors have advocated establishing universal neonatal screening protocols for determining maternal drug use during pregnancy.15

Urine Testing

Urine is a common specimen used for adult toxicology testing, and is widely used for newborn drug testing as well.2,16 However, the detection window is short and urine testing will typically only identify maternal drug use in the 3 to 7 days prior to delivery, depending on the particular drug half-life.2,17-20 A dilute urine specimen, or delayed collection can result in false-negative results; conversely, a urine specimen may be positive for drugs administered to the mother during labor and deliver, and to the newborn after delivery.17,18,20 Though urine is frequently used, some authors have suggested that it offers little diagnostic yield for detecting maternal non-medical drug use.15

Collecting urine from any newborn is difficult because a special collection device must be fitted over the genitals, which can cause irritation.14 The device can also fall off or become displaced resulting is loss or contamination of the specimen. Obtaining an adequate volume of urine is also difficult because urine output is only about 125 mL/day during the first 48 hours of life.18 Lastly, urine drug testing is typically based on immunoassays that have been designed for adults; neonatal drug metabolites may not be the same as those in adults, thus leading to false-negative results.2,18

Meconium Testing

Meconium is the first stool of a newborn, and the most commonly used specimen for newborn toxicology testing. Since the introduction of meconium testing it has become the “gold standard” for determining in utero drug exposures.2,17,23
Meconium begins to form around the 12th week of gestation, though 50% or more is formed during the final 8 weeks of pregnancy. In addition to metabolic waste products, drugs and drug metabolites accumulate in meconium resulting in a window of detection that includes the third trimester and, in certain instances, the latter part of the second trimester. Identification of drugs or drug metabolites in meconium in most cases indicates fetal exposure and metabolization by the fetus or neonate.

Although considered the “gold standard,” use of meconium is not without limitations. Meconium is passed in stages, typically during the first 1-3 days after birth; almost all full-term and post-term infants pass meconium during the first 24 hours of life. On the other hand, passage is generally delayed in preterm infants, with a longer delay the earlier the gestational age at birth. For example, approximately 80% of very low birth-weight infants have delayed meconium passage. Of preterm infants > 32 weeks’ gestation, around 37% pass meconium in the first 24 hours after birth, 32% after 48 hours, and 99% within 9 days.

Drugs given during pregnancy can also affect the timing of meconium passage: opioids given for pain control during labor (or illicit use by the mother) can delay passage; antenatal administration of betamethasone in the anticipation of preterm birth can lead to earlier meconium passage; magnesium sulfate given for preterm labor may delay meconium passage. When meconium passage is delayed, drugs and metabolites detected may reflect medications administered during labor and delivery or to the neonate after delivery, and not exposure during pregnancy.

Because meconium is heterogeneous, all that is passed must be collected, combined, and stored prior to providing a specimen for testing. The collection procedure across multiple hospital departments can be time consuming and potentially result in misidentification of specimens. In addition, prolonged or improper storage can affect the stability of some drugs and metabolites being detected.

Meconium can also be passed in utero, before or during delivery. This is not uncommon in late-term pregnancies, and is also associated with fetal distress during labor. Meconium passed in utero becomes mixed with the amniotic fluid and is not generally suitable for toxicology testing.

Overall, meconium is passed in utero in around 12% to 20% of pregnancies, and the rate is up to 40% in post-dates births. Thus, meconium may not be suitable for toxicology testing in 12% to 40% of pregnancies. In utero passage of meconium may indicate normal gastrointestinal maturation, or it may be a sign of acute or chronic fetal hypoxia, which can be associated with maternal drug use during pregnancy (i.e., drug use can cause placental abruption resulting in acute fetal hypoxia, or can cause intrauterine growth restriction associated with chronic fetal hypoxia). The inhalation of meconium by the fetus in utero or during delivery can result in meconium aspiration syndrome, which occurs in approximately 3% to 9% of infants delivered with meconium stained amniotic fluid. Meconium aspiration syndrome is an emergency than can require admission to the neonatal intensive care unit and mechanical ventilation.

