Umbilical Cord Tissue and Meconium May Not Be Equivalent for Confirming in Utero Substance Exposure

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Keywords

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In a retrospective study of 501 neonates with potential *in utero* substance exposure, the drug detection performance of a commercially available umbilical cord tissue toxicology test was evaluated against a commercially available gold-standard meconium toxicology test. Drugs detected in paired MEC and UCT samples were often discordant.

According to the 2016 National Survey on Drug Use and Health, nearly 20% of pregnant women aged 15–44 in the United States are estimated to have used alcohol, tobacco, or other illicit drugs during their pregnancy (1). Accurate assessment of substance exposure has implications for both the infant and mother (2–5). The American College of Obstetrics and Gynecology and the American Academy of Pediatrics recommend that screening of substance use in pregnancy be universal to avoid bias and utilize a standardized screening tool (eg, 4Ps or CRAFFT) (5,6).

Suspected substance use in pregnancy is commonly confirmed using toxicology tests on specimens from the infant (7,8). Meconium, the stool produced by the neonate during gestation, has a long window of drug detection and is often regarded as the gold standard test (7–10); however, collections can be challenging and MEC is not always available in sufficient quantities (11–13). Umbilical cord tissue (UCT) has been proposed as an

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alternative to MEC because it is readily available in large quantities at the time of birth (14–17); however, the window of drug detection in UCT is generally accepted to be shorter than that of MEC (7,9). Most studies assessing the comparability of UCT and MEC relied on small cohorts of paired samples where few subjects tested positive for drugs (15,18–21) or on large cohorts where samples were not paired (17).Larger cohorts with paired samples have been examined (9,14,22). Due to a combination of biochemical differences in drug or metabolite deposition and accumulation in UCT and MEC, cohort size, frequency of drug abuse in the test population, variation in drugs and metabolites included in the test panel and in test methodology, the existing literature is conflicted as to whether UCT is equivalent to MEC for confirming *in utero* substance exposure, and both tests are used in practice.

To evaluate the equivalence of the UCT and MEC toxicology tests in use at our institution for confirmation of *in utero* substance exposure, we examined results from a cohort of 501 neonates that had both UCT and MEC toxicology testing performed by a national reference laboratory.

Methods

This retrospective cohort study included a convenience sample of 501 infants cared for at the Monroe Carell Jr Children's Hospital at Vanderbilt between October 1, 2013 and February 1, 2016, who underwent screening for drug exposure using both MEC and UCT. Subjects were excluded from analysis for a specific drug group if toxicology results were not available in both UCT and MEC. This study was reviewed and approved by the Vanderbilt University Medical Center Institutional Review Board (IRB# 172002, 160434, 150839). The study population includes partial data from 217 subjects that was previously reported (22) and an additional 284 subjects.

Toxicology testing

All provider-ordered toxicology testing was performed by a national reference laboratory (Associated Regional and University Pathologists [ARUP], Salt Lake City, UT) using a combination of immunoassay and chromatography-mass spectrometry techniques (18,23–27). Both UCT and MEC tests were commercially available but were performed using different methodologies and had different limits of drug detection. In addition, the UCT and MEC tests detected different drugs, and for the purposes of this study, drugs that were only available in either the UCT or the MEC panel were excluded from analysis. The drugs analyzed in this study and the limits of detection in UCT and MEC can be found in Table 1.

Data collection

Results for UCT and MEC toxicology testing were retrospectively collected from the laboratory information system (Cerner Millennium). Subject demographics and length of stay were retrospectively collected from the laboratory billing system (McKesson HealthQuest). Additional clinical information was collected through medical record review.

Statistical analyses

We included 38 individual drugs from 8 groups in our analysis (Table 1). MEC results were defined as the gold standard for drug presence and measures of diagnostic utility were calculated for each drug group in UCT. Sensitivity, specificity, agreement, positive predictive value (PPV), negative predictive value (NPV), prevalence, and Cohen κ ($\kappa = p_o - p_e / 1 - p_e$, where p_o is the observed proportionate agreement and p_e is the probability of random agreement) were calculated using EP Evaluator software (Data Innovations).

