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Method Performance and Clinical Workflow Outcomes Associated with Meconium and Umbilical Cord Toxicology Testing

Ruth M. Labardee DNP, RNC, CNL[a], Jaime R. Swartzwelder MLS[b], Karen E. Gebhardt MLS[b], Justine A. Pardi MS MLS[b], Anna C. Dawsey PhD[c], R. Brent Dixon PhD[d] and Steven W. Cotten PhD[b]

[a] *Nursing Shared Services, Ohio State University Wexner Medical Center, Columbus, OH*

[b]*Department of Pathology, Ohio State University Wexner Medical Center, Columbus, OH*

[c]*Department of Quality, Elite Diagnostics, Charlotte, NC*

[d]*Public Health Laboratory, South Carolina Department of Environmental and Health Control, Columbia, SC*

Corresponding Author
Steven W. Cotten PhD
Department of Pathology
Ohio State University Wexner Medical Center
4164 Graves Hall
333 W 10th Avenue
Columbus OH 43210
Steven.Cotten@osumc.edu
614-688-5497

Abstract

Objective: Neonatal abstinence syndrome (NAS) is a rising concern with unknown long-term effects. It is apparent that higher cost of care, impact on the community and reduced quality of life are associated with similar etiologies (*e.g.*, fetal alcohol syndrome). Detection of drug exposure *in utero* allows for earlier intervention to potentially reduce undesired outcomes. Umbilical cord tissue (UCT) has been documented as a readily accessible specimen for detection of drug exposure and has emerged as an alternative specimen to meconium. Methods: The analytical and clinical impact of umbilical cord tissue relative to meconium was evaluated for assessment of *in utero* drug exposure. Quality metrics relating to turnaround-time and diagnosis of NAS were investigated after switching from meconium to UCT. Results: Umbilical cord tissue showed higher clinical sensitivity but lower specificity for prediction of NAS diagnosis. Birth to result time decreased with adoption of UCT. Conclusions: Birth to result time decreased by the switching to UTC as well as the number of missed collections. The clinical sensitivity and negative predictive value for NAS increased with UCT; however both meconium and UTC samples were negative for opiates for a significant percentage of newborns with a diagnosis of NAS.

Keywords: Meconium, Umbilical Cord, Drug Testing, Neonates, Newborns

Abbreviations: UCT (Umbilical Cord Tissue), 6-MAM (6- monoacetylmorphine), NAS (Neonatal Abstinence Syndrome)

Highlights:

- Processing efficiency was lower in umbilical cord tissue compared to meconium
- Our Umbilical Cord Tissue assay showed higher clinical sensitivity but lower specificity for predicting NAS than our meconium assay
- Both meconium and UCT samples were negative for opiates in a significant percentage of newborns with a diagnosis of NAS

1. Introduction

The rise in frequency of neonatal abstinence syndrome (NAS) has mirrored the rise in prescription and illicit opiate use in the United States over the last decade.[1] Overdose data from the Ohio Department of Health shows a steady rise in unintentional overdoses from prescription opiates that peaked in 2011 only to be eclipsed by heroin in 2012 and reaching >1,400 overdoses in 2015.[2] Fentanyl overdoses which have remained flat until 2013 rapidly spiked rising from <50 in 2013 to >1,100 in 2015.[2] NAS discharge data from Ohio hospitals has shown a ~10 fold increase in the frequency of NAS beginning at 14 per 10,000 births in 2004 to 134 per 10,000 in 2014.[3] Accurate and rapid detection of NAS by hospitals is crucial for therapeutic management and social services intervention.

Detection of NAS has conventionally relied on toxicology testing of the newborn to assess for drug exposure *in utero*.[4–8] Analysis of meconium has traditionally been the specimen of choice due to its long window of detection going back approximately 20 weeks.[7] However, meconium as a toxicology specimen comes with limitations that diminish its effectiveness. Collection of a complete meconium specimen for laboratory analysis is challenging for nursing staff and is time intensive. Additionally babies born to opiate addicted mothers often pass meconium slower which delays both collection and analysis. Affected neonates can present with post-natal symptoms that blur the picture between potential congenital disease crises such as metabolic disorders or withdrawal symptoms.[9] Providing a definitive test can speed the differential diagnosis for non-overt presentation of drug use by the mother. The delay in toxicology results impedes the ability for timely therapeutic and other social services interventions.