Testing Umbilical Cord for In Utero Drug Exposure

Testing umbilical cord tissue to determine drug exposure during pregnancy is a relatively new method. The umbilical cord is homogeneous (unlike meconium), is universally available at the time of delivery, and sample collection is relatively simple. For collection: 1) A sample of umbilical cord ≥ 6 inches is cut; 2) The specimen is drained of blood, rinsed with normal saline or sterile water, and patted dry; 3) The cord specimen is placed in a transport container and sealed.

Umbilical cord allows detection of drugs and metabolites in the pico-gram to nano-gram range. No guidelines have been set for detection limits of analytes in urine, meconium, or umbilical cord for newborn testing; laboratories develop reporting limits based on the instruments and testing methods used. Drugs and drug metabolites accumulate in umbilical cord resulting in a window of detection that includes the third trimester and the latter part of the second trimester. Due to its nature as a supply line between mother and fetus, drugs detected in umbilical cord tissue may come directly from the mother, either in original or metabolite form, or may have been metabolized by the fetus. In general, testing of umbilical cord tissue avoids detection of iatrogenic drugs administered during labor and delivery; however, in some instances drugs administered during the course of labor and delivery may be detected in the umbilical cord specimens. Of legal importance, the neonate, not the mother, is considered the “owner” of the umbilical cord, and a single collection helps to ensure the integrity of the chain of custody.
Advantages of Umbilical Cord Over Meconium or Urine

Using umbilical cord to determine drug exposure during pregnancy overcomes many of the disadvantages of urine or meconium.\textsuperscript{2,23,27} Comparison of the characteristics of urine, meconium, and umbilical cord when used for neonatal toxicology testing is shown in Table 2 and Figure 1.

Complete collection of all meconium is important because meconium is heterogeneous with respect to drugs and drug metabolites, i.e., they are not distributed evenly within and among meconium passages.\textsuperscript{2,17} Thus, multiple collections of meconium are typically necessary, which increases the time for collection (and prolongs the time to receive results), the risk of contamination, and can create logistic problems for hospital personnel.\textsuperscript{18} A study at a university hospital found the success rate of collecting meconium was about 88%.\textsuperscript{17}

TABLE 2  Comparison of the Characteristics of Urine, Meconium, and Umbilical Cord When Used for Neonatal Toxicology Testing\textsuperscript{2,23}

<table>
<thead>
<tr>
<th></th>
<th>UMBILICAL CORD</th>
<th>MECONIUM</th>
<th>URINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ease of Collection</td>
<td>Easy</td>
<td>Moderate</td>
<td>Difficult</td>
</tr>
<tr>
<td>Window of Detection</td>
<td>2nd &amp; 3rd Trimester</td>
<td>2nd &amp; 3rd Trimester</td>
<td>Days</td>
</tr>
<tr>
<td>Specimen Availability</td>
<td>Universal</td>
<td>80-90% of the time</td>
<td>Universal</td>
</tr>
<tr>
<td>Specimen Size</td>
<td>6 inches</td>
<td>Variable (grams)</td>
<td>Variable (milliliters)</td>
</tr>
<tr>
<td>Composition</td>
<td>Homogeneous</td>
<td>Heterogeneous</td>
<td>Homogenous</td>
</tr>
<tr>
<td>Stability</td>
<td>More stable</td>
<td>Noted problems with some drugs</td>
<td>More stable</td>
</tr>
<tr>
<td>Testing Turnaround Time*</td>
<td>Negative: 1 day</td>
<td>Negative: 2 days</td>
<td>Hours (if performed in same hospital as birth)</td>
</tr>
</tbody>
</table>

* Turnaround time at NMS Labs.

