Meconium Drug Testing in Multiple Births in the USA

Kelly E. Wood1*, Matthew D. Krasowski2, Frederick G. Strathmann3,4 and Gwendolyn A. McMillin3,4

1Stead Family Department of Pediatrics, University of Iowa Children’s Hospital, Iowa City, IA 52242, USA, 2Department of Pathology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242, USA, 3ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT 84108, USA, and 4Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT 84112, USA

*Author to whom correspondence should be addressed. Email: kelly-wood@uiowa.edu

Little is published about newborn drug testing in multiple gestations. The objective of this study was to review the results of meconium drug screening in multiple births to compare drug(s) and/or drug metabolite(s) detected. A retrospective analysis was conducted using data from a national reference laboratory and an academic medical center. The data were de-identified for the reference laboratory dataset. For the academic center data, a detailed chart review of the newborn and mother’s medical record was performed on cases for which one or more drug(s) and/or metabolite(s) were identified and confirmed in meconium. Meconium was analyzed for amphetamine, methamphetamine, barbiturates, benzodiazepines, cannabinoid metabolites, cocaine metabolites, methadone, opiates, oxycodone, phencyclidine and propoxyphene. One hundred and forty-two of 1,084 sets of twins and 2 of 20 sets of triplets had mismatched results. The incidence of mismatched results among the individual drug or drug classes tested was 0.9% (208 of 23,848 total results). For the panel of drug testing performed, mismatches were seen in 13% (142 of 1,084) sets of twins and 10% (2 of 20) sets of triplets. Barbiturates (33%), opiates (30%) and benzodiazepines (28%) were the most common mismatches in the national reference laboratory dataset. Benzodiazepines (89%) and opiates (91%) were most common in the academic medical center dataset with most explained by iatrogenic medications administered to one infant but not the other. Mismatches for cannabinoids most often occurred when tetrahydrocannabinol metabolites were present at a low concentration (near lower reporting limit) in one infant but not the other. Mismatched results of meconium drug testing in multiples explainable by differences in prescribed medications are uncommon and most often occur when an analyte is barely above reporting cutoff in only one infant. Administration of iatrogenic medications to one infant but not the other(s) is another frequent cause of such mismatches.

Introduction

Maternal non-medical drug use is estimated to occur in 5% of pregnancies in the USA (1). Meconium analysis has traditionally been the ‘gold standard’ for newborn drug screening but other biological specimens are available for testing including hair, urine and umbilical cord (2). Analysis of meconium theoretically detects maternal drug use for the last two trimesters of a full-term birth, with a retrospective review suggesting better detection in the third versus second trimester (3). Meconium is not always available for testing, such as when it is expelled in utero, or when collection is not well coordinated and the meconium is disposed of with a diaper.

Little is known regarding newborn drug screening results in multiple gestations. Most published literature involves twin births. Of the specimens reported as testing positive, the majority had the same drug(s) detected in the meconium from both infants, but at different concentrations (4–7). Lewis et al (6) published the largest study to date involving 21 sets of twins and 2 sets of triplets. Different concentrations may reflect variation in the bowel movements and quantity collected from each of the twins, rather than differences in prenatal exposure between the infants. Qualitative discrepancies between drug test results may reflect concentration differences for which one result falls below the reporting limits of the assay (e.g., cutoff concentration) and one result falls above reporting limits, or may reflect the quality of the specimen, such as if one specimen is meconium and the other is primarily milk stool. Qualitatively discrepant results may also raise questions about specimen mix-up, tampering or the validity of testing.

The objective of this study was to review the results of meconium drug screening in multiple births to compare drug(s) and/or drug metabolite(s) detected. We examined de-identified data from a national reference laboratory and also data from an academic medical center. The national reference laboratory data are very extensive, but clinical details are inaccessible. The academic medical center data are more limited in size, but a detailed chart review was possible.