Recently umbilical cord tissue (UCT) has emerged as an alternative to meconium for assessment of *in utero* drug exposure.[10–13] The adoption of UCT testing has the potential to solve several issues regarding collection and clinical workflows associated with meconium testing. The following project investigated the performance of meconium and UCT in 2 phases. Phase 1 compared the analytical detected of prescription and illicit drugs for both specimen types using paired specimens. Phase 2 evaluated the impact of switching from meconium to UCT testing on clinical nursing workflow as it relates to ordering, collection and test resulting. Finally we calculated the clinical sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of an opiate positive test result for a diagnosis of NAS in the patient's discharge summary or problem list. The results suggest that UCT testing has a higher clinical sensitivity and negative predictive value than our current meconium assay for predicting a diagnosis NAS but neither specimen had a clinical sensitivity >80%.

2. Materials and Methods

2.1 Sample Collection

Meconium specimens were collected in accordance with hospital policy. Upon admission to the hospital, all pregnant patients are routinely asked about their use of drugs and alcohol, including prescription opioids and other medications used for nonmedical reasons. These questions along with the organization's *Drug Screening/Referral* policy, guide the practitioners as to which patients are tested. Remnant paired UCT was used for the validation using the same testing criteria for meconium and was deemed IRB-exempt by the institutional IRB. Results of paired meconium and umbilical cord specimens were compared for detection of different drug classes from the same individual.

2.2 Sample Preparation

For meconium testing, 0.5 g of meconium was homogenized in 1.5 mL of 100% methanol for 5 minutes followed by centrifugation at 13,000 rpm for 5 minutes. 1.0 mL of supernatant was transferred to a 2 mL SLE column (Biotage, Charlotte NC) and allowed to absorb for 5 minutes. Drugs were eluted with two additions of 2.5 mL of ethyl acetate:isopropanol (90:10) into a 13x100 mm glass tube followed by evaporation at 40°C under nitrogen to complete dryness. Samples were reconstituted in 1.0 mL of water, analyzed via immunoassay and confirmed by mass spectrometry.

For UCT testing, approximately 2.0 g of tissue was homogenized in 3 mL methanol: water (90:10) using a Bead Ruptor (Omni International, Kennesaw GA) using the following conditions: speed 5, time 1.5 minutes, dwell time 2 minutes, cycles 4. The homogenized tissue was centrifuged for 10 minutes at 3500 rpm and 1.0 mL of the supernatant was transferred to an SLE column and allowed to absorb for 5 minutes. Drugs were eluted with two additions of 2.5 mL of ethyl acetate:isopropanol (90:10) into a 13x 100 mm glass tube followed by evaporation at 40°C under nitrogen to complete dryness. 100 µL of the extract was combined with 100 µL of internal standard (I.S.) (100 ng/mL of D_5-diazepam and D_3-doxepin in acetonitrile), vortexed and centrifuged for 1 min at 13,000 rpm. 100 µL of the sample/I.S. mixture was diluted into 400 µL of water and qualitatively analyzed by liquid chromatography mass spectrometry.

2.3 Analysis and Chromatography Conditions

For immunoassay testing, aqueous 1mL extracts from 0.5 g meconium were screened on the Beckman Coulter (Brea, CA) AU680 analyzer using the following reagents and cutoffs, Amphetamines (EMIT II PLUS-cutoff 1000 ng/mL), Barbiturates (EMIT II PLUS-cutoff 300 ng/mL, Benzodiazepines (CEDIA ThermoFisher cutoff 200 ng/mL), Cannabinoid (EMIT II PLUS cutoff 50 ng/mL), Cocaine metabolite (EMIT II PLUS cutoff 300 ng/mL), Methadone

(EMIT II PLUS cutoff 300 ng/mL), Opiates (EMIT II PLUS cutoff 300 ng/mL), Oxycodone (ThermoFisher DRI 100 ng/mL), PCP (EMIT II PLUS cutoff 25 ng/mL), and Propoxyphene (EMIT II PLUS cutoff 300 ng/mL).