FIGURE 1  Windows of Drug Detection for Urine, Meconium, and Umbilical Cord\textsuperscript{2}

- **Urine**
  - Recent Use/Exposure
    - Hours
    - Days
    - Weeks
    - Months
    - Years

- **Umbilical Cord Tissue**
  - Long-Term Use/Exposure
    - Hours
    - Days
    - Weeks
    - Months
    - Years

- **Meconium**
  - Long-Term Use/Exposure
    - Hours
    - Days
    - Weeks
    - Months
    - Years

Note: Actual detection window is drug-dependent and also reflects patterns of use, dose, and performance of laboratory testing.
On the other hand, an umbilical cord specimen is collected immediately after birth. Thus, results are available faster than when meconium or urine is used. In addition, the chances of contamination are virtually eliminated, and the staff time required for collection is markedly less than that for meconium or urine. There are also fewer collection errors and contamination issues at the hospital with umbilical cord because only a single sample is needed.

Umbilical cord offers a much longer window of detection for identification of drug use as compared to urine, and a detection window similar to, or possibly longer in certain instances, than that of meconium. Importantly, when umbilical cord is used, the turnaround time for a negative result can be as short as 24 hours, and confirmation of a positive result can be obtained within 72 hours, a timeframe much shorter than that of meconium and potentially other specimen types.

**Umbilical Cord Compared to Meconium: Are They Equivalent?**

Comparing the equivalence of toxicology testing using meconium and umbilical cord is somewhat difficult because their characteristics are so different. However, overall studies have indicated that umbilical cord provides results that are consistent with meconium when used for neonatal toxicology testing.

While not a paired sample study, Palmer et al.28 evaluated the change from using meconium to umbilical cord for neonatal toxicology testing at an academic medical center. A review of the records of approximately 2,000 newborns who received toxicology testing indicated that umbilical cord testing yielded a similar detection rate as meconium testing. Importantly, differences noted were primarily from drugs administered during labor and at delivery; thus, differences could be accounted for by reviewing the patient’s medical history. Montgomery et al.23 tested paired samples of meconium and umbilical cord for drugs of abuse, and reported agreement was > 90% for the presence of amphetamines, opiates, cocaine, and cannabinoids.

Larbardee et al.29 studied using meconium or umbilical cord for toxicology testing with respect to the turnaround time to receive results and for a diagnosis of NAS. The median time from birth to meconium test results was approximately 25 hours, and after switching to umbilical cord the median time from birth to test results improved to 9.7 hours. Meconium testing had a clinical sensitivity of 65% and a clinical specificity of 85% for a diagnosis of NAS. For umbilical cord, the clinical sensitivity and specificity for NAS were 79% and 76%, respectively. The authors pointed out that neither specimen type was able to predict NAS in all cases, and that both meconium and umbilical cord tissue samples were negative for opiates in a significant percentage of newborns with a diagnosis of NAS, 35% and 21%, respectively. They suggested reasons for this including challenges of matrix effects (where other substances interfere) of both sample types (Table 3), limitations with their scope of testing, the exclusion of fentanyl as a “positive” opioid result (due to its presence as a common labor and delivery drug), and nicotine withdrawal mimicking opioid withdrawal. It is also important to note that NAS diagnosis is based on a combination of clinical observation and Finnegan Scoring of withdrawal symptoms; thus, it is possible for newborns to exhibit symptoms which mimic opioid withdrawal and not be experiencing NAS.

A 2017 retrospective study by Colby30 compared the results of umbilical cord tissue and meconium for the confirmation of in utero drug exposure. Overall, the author reported that meconium provided greater sensitivity for detection of the drugs examined. However, the author acknowledged that umbilical cord and meconium testing were conducted using different extraction procedures and analytical techniques, which resulted in differences in the ability of the 2 methods to detect low drug concentrations.