Methods

Analytical methods

Meconium testing was performed by the traditional two-step process including a broad immunoassay-based drug screen, followed by confirmatory testing of specimens that tested positive in any of the individual screening tests. The drug screen was performed with 11 unique enzyme-linked immunosorbent assays (ELISAs) that were obtained from Immunal (Pomona, CA, USA), and modified as described previously (8). Briefly, meconium (0.25 g) was homogenized in buffer and centrifuged. Hydrolysis was not performed in the screening (ELISA) steps. The supernatant along with matrix-appropriate controls and calibrators, using test plates and reagents designed to detect amphetamine, methamphetamine, barbiturates, benzodiazepines, cannabinoid metabolites, cocaine metabolites, methadone, opiates, oxycodone, phencyclidine (PCP) and propoxyphene. There were nine corresponding confirmatory tests that were performed using gas chromatography/mass spectrometry for barbiturates, cocaine metabolites and PCP, and liquid chromatography-tandem mass spectrometry for all other drug classes. Hydrolysis was used for the benzodiazepine and cannabinoid confirmations. Methamphetamine and amphetamine were confirmed in a single test, as were opiates and oxycodone. The cutoff concentrations, ELISA calibrators and specific analytes detected by confirmation testing are shown in Table 1.
Retrospective analysis was conducted of the medical records of University of Iowa data. Likewise, it is possible that some twins were missed, if meconium in meconium was not collected for one of the three infants. Chart review included zygosity, chorionicity and identification of maternal, delivery and newborn risk factors. Chart review included zygosity, chorionicity and identification of parameters associated with multiple births (twins or triplets). To identify the results corresponding to multiple births, a unique identification string was generated for each patient using the last name, birth date and client-specific identification number. Multiple births were identified by exact matches of the unique string. Identified multiple births with exact first and last name matches were excluded to eliminate multiple collections from a single patient submitted as independent samples. Within the multiple birth data subset, twins were distinguished from triplets by determining if two or three unique first and last name combinations, respectively, occurred among the exact matches of each unique string. Concordance for all included analytes was determined for each set of multiples with exclusion of any discordant results due to insufficient sample available for confirmatory testing. After verification of data integrity, the unique identification string for each multiple set was used to assign a unique numeric string. Identified multiple births with exact first and last name matches were excluded to eliminate multiple collections from a single patient submitted as independent samples. Within the multiple birth data subset, twins were distinguished from triplets by determining if two or three unique first and last name combinations, respectively, occurred among the exact matches of each unique string. Concordance for all included analytes was determined for each set of multiples with exclusion of any discordant results due to insufficient sample available for confirmatory testing. After verification of data integrity, the unique identification string for each multiple set was used to assign a unique numeric string to allow for de-identification of samples prior to data analysis. Reported concentrations of all analytes determined in the confirmation testing were used to identify whether the results were qualitatively equivalent for the infants within each set of twins and triplets. If a result for one infant was negative, and the corresponding result for the other infant(s) in the multiple birth set was positive, those results were considered discrepant and referred to as ‘mismatched.’ Due to limitations of the reference laboratory relationship with clients who ordered the testing, charts could not be reviewed, making it impossible to determine if some presumed twins were actually triplets, where-in meconium was not collected for one of the three infants. Likewise, it is possible that some twins were missed, if meconium from only one of the two infants was submitted for drug testing.

### Table I

Overview of Analytes Detected for Meconium Drug Testing (Screen with Reflex to Confirmation)