Positive results for amphetamines, benzodiazepines, cocaine, methadone, opiates, oxycodone, PCP and propoxyphene were qualitatively confirmed via mass spectrometry using a SCIEX 3200 Q-TRAP LC/MS/MS System (Framingham, MA). Chromatographic separation was performed using a Restek (Bellefonte, PA) PFP propyl column 50 x 2.1 mM with a 5 µM particle size installed in a Shimadzu Prominence LC System. The mobile phase system used for separation consisted of LCMS grade water (mobile phase A) and LCMS grade acetonitrile (mobile phase B) both with 2 mM ammonium formate and 0.2% formic acid. The chromatographic method included a gradient elution of mobile phase B from 10% to 90% over 10 minutes followed by 5 minutes at 90% mobile phase B. The column was re-equilibrated back to 10% mobile phase B over 2.5 minutes. Flow rates ramped from 0.5 to 1.0 over 10 minutes then back to 0.5 during the re-equilibration phase. The total analytical run time is 17.5 minutes. The LC column was maintained at 40°C and with an injection volume of 30 µL.

Mass spectra were acquired in positive mode using ESI with a collision energy of 35 V and a collision energy spread of 15V. Acquired spectra were matched against a compound library for target identification using Cliquid Software® (Version Number 3.2.1.). Similar broad drug screening methods with the SCIEX 3200 Q-TRAP LC/MS/MS System have been described previously.[14] The compound library and respective cutoffs are listed in Supplemental Table 1. UCT extracts were reconstituted as described in section 2.2 and analyzed directly via mass spectrometry as described above.

2.4 Matrix Effects and Extraction Efficiency

The effect of ion suppression or enhancement for umbilical cord and meconium extracts were evaluated in a similar approach described by Matuszewski et al.[15] Unextracted sample sets containing all compounds near the limit of detection were generated by adding 1.5 mL of drug standard stock solutions into 300 uL of mobile phase B in the absence of UCT lysate. Post-extracted sample sets were generated by adding 1.5 mL of drug standard stock solutions into 1.0 mL of drug-free UCT lysate dried and resuspend in 300 uL of mobile phase B. Pre-extracted sample sets were generated by adding 1.5 mL of drug standard stock solutions into intact UCT followed by homogenization and extraction as previously described. The resulting eluent was dried down and resuspended in 300 uL of mobile phase B. Each sample set was analyzed and raw peak areas for each compound were used to determine overall matrix effects (calculated difference of post-extracted samples to unextracted samples), extraction efficiency (calculated difference of pre-extracted samples to post-extracted samples) and processing efficiency (calculated difference of pre-extracted samples to unextracted samples).

2.5 Concordance of Meconium and UCT Results

Paired specimens of meconium and UCT were collected and analyzed as described previously for assay validation. The study consisted of 197 sets of specimens that were analyzed for detection of key drug classes. The agreement between detected drugs was calculated for negative results and positive results independently. Additionally, Cohen's Kappa was calculated to estimate the possible impact of random error on specimen concordance.

2.6 Impact of Meconium and UCT on laboratory metrics and NAS diagnosis

In January of 2016, after the method comparison study was completed and reviewed by relevant clinical stakeholders, meconium toxicology analysis was removed from the Labor and Delivery order set and replaced with umbilical cord tissue toxicology analysis. Meconium testing was still

available for order but the default specimen type was for umbilical cord tissue. After 1 year, data related to order time, specimen collection time, result reporting time, and NAS diagnosis were retrieved for all umbilical cord specimens analyzed in 2016 as well as all meconium specimens from the year prior in 2015. Results for meconium testing from 2015 were compared with UCT testing from 2016. All toxicology specimens regardless of specimen type were collected in accordance with the hospital's policy for risk-based evaluation of neonatal drug exposure.