Another retrospective study by Colby et al.31 in 2019 also compared the results of meconium and umbilical cord neonatal toxicology testing. The results between the 2 specimen types were often discordant, although the overall prevalence of drugs in umbilical cord and meconium was similar for several groups of drugs and the agreement between both specimen types ranged from 80% to 100%. When interpreting the results of the aforementioned studies, it has been pointed out that the concentrations of drugs in umbilical cord are much lower than in meconium and require much lower limits of detection and/or cut-offs than traditional meconium testing methods,2,32 which may explain the discordant results reported by Colby et al. in 2019.

**NMS Labs Umbilical Cord Drug Testing**

NMS Labs offers comprehensive umbilical cord testing that can identify up to 57 drugs and drug metabolites. Drugs and drug metabolites tested for include opioid analgesics, cannabinoids, stimulants, benzodiazepines, and other common psychoactive substances. An ethyl glucuronide test is available separately. NMS Labs also offers meconium toxicology testing with up to 41 analytes. A summary of umbilical cord and meconium toxicology assays available from NMS Labs is shown in Table 4. Note that NMS Labs does not offer neonatal urine toxicology testing.
Advantages of NMS Labs Umbilical Cord Testing Include:

- High sensitivity and specificity with ease and speed of collection allowing the delivery of results faster than meconium, and a longer window of detection compared to urine.
- Simplified specimen collection. NMS Labs provides Umbilical Cord Testing (UCT) Collection Kits. Only a few minutes are required to obtain a sample, rinse, pat dry, and place the sample in the collection container.

Six simple steps of sample collection

1. Collect at least 6 inches of umbilical cord.
2. Drain and discard any blood.
3. Rinse the exterior of the cord with normal saline or sterile water.
4. Pat the cord dry.
5. Place specimen in container provided by NMS Labs, and apply tamper seal and patient label.
6. Place container in specimen bag, fill out chain of custody and requisition form, and send to NMS Labs with a cold pack.

- Turnaround time of less than 24 hours for a negative result; less than 72 hours if positive.
- Use of mass spectrometry provides high sensitivity and greater specificity compared to immunoassay screening, and confirmation testing adds confidence to these critical results.
- Detailed protocol for monitoring the specimen at every stage of testing, thus assuring a legally binding chain of custody.

Indications for NMS Labs Umbilical Cord Toxicology Testing

Umbilical cord toxicology testing detects prenatal exposure to drugs in umbilical cord tissue, and maternal and neonatal indications include, but are not limited to:

**Maternal**
- History of past or current drug use
- Little or no prenatal care
- Unexplained complications of pregnancy (i.e., preterm labor, placental abruption)

**Neonatal**
- Drug withdrawal signs and symptoms (i.e., NAS)
- Unexplained intrauterine growth retardation
- Unexplained neurological complications

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Common Reasons for False-Positive and False-Negative Results of Neonatal Toxicology Testing²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FALSE-POSITIVE RESULTS</strong>²</td>
<td><strong>FALSE-NEGATIVE RESULTS</strong></td>
</tr>
<tr>
<td>• Cross-reactivity, primarily with immunoassays. Antibody based immunoassays look for classes of drugs, unlike mass spectrometry assays that detect specific compounds</td>
<td>• Poor reactivity, primarily with immunoassays such those for benzodiazepines where one benzodiazepine may react strongly and another may not be detected in low amount</td>
</tr>
<tr>
<td>• Interfering substances limit ability to detect compounds</td>
<td>• Matrix effectsb where sample types have different requirements for proper testing</td>
</tr>
<tr>
<td>• Specimen contamination, such as urine, milk, stool may impact results</td>
<td>• Concentrations below cut-off values</td>
</tr>
<tr>
<td></td>
<td>• Improper sample type for compound of interest</td>
</tr>
</tbody>
</table>

² Performing separate screen and confirmation testing minimizes the chances of false-positive results.