A κ of 100% indicates perfect agreement, and a value of 0% indicates random agreement. For the purposes of this analysis, a κ of 75% was deemed acceptable (22). 95% confidence intervals were calculated using the Wilson Score method (EP Evaluator).

Results

A total of 501 subjects were included in this single-center study. The study population was predominantly Caucasian (69.9%). More than 37% of study subjects had a neonatal intensive care unit (NICU) stay with a median length of stay of 15 days. The results of each subject's UCT and MEC toxicology tests were compared by drug group (Table 1 and Table 2). Although overall prevalence of drugs in UCT and MEC was similar for many drug groups, UCT had slightly higher prevalence of amphetamines, barbiturates, and benzodiazepines. The overall agreement between UCT and MEC ranged from 80–100%. Cohen's κ, which accounts for agreement due to chance, ranged from 40–88%. Only two drug groups, amphetamines and methadone, had acceptable κ values of 75%. The sensitivity of UCT ranged from 41% for cannabinoids (95% CI 33.3 – 49.6%) to 100% for barbiturates (95% CI 34.2 – 100.0%). The sensitivity of UCT for opioids ranged from 53% for other opioids (95% CI 43.9 – 62.3%) to 75% for methadone (95% CI 50.5 – 89.8%; Table 2).

Discussion

Our study builds upon several smaller studies which previously used paired samples to assess confirmation of *in utero* substance exposure using UCT and MEC testing (14,15,18,22). Our results demonstrate that detection of drugs is variable between paired UCT and MEC samples as evidenced by lack of concordance and relatively low κ values (Table 2). Our findings support other studies which demonstrated that paired UCT and MEC results are often discordant (9,14,22). Differences in analytical detection limits of the tests may play a role in some of the discrepancies, as may administration of drugs close to or during labor. In addition, commercially available UCT testing may not include drug metabolites that preferentially accumulate in UCT, thus may contribute to false negative UCT results.

Our study does have limitations that merit mentioning. The drugs detected in study subjects are representative of use patterns observed in our setting; they may not be generalizable to other settings with different drug prevalence. The UCT and MEC tests rely on different but related analytical principles. Both tests underwent improvements during the study period. Because there is no FDA-approved toxicology test for MEC or UCT, each laboratory's test

will be different, and the tests used in this study may not be equivalent to tests performed by other laboratories. Both tests we used are commercially available, offer analytes and detection limits similar to tests offered by other commercial laboratories, and have been used in a number of previous publications (15,17,22). The choice to order MEC or UCT testing should be based on a number of factors, some of which are scientific (e.g. window of detection) and some of which are practical (e.g. specimen availability). Both UCT and MEC have a place in confirmation of *in utero* substance exposure, provided that those interpreting the results are aware that UCT and MEC may not produce equivalent results.

Acknowledgements:

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Table 1.

Drug group analytes with screening limits of detection in meconium (MEC) and umbilical cord tissue (UCT).

	Limit of detection (ng/g)	
	MEC	**UCT**
	Screen	Screen
Amphetamines	30	8
amphetamine	30	8
methamphetamine	30	8
MDMA	30	8
MDA	30	8
MDEA	30	8
Barbiturates		
butalbital	75	75
phenobarbital	75	75
secobarbital	75	75
Benzodiazepines		
alprazolam	75	5
alpha-OH-alprazolam	75	5
clonazepam	75	5
7-aminoclonazepam	75	5
diazepam	75	5
desalkylflurazepam	75	10
alpha-OH-ethylflurazepam	75	10
lorazepam	75	5
midazolam	75	5
nordiazepam	75	5
oxazepam	75	5
temazepam	75	5
alpha-OH-triazolam	75	5
Buprenorphine		
buprenorphine	40	1
norbuprenorphine	40	0.5
Cannabinoids		
THC-COOH	30	150[1]
Cocaine		
cocaine	30	8
benzoylecgonine (BE)	30	8
meta-OH-BE	30	8
Methadone		
methadone	40	10
EDDP	40	10
Other Opioids		

	Limit of detection (ng/g)	
	MEC	**UCT**
6-monoacetylmorphine	30	4
codeine	30	6
heroin	30	2
hydrocodone	30	6
hydromorphone	30	4
morphine	30	4
oxycodone	30	4
oxymorphone	30	4
propoxyphene	75	10

[1] The limit of detection for this analyte changed to 1 ng/g on 11/16/15.