2.7 Quality Metrics and Outcomes

All quality metrics related to order time, specimen collection time, result reporting time, and NAS diagnosis were obtained through the institution's Quality Data Release request system in accordance with hospital policy.

3. Results

3.1 Concordance between specimen types

Table 1 shows the agreement for drugs detected in both meconium and UCT. Negative agreement was greater than 95% for all drug classes (partitioned by immunoassay drug class). The positive agreement calculated ranged from 40-100%. Out of 197 umbilical cord specimens 9 new instances of cocaine, 7 new instances of methadone, 3 new instances of oxycodone, 4 new instances of benzodiazepines, and 3 new opiates were detected. Two drug classes did show decreased detection of target drug; oxycodone (n=9) and opiates (n=8) were not detected in a paired cord specimen. Subsequent investigation into the alteration of the organic solvent to water ratio of 90:10 during homogenization has improved recovery of these drug classes. Two umbilical cord samples were found to be positive for methamphetamine but had negative corresponding results in the paired meconium sample.

3.2 Matrix effects and efficiency

Figure 1 shows the overall processing efficiency calculated from raw peak area ranged from 19-51% for meconium specimens and 17-39% for UCT testing. The largest decreases in processing efficiency were observed for codeine, oxycodone, and methadone. Interestingly, 6-monoacetylmorphine (6-MAM) showed increased processing efficiency from 26 to 39%. Small improvements in processing efficiency were also observed for oxymorphone and hydromorphone in the umbilical cord extraction.

3.3 Impact of switching specimen types on Birth to Order and Birth to Test Result time

Prior to initiation of the project, the average time from birth to meconium toxicology result was >29 hours. After switching from liquid-liquid extraction to SLE extraction the average time was 12.6 hours (Figure 2). Average time of birth to meconium test ordering by the physician also dropped 50% from 10.4 hours to 4.1 hours. After switching to UCT testing from meconium testing, median birth to toxicology result was 9.7 hours.

3.4 Impact of switching specimen types on Opiate Positive Test Results and NAS diagnosis

ICD9 (779.5) and ICD10 (P96.1 and P96.2) diagnosis codes for neonatal abstinence syndrome were retrieved from the medical record and compared against positive toxicology results in key drug classes (opiates, oxycodone, methadone, buprenorphine, and cocaine) to evaluate each specimen performance in predicting NAS. One year of meconium testing had a clinical sensitivity and clinical specificity for NAS of 65% and 85% respectively (Table 2). Twenty-two newborns had no meconium results but had a diagnosis of NAS. Negative predictive value (NPV) and positive predictive value (PPV) for NAS were 58% and 88% respectively. One year of UCT testing showed a clinical sensitivity and clinical specificity for NAS of 79% and 76% respectively (Table 3). Three newborns had no umbilical cord toxicology results but had a

diagnosis of NAS. Negative predictive value (NPV) and positive predictive value (PPV) for NAS were 97% and 30% respectively.

4. Discussion

The assessment of neonatal drug exposure has become increasingly important as opiate addiction increases in the United States.[3] The approach to assessment is largely institution dependent due to the lack of national guidelines for evaluation of drug use during pregnancy. Several position statements have been drafted from key stakeholders such as The American Congress of Obstetricians and Gynecologist (ACOG) and The Association of Women's Health, Obstetric and Neonatal Nurses (AWHONN).[16,17] The use of the word "screening" as it related to drug use during pregnancy can refer to both laboratory testing and verbal questioning related to drug use. These differences highlight the lack of standardization for neonatal assessment of drug exposure. Meconium is generally preferred over neonatal urine due to its longer window of detection, going back almost 20 weeks. However, the choice of the specimen collected greatly impacts the clinical workflow across multiple units of service. This in turn has a cascading impact on the timeline for possible therapeutic or social services interventions by providers. Collection of the first urine void could happen shortly after delivery and requires coordination from multiple nursing units depending on the transition of care (L&D, OB-GYN, NICU). Missing the first urine void can diminish the likelihood of identifying drugs from subsequent collection. As patients move out of delivery to the well-baby nursery or the neonatal intensive care unit, communication is needed to avoid missing collection of either urine or meconium specimens. Logistical challenges with the collection of urine and meconium have been previously described and can be barriers to efficient interventions. Umbilical cord tissue has emerged as an alternative to meconium and offers a simplified collection procedure that can be standardized across health