<table>
<thead>
<tr>
<th>Target drug</th>
<th>ELISA cutoff (ng/g)</th>
<th>ELISA calibrator</th>
<th>Confirmation cutoff (ng/ml)</th>
<th>Analytes detected by confirmation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>30</td>
<td>d-Amphetamine</td>
<td>20</td>
<td>Amphetamine, MDA, MDMA, MDE, methamphetamine</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>30</td>
<td>d-Methamphetamine</td>
<td>20</td>
<td>Butalbital, phenobarbital</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>75</td>
<td>Secobarbital</td>
<td>50</td>
<td>Alprazolam, a-hydroxyalprazolam, clonazepam, 7-aminoctazolam, desalkylflurazepam, 2-hydroxyethyl-flurazepam, diazepam, lorazepam, midazolam, nordiazepam, oxazepam, temazepam, a-hydroxytriazolam</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>75</td>
<td>Clonazepam</td>
<td>20</td>
<td>Butalbital, phenobarbital</td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>30</td>
<td>9-COOH-THC</td>
<td>5</td>
<td>9-COOH-THC</td>
</tr>
<tr>
<td>Cocaine</td>
<td>30</td>
<td>Benzylecgonine</td>
<td>20</td>
<td>Benzylecgonine, coacachylene, cocaine, m-hydroxybenzylecgonine</td>
</tr>
<tr>
<td>Methadone</td>
<td>40</td>
<td>Methadone</td>
<td>10</td>
<td>Methadone, EDDP</td>
</tr>
<tr>
<td>Opiates</td>
<td>30</td>
<td>Hydrocodeine</td>
<td>20</td>
<td>6-MAM, codeine, dihydrocodeine, hydrocodone, hydromorphone, morphine, oxycodone, oxymorphone</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>30</td>
<td>Oxycodone</td>
<td>10</td>
<td>Phencyclidine</td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>15</td>
<td>Phencyclidine</td>
<td>10</td>
<td>Phencyclidine</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>25</td>
<td>Propoxyphene</td>
<td>10</td>
<td>Propoxyphene, norpropoxyphene</td>
</tr>
</tbody>
</table>

*MDA, methylenedioxamphetamine; MDE, methylenedioxymethamphetamine (Eve), MDMA, methylenedioxymethamphetamine (Ecstasy); 9-COOH-THC, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol; 6-MAM, 6-monoacetylmorphine (heroin metabolite); EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (methadone metabolite).

### National reference laboratory data

Historical data were retrieved and de-identified at ARUP Laboratories, a national clinical reference laboratory (Salt Lake City, UT, USA), according to protocols approved by the University of Utah Institutional Review Board (IRB). Results represented all routine laboratory drug testing of meconium in the five-year time period 26 March 2007 to 29 June 2012. The data analysis was conducted in R using the ggplot2, gdata and xlsx packages (9–12). The data were interrogated to seek specimens that were associated with multiple births (twins or triplets). To identify the results corresponding to multiple births, a unique identification string was generated for each patient using the last name, birth date and client-specific identification number. Multiple births were identified by exact matches of the unique string. Identified multiple births with exact first and last name matches were excluded to eliminate multiple collections from a single patient submitted as independent samples. Within the multiple birth data subset, twins were distinguished from triplets by determining if two or three unique first and last name combinations, respectively, occurred among the exact matches of each unique string. Concordance for all included analytes was determined for each set of multiples with exclusion of any discordant results due to insufficient sample available for confirmatory testing. After verification of data integrity, the unique identification string for each multiple set was used to assign a unique numeric string to allow for de-identification of samples prior to data analysis. Reported concentrations of all analytes determined in the confirmation testing were used to identify whether the results were qualitatively equivalent for the infants within each set of twins and triplets. If a result for one infant was negative, and the corresponding result for the other infant(s) in the multiple birth set was positive, those results were considered discrepant and referred to as ‘mismatched.’ Due to limitations of the reference laboratory relationship with clients who ordered the testing, charts could not be reviewed, making it impossible to determine if some presumed twins were actually triplets, where-in meconium was not collected for one of the three infants. Likewise, it is possible that some twins were missed, if meconium from only one of the two infants was submitted for drug testing.

### University of Iowa data

Retrospective analysis was conducted of the medical records of all multiple birth newborns who had meconium drug analysis studies performed over a four-year period (1 June 2008–31 May 2012) at the University of Iowa Hospitals and Clinics (UIHC) using an IRB-approved protocol. UIHC is a state academic medical center that serves as a tertiary care center. The medical center includes high-risk obstetric services and a level IV neonatal intensive care unit. By institutional practice, the decision to perform newborn drug screening is based on the assessment of maternal, delivery and newborn risk factors.

A detailed chart review was performed on all multiple births (n = 115 sets of multiples; 109 twins and 6 triplets) born during the study period, including both those for which one or more drug(s) or metabolite(s) were identified and confirmed in meconium and those without any drugs identified in meconium. The chart review included zygosity, chorionicity and identification of prescribed medications for mother and for infant prior to meconium collection. The review of infant and maternal medications sought to determine whether any drug(s) or metabolite(s) detected in meconium could be reasonably assigned to maternal prescriptions during pregnancy or to medications administered in the perinatal and/or postnatal period to mother or infant.