Table 2.

Comparison of paired umbilical cord tissue (UCT) and meconium (MEC) results by drug group.

Amphetamines							

			95% CI			
Agreement	99%	98.2 – 99.8%	Positive Predictive Value	85%
Cohen's κ	88%	73.8 – 101.6%	Negative Predictive Value	100%
Sensitivity	92%	64.6 – 98.5%	Prevalence in UCT	3%
Specificity	100%	98.5 – 99.9%	Prevalence in MEC	2%

UCT / MEC table: MEC + : UCT + 11, UCT − 1; MEC − : UCT + 2, UCT − 485

Barbiturates

		95% CI		
Agreement	100%	98.6 – 99.9%	Positive Predictive Value	50%
Cohen's κ	67%	20.1 – 112.8%	Negative Predictive Value	100%
Sensitivity	100%	34.2 – 100.0%	Prevalence in UCT	1%
Specificity	100%	98.6 – 99.9%	Prevalence in MEC	0%

UCT / MEC table: MEC + : UCT + 2, UCT − 0; MEC − : UCT + 2, UCT − 496

Ben zodiazepines

		95% CI		
Agreement	93%	90.2 – 94.8%	Positive Predictive Value	33%
Cohen's κ	40%	21.5 – 59.1%	Negative Predictive Value	98%
Sensitivity	64%	43.0 – 80.3%	Prevalence in UCT	8%
Specificity	94%	91.7 – 95.9%	Prevalence in MEC	4%

UCT / MEC table: MEC + : UCT + 14, UCT − 8; MEC − : UCT + 28, UCT − 451

Buprenorphine

		95% CI		
Agreement	88%	81.8 – 92.1%	Positive Predictive Value	84%
Cohen's κ	66%	51.0 – 80.0%	Negative Predictive Value	89%
Sensitivity	65%	49.5 – 77.9%	Prevalence in UCT	20%
Specificity	96%	90.3 – 98.1%	Prevalence in MEC	26%

UCT / MEC table: MEC + : UCT + 26, UCT − 14; MEC − : UCT + 5, UCT − 111

Cannabinoids

		95% CI		
Agreement	80%	76.1 – 83.1%	Positive Predictive Value	73%
Cohen's κ	41%	30.7 – 51.3%	Negative Predictive Value	81%
Sensitivity	41%	33.3 – 49.6%	Prevalence in UCT	15%
Specificity	94%	91.3 – 96.2%	Prevalence in MEC	27%

UCT / MEC table: MEC + : UCT + 56, UCT − 80; MEC − : UCT + 21, UCT − 343

Cocaine

		95% CI		
Agreement	96%	93.5 – 97.2%	Positive Predictive Value	89%
Cohen's κ	58%	40.9 – 75.8%	Negative Predictive Value	96%
Sensitivity	46%	30.5 – 61.8%	Prevalence in UCT	4%
Specificity	100%	98.4 – 99.9%	Prevalence in MEC	7%

UCT / MEC table: MEC + : UCT + 16, UCT − 19; MEC − : UCT + 2, UCT − 453

Methadone

	UCT			95% CI			
	+	-	Agreement	99%	98.0 – 99.7%	Positive Predictive Value	100%
MEC +	12	4	Cohen's κ	85%	71.0 – 99.6%	Negative Predictive Value	99%
MEC -	0	484	Sensitivity	75%	50.5 – 89.8%	Prevalence in UCT	2%
			Specificity	100%	99.2 – 100.0%	Prevalence in MEC	3%

Other Opioids

	UCT			95% CI			
	+	-	Agreement	86%	82.9 – 89.0%	Positive Predictive Value	76%
MEC +	58	51	Cohen's κ	55%	44.6 – 64.5%	Negative Predictive Value	88%
MEC -	18	374	Sensitivity	53%	43.9 – 62.3%	Prevalence in UCT	15%
			Specificity	95%	92.9 – 97.1%	Prevalence in MEC	22%