systems. Studies have been mixed on the performance of UCT relative to meconium with some showing equal detection of drug classes and others showing reduced detection of drugs in umbilical cord tissue.[18–21]

Initial assay development for our UCT assay utilized paired meconium and umbilical cord specimens. Drugs detected in meconium via immunoassay were compared with drugs detected in UCT (via mass spectrometry). Drugs from UCT were grouped into their respective drug class and compared with the corresponding meconium immunoassay. Table 1 shows that positive agreement was similar to a recent study showing reduced detection of drugs in UCT.[18] Discordant results, specifically false negatives, were investigated by the laboratory. Nine (9) oxycodone results and eight (8) opiate results were initially found to be below the limit of the detection confirmed via a reference lab, but after minor instrument maintenance (cleaning of the mass spectrometer ion source), detection improved for both target compounds. However, these samples were deemed quantity-not-sufficient (QNS) and could not be reanalyzed. Subsequent external sample exchange for alternative proficiency has demonstrated equivalent detection with an outside laboratory (data not shown). Detection rates improved for Amphetamine/Methamphetamine, Cocaine, Methadone, and Benzodiazepine drug classes with the umbilical cord tissue compared to meconium (Table 1). Agreement of the meconium and UCT drug detection rates were evaluated by all stakeholders of the project and found to meet clinical needs.

When processing efficiency was investigated it was calculated that overall extraction recovery of key compounds was generally less than 50% for umbilical cord specimens. (Figure 1) This recovery speaks to the challenging matrix of UCT and the single extraction technique chosen for multiple drug classes. Targeted extraction of chemically similar compounds would likely

improve recovery from the tissue. Despite the low recovery, subsequent diagnosis data revealed the detection of opioids exceeded the detection of NAS. (Tables 2 and 3)

The impact that UCT analysis has on the clinical nursing workflow and the clinical sensitivity for a diagnosis of NAS was investigated using several key quality metrics. Slow passage of meconium can delay collection and therefore receipt by the laboratory up to 48 hours. We evaluated Birth to Collect and Birth to Result times for both meconium and umbilical cord specimens over the course of 1 year. Median hours of birth to result decreased from 24 hours with meconium to 10 hours using UCT specimens. (Figure 2) This decrease is largely attributed to the collection of UCT at birth without the delay of waiting for infants to pass meconium. Additionally, post-extraction evaporation time is significantly less for UCT which contributes to the improvement in turn-around-time.

The ability of a positive opioid result to predict a later diagnosis of NAS was investigated for meconium and UCT. Previous performance of meconium testing showed somewhat unfavorable clinical sensitivity and specificity of 65 and 85% respectively for NAS diagnosis. A calculated NPV of 88% suggested meconium worked more effectively as a rule out test. When UCT performance was investigated, clinical sensitivity for NAS increased but clinical specificity for NAS decreased relative to meconium testing. Furthermore, PPV for NAS decreased from 58 to 30%. This decrease can be attributed to the detection of more opioid positive samples without a NAS diagnosis. It is important to note that fentanyl positive samples were not considered "opioid positive" due the presence of fentanyl in epidural medications that may be administered during labor. The NPV of UCT for NAS increased to 97% improving its performance as a rule out test for detection of NAS after delivery.