### Results

#### National reference laboratory data

There were a total of 2,454 infants identified as twins or triplets in the reference laboratory dataset, including 20 sets of triplets for which meconium was submitted for all three infants in each set. The specimens originated from 35 different states, with ~50% of specimens originating in Texas, California, Iowa, Kentucky, New York or South Dakota. Of the twins, there were approximately equal numbers of female/female pairs, male/female pairs and male/male pairs. Gender was unknown for two sets of twins. Of the triplets, there were seven sets of all female, five sets each of all male, male/male/female and three sets of male/female/female triads.

Excluding the UIHC data, there were 2,168 referred meconium samples evaluated by the 11 ELISA and corresponding confirmation tests, thus generating 23,848 results. As shown in Table II, the two most commonly identified (confirmed) drug classes were cannabinoids and opiates. The positivity rate was 19.3% for cannabinoids (n = 419) and 17.3% for opiates (n = 374). The proportion of specimens positive for methamphetamine was 7.1% (n = 153), 4.6% for cocaine (n = 99), 3.7% for...
benzodiazepines (n = 81), 2.8% for methadone (n = 60), 2.8% for amphetamine (n = 60), 1.5% for propoxyphene (n = 33) and 1.3% for barbiturates (n = 27). Only one set of twins was positive for PCP.

The overall incidence of results that were not concordant or 'mismatched', defined here as results that were qualitatively different for one infant within a set of twins or triplets, was low, representing ~0.9% of all meconium results (including all individual components within the meconium drug panel). Thus, there were 208 mismatched results among the 23,848 total results generated for samples tested. There were two sets of triplets with mismatched results, and 142 sets of twins with mismatched results (with some multiple sets showing more than one mismatch), translating to 13% (142 of 1,084) of twins showing one or more mismatches. The proportion of positive results that were mismatches, along with the related analytes for the twins, is shown in Table II. There were no mismatches for PCP or propoxyphene. The mismatch rate for triplets was 10% (2 of 20), with mismatches for opiates for one set and lorazepam in the other set.

Mismatches for cannabinoids were observed in 9% of twins in which either or both tested positive for cannabinoids (total n = 39). Mismatched cannabinoid results were evaluated based on the concentrations of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (9-COOH-THC) that were detected. As shown in Figure 1, the concentrations of 9-COOH-THC in the mismatched results were significantly lower than with concordant results between twins or triplets. The median concentration of the 9-COOH-THC mismatches was 22 ng/g, which is low, compared with a median concentration for matched results of 75 ng/g.

The most common mismatch within a drug class occurred with 33% of positive barbiturate results, wherein seven of the nine mismatches identified phenobarbital in one infant at concentrations that exceeded the upper limit of quantitation for this test (>5,000 ng/g). Medical charts were not available to evaluate the infant medication history to see if discrepancy was due to medication administered to one infant but not the other. A similar challenge exists with benzodiazepines; 13 of the 23 mismatched results related to midazolam. There were also mismatched results for diazepam and metabolites (n = 6), alprazolam and metabolite (n = 3), and lorazepam (n = 1). There were four mismatches for cocaine that affected two sets of twins. One twin’s meconium contained 68 ng/g cocaine, 248 ng/g benzoylecgonine and 143 ng/g m-hydroxybenzoylecgonine, while the meconium from the sibling twin did not contain any biomarkers of cocaine use. In a second set of twins, meconium from one infant contained 76 ng/g m-hydroxybenzoylecgonine, while the meconium sample submitted for the sibling twin did not contain any biomarkers of...
cocaine use. There were three sets of twins with methadone mismatches (i.e., methadone ± metabolites detected in one infant but not the sibling), one of which was associated with 534 ng/g methadone (no metabolite). The other two sets of twin mismatches included both methadone and the methadone metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) at concentrations of 285 and 715 ng/g; and 99 and 2,622 ng/g of methadone and EDDP, respectively, thus accounting for the five mismatches that were observed.