b Matrix effects are similar to the effects of interfering substances. Some matrices (i.e., meconium vs. umbilical cord) have higher background noise with respect to certain tests than others. This effect can make it challenging to detect low concentrations of some compounds using those tests.
<table>
<thead>
<tr>
<th>Test Code</th>
<th>UMBILICAL CORD</th>
<th>MECONIUM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Comprehensive</td>
<td>Expanded</td>
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<tr>
<td>Test Code</td>
<td>9145UC</td>
<td>9352UC</td>
</tr>
<tr>
<td>Number of Analytes</td>
<td>57</td>
<td>31</td>
</tr>
<tr>
<td>Amphetamines/ Methamphetamines</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cocaine</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Opiates</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>PCP</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Benzodiazepines</td>
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<td>X</td>
</tr>
<tr>
<td>Methadone</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Buprenorphine</td>
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<tr>
<td>Zolpidem</td>
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<tr>
<td>Depressants</td>
<td>X</td>
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</tr>
<tr>
<td>Cathinones</td>
<td>X</td>
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<tr>
<td>Fentanyl</td>
<td>X</td>
<td></td>
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<tr>
<td>Muscle Relaxants</td>
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<td></td>
</tr>
<tr>
<td>Ethyl Glucuronide</td>
<td>Add-on</td>
<td>Add-on</td>
</tr>
<tr>
<td>Screen method</td>
<td>Mass spectrometry</td>
<td></td>
</tr>
<tr>
<td>Confirmation method</td>
<td>Mass spectrometry</td>
<td></td>
</tr>
<tr>
<td>Average turnaround time (from receipt of sample to report)</td>
<td>Negative: 1 day</td>
<td>Positive: 3 days</td>
</tr>
</tbody>
</table>
Interpretation of Results
As with all laboratory testing, the results of neonatal toxicology testing must be interpreted in light of other clinical and laboratory data.

A positive result indicates that the drug or drug metabolite was detected in the umbilical cord tissue, while a negative result indicates the substance was not detected at or above the reported limit validated for the assay. As with any analytical test, false-positive or false-negative results can occur (Table 4). Accuracy is improved by performing the secondary confirmation test – it ensures the accuracy of the result obtained from initial test performed.

While the clinical specificity and sensitivity of umbilical cord testing are high, a negative result does not completely exclude the possibility of maternal drug use during pregnancy as levels of drugs or drug metabolites may be below the assay limits of detection. Detection of drugs in umbilical cord tissue depends on the extent of maternal drug use, drug and drug metabolite stability, characteristics of deposition in umbilical cord tissue, and the performance of the analytical method.

Each drug and drug metabolite tested is reported as positive or negative based on a minimum reporting limit for each compound; quantitative results are not reported for meconium or umbilical cord. Qualitative (positive or negative) rather than quantitative results are reported for a number of reasons including, but not limited to: 1) Quantitative values are not necessarily correlated with the degree of drug exposure. For example, it has been shown that the concentration of THC (tetrahydrocannabinol) in meconium is not correlated with maternal consumption nor is the concentration of nicotine and metabolites in meconium correlated with the number of cigarettes smoked; 2) Quantitative values are not necessarily correlated with infant outcomes, as has been shown in studies of babies born to women in opioid maintenance programs; 3) Both meconium and umbilical cord are reservoirs where drug and drug metabolites accumulate over weeks and months, and this along with other factors such as variable pharmacokinetic profiles and placental permeability changes during pregnancy, make determining the value of specific concentrations difficult.

Screening pregnant women and neonates for illicit drug use has important legal, ethical, and social implications, and clinicians should be aware of relevant state and local laws. 33

Contact NMS Labs
At NMS Labs, we are pleased to provide you with additional information and answer any questions you have regarding our testing.

Visit www.nmslabs.com/umbilical-cord-testing for resources, including requisition forms.

Contact Customer Support
800.522.6671 or Clinical@nmslabs.com to request the Neonatal Umbilical Cord Drug Testing Kit©.

References