It is important to note that neither specimen type was able to detect NAS diagnosis in all cases. When meconium was used, results were negative for opioids in 35% of NAS cases. Conversely when UCT was used, results were negative for opioids in 21% of NAS cases. There are several limitations which may contribute to these observations. Low recovery from meconium or UCT may contribute to "false negative" results for opioids where detection was missed and the patient ultimately developed NAS. Although possible, the authors find this scenario unlikely because 70% of opioid positive UCT samples were negative for NAS. Such trace or distant opioid exposure would be an unlikely cause of NAS given the high percentage of positive opioid results in UCT without NAS. Another possible limitation may be the exclusion of fentanyl in our definition of an opioid positive result. Fentanyl–laced heroin emerged in Ohio in 2014-2015 and fentanyl related deaths accounted for more overdoses than heroin in 2016.[22] However, the presence of fentanyl in epidural medications makes distinguishing illicit drug exposure from therapeutic medication difficult. Detection of fentanyl and lidocaine together often indicated epidural administration but the authors chose to exclude any fentanyl as an "opioid positive result" due to its high prevalence in the specimens. This analysis may have reduced the calculated clinical sensitivity for NAS in our study. Additionally there may be other opioid compounds present in the specimens that are not included in the mass spectrometry panel. Emerging drugs such as carfentanil or acetyl fentanyl may be adulterants in heroin and could contribute to *in utero* exposure and contribute to subsequent NAS withdrawal. These emerging substances would not be detected by our assay. Lastly, nicotine exposure has been linked to increased Finnegan Scoring of withdrawal for newborns and may mimic withdrawal symptoms from opioids in the first 72 hours after birth. [23–25] The prevalence of cotinine (nicotine metabolite) positive UCT specimens analyzed by our laboratory is >50%. Because the diagnosis

of NAS is made entirely from clinical observations and the Finnegan Scoring System; the possibility of nicotine withdrawal mimicking opioid withdrawal cannot be excluded.

5. Conclusions

Umbilical cord tissue testing for neonatal drug exposure enables proactive intervention to mitigate withdrawal symptoms and potential adverse effects in neonatal abstinence syndrome. Switching from meconium to UCT demonstrated reductions in collect to result time and positively impacted the nursing clinical workflow. Additionally the number of QNS specimens and missed collections was minimized compared to meconium. The clinical sensitivity and specificity evaluated through ICD9 and ICD10 codes showed mixed results for both specimen types. UCT showed increased clinical sensitivity and NPV for NAS relative to meconium. However, neither specimen showed a calculated sensitivity for NAS >80%. The choice of specimen will be dictated largely by the how the results will be used. Testing algorithms that incorporate meconium or urine analysis should be considered for other clinical applications such as assessment of drug compliance during pregnancy or legal testing for social services for the highest clinical sensitivity due to varied disposition of drugs in UCT.

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Table 1: Concordance between specimen types

Cord	Meconium									
	Cocaine		Methadone		Oxycodone		Benzodiazepines		Opiates	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Pos	8	6	16	7	6	3	1	4	12	3
Neg	1	182	0	174	9	179	0	192	8	174

	Negative Agreement	96.8%	95.6%	97.8%	97.5%	97.8%
	Positive Agreement	90.0%	100%	40.0%	100%	60.0%
	Cohen's Kappa	70.2	77.9	44.6	27.9	63.4%

Table 1: Comparison of detected drug classes with paired meconium and umbilical cord samples. Negative agreement was >95% for all drug classes analyzed but positive agreement was less than or equal to 60% for detection of opiates using umbilical cord tissue.