Methamphetamine and amphetamine were observed together in mismatched results for three sets of twins, in each case found in one infant but not the sibling. The concentrations did not follow a strong pattern: 2,500 and 500 ng/g, 43 and 31 ng/g, and 33 and 176 ng/g for methamphetamine and amphetamine, respectively. For one mismatch, methamphetamine was observed (96 ng/g) along with methylenedioxymethamphetamine (MDMA, 2,102 ng/g) and the MDMA metabolite methylenedioxymethamphetamine (MDA, 133 ng/g). Methamphetamine was observed alone with one other twin mismatch, at a low concentration of 26 ng/g. Amphetamine was observed alone with five sets of twin mismatches, at concentrations that ranged from 85 to 500 ng/g. Perhaps, the most difficult scenarios to interpret relate to the wide range of patterns observed with opioid detection. There were 114 mismatches in the reference laboratory dataset (30% of results positive for opiates), involving morphine \( n = 40 \), hydromorphone \( n = 17 \), hydrocodone \( n = 19 \), oxycodone \( n = 10 \), oxymorphone \( n = 12 \), codeine \( n = 11 \) and dihydrocodeine \( n = 5 \). The distribution of concentrations for matching and mismatched samples is shown in Figure 2.

**University of Iowa data**

In the University of Iowa dataset, there were a total of 109 sets of twins, and 6 sets of triplets. The positivity rate was 19.5% for opiates \( n = 46 \), 2.5% for cannabinoids \( n = 6 \) and 3.8% for benzodiazepines \( n = 9 \) (Table II). There were no positive results for amphetamine, barbiturates, cocaine, methadone or PCP. The data are presented in Table III by twin/triplet.

The most common mismatches were with lorazepam (89%) and opiates (52%). There was one mismatch of cannabinoids (17%) which involved 19 ng/g 9-COOH-THC detected in one infant but not the other. A detailed chart review revealed that most mismatches in the Iowa dataset \( n = 21 \) of 31 total) were accounted for by prescription medications given to one infant but not the other prior to meconium collection. This explanation accounted for all lorazepam mismatches and all mismatches that
involved morphine alone (without other opiates) detected in one infant but not the other. There were four sets of twins with mismatches involving codeine ± morphine detected in one infant but not the other: codeine 124 ng/g and morphine 64 ng/g; codeine 33 ng/g and morphine 60 ng/g; codeine 3 ng/g and codeine 73 ng/g.

The mismatches likely due to differences in infant medications provide an opportunity to estimate how far in advance medication needs to be administered to be detectable in meconium. The absence of the analyte in one twin reduces the probability of maternal medication use (or another source such as dietary poppy seeds). Of the 21 cases that involved mismatches likely due to infant medications, 20 involved medications administered at least 48 h prior to meconium collection. Only one case involved morphine administered ~24 h prior to meconium collection, and only two cases involved morphine or lorazepam administered ~48 h prior to sample collection. The remainder ranged from ~72 to 196 h prior to meconium collection.

There were also examples of more complex opiate patterns that showed minor differences between twins that would not change most likely interpretation of results. In one set of twins, the following results were obtained (Twin A first and Twin B second): codeine 2,241 and 216 ng/g; hydrocodone 14 and 8 ng/g; morphine 769 and 54 ng/g, and hydromorphone 15 ng/g and not detected. In another set of twins, the following results were obtained: codeine 136 and 151 ng/g; hydrocodone 2 ng/g and not detected; morphine 332 and 20 ng/g; and hydromorphone 10 ng/g and not detected. In a third set of twins, the results were: codeine 86 and 46 ng/g; and hydrocodone 3 ng/g and not detected. In a fourth set of twins, the following results were obtained: codeine 1,171 and 507 ng/g; hydrocodone not detected and 4 ng/g; dihydrocodeine detected (qualitative only) and not detected; morphine 350 and 42 ng/g; and hydromorphone 6 and 3 ng/g. In all four cases, there were documented prescriptions of codeine to the mother during pregnancy and no known prescriptions or misuse of other opiates. Maternal use of codeine alone would explain the findings (with metabolism to morphine, hydrocodone and hydromorphone), although undocumented use of additional opiates cannot be ruled out. The presence of hydrocodone and hydromorphone at lower concentrations relative to codeine (and in some instances morphine) in these infants would be consistent with these opiates being minor metabolites of codeine detectable at low level in meconium. Of note, the confirmatory method employed here for opiates in meconium does not include a hydrolysis step. As such, the concentration of hydrocodeine relative to codeine is expected to be higher than what has been reported in urine, because codeine is largely eliminated as a glucuronide conjugate (not detected by this assay), whereas hydrocodone is eliminated as largely parent drug, hydromorphone and nor metabolites.