Figure 1: Processing Efficiency for Meconium and Umbilical Cord

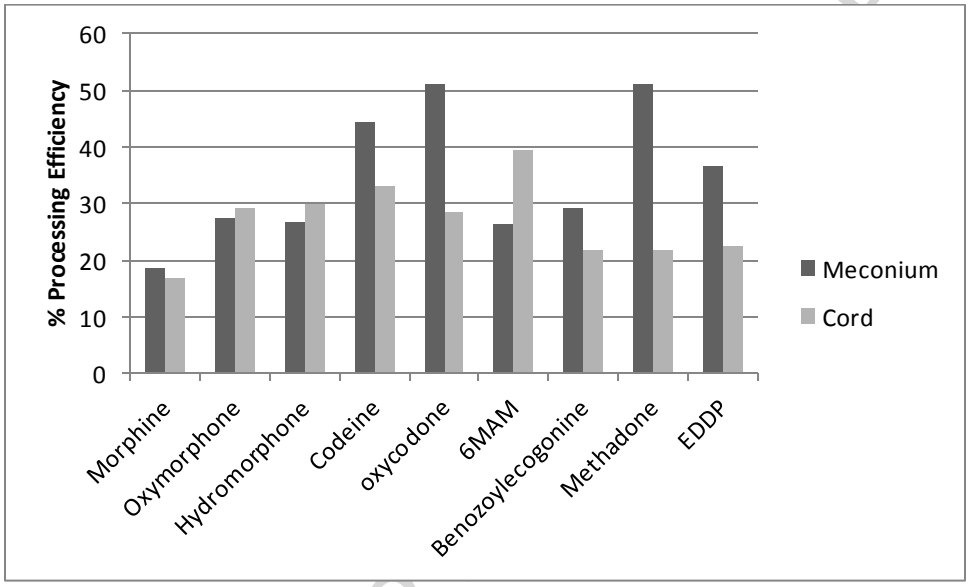

Figure1: Calculated processing efficiency for meconium and umbilical cord tissue for key opioid drugs. Processing efficiency varied greatly from 50% to <20% for various compounds. There was mixed recovery between meconium and umbilical cord tissue however most drugs showed a decreased processing efficiency from the umbilical cord tissue except oxymorphone, hydromorphone, and 6-monoacetylmorphine however these differences are not considered significant.

Figure 2: Meconium and Umbilical Cord Birth to Order and Birth to Result

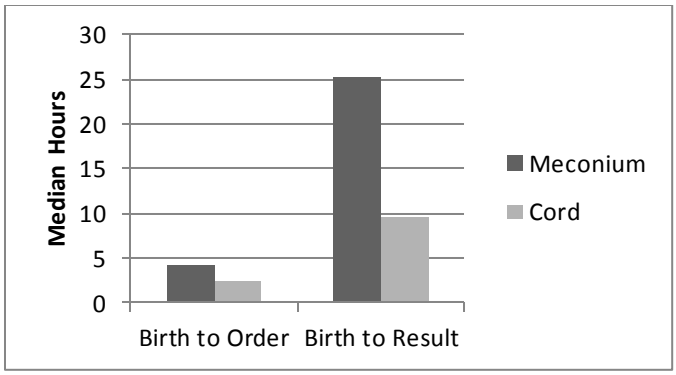

Figure 2: Comparison of Birth to Order and Birth to Result Times for Meconium and UCT. Birth to order and birth to result time decreased dramatically after transitioning from meconium to umbilical cord tissue as the default specimen type in the labor and delivery order set.

Table 2: NAS diagnosis with an opioid positive meconium test result

					Percent
Meconium		NAS ICD9		Sensitivity	65
		Positive	Negative	Specificity	85
Opioid Test Result	Positive	65	47	PPV	58
	Negative	35	260	NPV	88

Table 2: Detection of positive opioid meconium results with ICD9 diagnosis of NAS was moderate (<65%). Thirty five percent of all NAS diagnosis were associated with negative opioid results as defined by the study.

Table 3: NAS diagnosis with an opioid positive umbilical cord test result

					Percent
Umbilical Cord Tissue		NAS ICD10		Sensitivity	79
		Positive	Negative	Specificity	76
Opioid Test Result	Positive	75	177	PPV	30
	Negative	20	559	NPV	97

Table 3: Detection of positive opioid umbilical cord results with an ICD10 diagnosis of NAS was 79%. Sensitivity and specificity were improved relative to the meconium results in the year prior but a significant number (21%) of all NAS diagnosis were associated with negative opioid results as defined by the study.