Table III presents the data broken down by the degree of separation as obtained from the chart review. This analysis revealed that three of the ‘twins’ (as inferred if only de-identified data were available) were in fact two infants from a set of triplets, with one infant not tested. All five mismatches not explainable by iatrogenic medications occurred in dichorionic diamniotic twins.

**Discussion**

In the USA, the incidences of twins and triplets are 3.3 and 0.1%, respectively (13, 14). There has been very limited study of newborn drug screening in multiples. Our literature search showed only five cases of mismatched results of newborn drug screening among twins (three dizygotic, one monozygotic, one not specified) that have been reported (4, 6, 7). Of the monozygotic twin pair, meconium analysis detected benzoylecgonine and m-hydroxybenzoylecgonine (cocaine metabolites) in specimens from both twins but the parent compound was present in only one twin’s specimen (5). Mismatched results from the analysis of meconium in three cases and hair in two cases involved either cannabinoids or cocaine (4–6).

Multiples differ in the degree of genetic similarity and placenta which may alter the fetuses’ prenatal drug exposure and subsequent testing results. In twin pregnancies, both fetuses are theoretically exposed to similar maternal drug concentrations but drug testing results may vary. Dizygotic twins are genetically different and always have separate placenta (dichorionic) (15). A single placenta (monochorionic) only occurs in monozygotic twins (15). Separate placentas are postulated to explain discordant results in drug concentration and drugs detected in dizygotic twins (4, 7). In one study, nearly identical concentrations of cocaine were detected in the hair of monozygotic twins, while concentrations in dizygotic twins varied.

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**Table III**

Concordance in Meconium Drug Analysis Between Twins and Triplets Sorted by Degree of Separation (University of Iowa Dataset with Chart Review)

<table>
<thead>
<tr>
<th>Meconium status</th>
<th>Di/Di twins*</th>
<th>Mono/Di twins*</th>
<th>Mono/Mono twins*</th>
<th>Twins—unknown degree of separation</th>
<th>Triplets (all three infants tested)</th>
<th>Only two sets of triplets tested</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meconium negative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All multiples negative for drug analysis</td>
<td>45</td>
<td>22</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td><strong>Meconium positive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete agreement between multiples</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Agreement in overall interpretation, but minor difference in opiate metabolites</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Difference due to iatrogenic medications to infant</td>
<td>14</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Codeine ± morphine in one but not other</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other difference in drugs detected</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Di/di, dichorionic/diamniotic; mono/di, monochorionic/diamniotic; mono/mono, monochorionic/monoamniotic.

†Difference was only in the pattern of opiates that could be explained by metabolite(s) seen in one multiple but not other.

‡All cases involved morphine and/or lorazepam.

§This was case where THC was detected in one twin but not other infant.
substantially (4). In the University of Iowa dataset, all definitive mismatched results occurred in dichorionic twins. The three mismatches involving monochorionic twins detected minor differences in opiate metabolites that would be unlikely to change the interpretation of the results.

Alternatively, discrepancies in drug testing results in multiples may be related to inconsistent drug metabolite distribution in the heterogenous meconium matrix, timing or quality of specimen collection, or specimen mix-up. Serial meconium samples collected over the first several days have a 60% likelihood of subsequent positive results if the first sample was positive, due at least in part to variable drug metabolite distribution in a heterogeneous specimen matrix (2). The sensitivity of newborn drug testing is dependent on the screening cutoff values used. Wang et al (7) reported a case of one dizygotic twin testing positive for benzodiazepines on screening meconium analysis, while the other twin tested negative. Confirmatory testing using a lower cutoff concentration detected benzodiazepines in both samples.

Our study reveals that mismatches in meconium drug testing results in multiples are uncommon, affecting only ~1% of results for individual drug or drug classes tested. However, for the panel of drug testing performed, one or more mismatches were seen for 1.3% of multiple sets in the reference laboratory dataset. Many mismatches occur with low concentrations of the analyte detected in one twin but not the other, suggesting the possibility that drug may be present in both infants, but below the reporting cutoff in one. The University of Iowa dataset allowed for detailed chart review which showed that differences in prescription medications (particularly benzodiazepines and opiates) administered to multiples can account for some mismatches. Differences in infant medications likely explain many mismatches in the national reference laboratory de-identified dataset, especially those involving phenobarbital, midazolam, lorazepam and morphine. Such differences would be more likely in a neonatal intensive care unit population where such medications are commonly used for sedation, intubation, and pain control. In addition, premature infants are more likely to have delayed passage of meconium providing greater time window for infant medications to distribute into meconium (16). This finding highlights the importance of thorough pharmacy review when interpreting newborn drug findings. An alternative specimen for drug testing such as umbilical cord tissue may overcome these challenges because the specimen is available at birth, and would not detect drugs administered directly to an infant (17).

The mismatches due to infant medication also provided an opportunity to estimate how fast medications can penetrate meconium following administration. The shortest time from medication administration to meconium collection was 24 h observed in one infant. Two infants had ~48 h between morphine or lorazepam administration and meconium collection. There is the caveat that undocumented maternal exposure to opiates (e.g., dietary poppy seeds) may have occurred. However, these three cases had thorough obstetric and pharmacy records. To our knowledge, there are no other previous studies that have tried to assess how fast medications can appear in meconium.

Mismatches in opiates were seen in both the national reference laboratory and the University of Iowa datasets. Complex patterns involving codeine and the presence of possible codeine metabolites (morphine and hydrocodone, along with their additional downstream metabolites) may be especially hard to interpret. Analysis of urine in adults administered codeine in controlled research settings has shown that morphine, hydrocodone and hydromorphone are possible metabolites of codeine (18, 19). The pattern of metabolites in meconium resulting from maternal codeine use has not been reported. Our analysis of the University of Iowa dataset found four set of twins where hydrocodone and/or hydromorphone were detected at low concentrations relative to codeine in mothers with documented prescriptions for codeine and no identified use of other opiates. In all the four sets, there were differences in detection of hydrocodone or hydromorphone in a given twin pair. These discrepancies all involved an analyte detected barely above cutoff in one infant but not the other.

Codeine is a very common prescription medication (and even available over-the-counter in some states) and additionally may be present in dietary poppy seeds. Due to the risk of adverse events possibly in infants of mothers who are cytochrome P450 2D6 ‘ultra-rapid’ metabolizers, some authorities now discourage codeine use in children or lactating mothers (20, 21). There is also some concern over codeine use in late pregnancy (22). If these recommendations and reports decrease codeine use in pregnancy and lactation, differences in meconium test results involving codeine may also decline.

Limitations of our study include the inability to perform chart review of the de-identified national reference laboratory dataset. An additional limitation was that the University of Iowa dataset, while allowing for detailed chart review, had an overall low rate of non-medical drug use. False-negative tests may have occurred if sample drug concentrations fell below testing cutoffs or if interfering substances were present that reduced immunoassay cross-reactivity. As a consequence of delayed collection, milk stools rather than meconium may have been analyzed in some cases. Differences in day of stool collection could result in some mismatched results among multiples, even when all infants within a multiple set had the same drug exposure. Specimens were not collected for all infants with drug testing ordered. The complex metabolic pathways of opiates made interpretation of mismatches involving opiates challenging.

Conclusions
To our knowledge, the present study is the largest to date examining newborn drug testing results differences between multiples. Mismatched results of meconium drug testing in multiples are uncommon, with iatrogenic medication administered to one infant but not the other being a frequent cause of such mismatches. This was particularly true with lorazepam and morphine in our study. Finally, our study supports separate placenta as a possible explanation of mismatched results. Future studies using umbilical cord (or other specimens) for newborn drug testing would be of interest to compare with the findings in meconium. Use of umbilical cord would avoid detection of infant medications, limiting factors that may lead to mismatched drug testing results.

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