False-Positive and False-Negative Rates in Meconium Drug Testing
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To determine the number of false-negative results produced by inefficient extraction of drugs from meconium, three published procedures were compared by using previously confirmed positive and negative meconium specimens. The methods were not equivalent in their ability to extract drugs from the matrix. To determine the number of false positives reported by the use of screen-only (unconfirmed) results, 535 screen-positive meconium specimens were subjected to confirmation by gas chromatography–mass spectrometry. Fifty-seven percent of the samples were confirmed positive for one or more of the drugs under investigation, showing that a false-positive rate as high as 43% may exist when unconfirmed screening results are used.

Indexing Terms: gas chromatography–mass spectrometry/pediatric clinical chemistry/drug metabolites/solvent extraction/solid-phase extraction

Analysis of meconium as an alternative to the analysis of neonatal urine to determine fetal drug exposure is becoming increasingly popular. Meconium provides a longer history of drug exposure than urine (1), contains higher drug concentrations than urine, and is easier to collect. However, unlike National Institute on Drug Abuse urine testing, the analysis of meconium is not government regulated. The actual extraction and analysis procedures are perceived as having no significant impact on the results. Our laboratory undertook two experimental protocols to estimate the false-negative and false-positive rates associated with current meconium testing procedures to determine (a) whether differences exist between published screening procedures and (b) whether the reporting of screen-only data is acceptable.

First, our laboratory compared three published procedures for the determination of abused drugs in meconium to determine the false-negative rate associated with some screening methods.

Second, we determined the actual number of immunoassay screen-positive samples, subsequently confirmed by gas chromatography–mass spectrometry (GC-MS), to determine the false-positive rate of screen-only data.3

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4 Nonstandard abbreviations: GC-MS, gas chromatography–mass spectrometry; EMIT, enzyme-multiplied immunoassay technique; FPIA, fluorescence polarization immunoassay; THC, tetrahydrocannabinol; and m-OH-BZP, m-hydroxybienzoylegone.

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Experiment 1

We confirmed by GC-MS analysis that 100 meconium samples contained drugs after homogenization in organic solvent and then solid-phase or solvent extraction, and 26 meconium samples contained no drugs. We did not screen the samples before GC-MS analysis. All the samples were then reextracted according to three published methods.

Method 1

In method 1a, water (10 mL) and concentrated HCl (1 mL) were added to meconium (1 g). The mixture was vortex-mixed, filtered, and centrifuged. The supernatant was analyzed for opiate and cocaine metabolites by enzyme-multiplied immunoassay technique (EMIT). In method 1b, methanol (0.4 mL) was added to meconium (0.1 g), mixed, and left at room temperature for 10 min. The mixture was centrifuged and analyzed by EMIT for cannabinoids (2).

Method 2

To meconium (1 g), 5 mL of 0.1 mol/L phosphate buffer–methanol (4:1) were added. The mixture was centrifuged and filtered, and the supernatant was analyzed by EMIT for amphetamines, cannabinoids, cocaine, and opiate metabolites. The screening cutoff values for methods 1 and 2 were 50 μg/L, as recommended in the literature (3).

Method 3

Meconium (1 g) was homogenized in glacial acetic acid (3 mL). Diphenylamine in acetone (1.67 mg/L; 6 mL) was added, and the resultant solution was mixed and centrifuged. The top layer was evaporated to dryness (a drop of 1% H2SO4 was added to prevent amphetamine loss). The sample was reconstituted in Abbott ADx buffer–methanol (50:50; 0.7 mL) and centrifuged. The top lipid layer was aspirated, and the concentrated extract was analyzed for drugs by fluorescence polarization immunoassay (FPIA) (4). For method 3, the screening cutoff values were 50 μg/kg for all compounds. All screen-positive rates were subsequently confirmed by GC-MS at cutoff values of 5 μg/kg for cocaine metabolites, opiates, and amphetamines and 2 μg/kg for tetrahydrocannabinol (THC) metabolites.

Experiment 2

Many hospital laboratories rely on immunoassay screening results to determine positive and negative results. The use of screen-only data to report any drug use is unethical and highly dangerous. False-positive
results may cause a mother to lose her child or even be imprisoned for child endangerment. False-negative results may result in denial of specialized treatment for newborns.

Methods

For 2 months (939 total meconium samples), our laboratory correlated the number of screen-positive samples against the number of results confirmed by GC-MS. The samples were homogenized in organic solvent and centrifuged, and the supernatant was reextracted. The final solvent was evaporated to dryness, reconstituted in buffer, and screened by FPIA.

An aliquot of each meconium sample that screened positively was reextracted [solid-phase method for cocaine and its metabolites; solvent extraction for opiates (5), amphetamines, and THC-COOH (6)]. The drugs were confirmed by selected-ion monitoring GC-MS at the following cutoffs: codeine, morphine, amphetamine, methamphetamine, cocaine, and metabolites, 5 ng/g (5 μg/kg); THC metabolite, 2 ng/g (2 μg/kg). All GC-MS analyses were carried out in splitless injection mode, to maximize sensitivity, on an HP 5890 gas chromatograph connected to a 5971A mass selective detector (Hewlett Packard, Naperville, IL) and equipped with a DB-5 MS column [25 m × 0.22 mm (i.d.) × 0.33-μm film thickness] (J & W Scientific, Folsom, CA); the carrier gas was helium. All compounds were quantified by deuterated internal calibrators (Radian Corp., Austin, TX).

Benzoylecgonine, m-hydroxybenzoylecgonine (m-OH-BZE), and THC-COOH were analyzed as the tertiary butyldimethylsilyl derivatives (benzoylecgonine ions 403, 282, 346; m-OH-BZE 533, 476, 282; THC-COOH 572, 515, 413). Opiates were analyzed as trimethylsilyl derivatives (codeine ions 371, 343, 234; morphine 429, 430, 401). Amphetamines were analyzed as heptafluorobutyl derivatives (amphetamine ions 240, 118, 91; methamphetamine 254, 210, 118). Derivatizing reagents were obtained from Regis Technologies, Morton Grove, IL). Other procedures for GC-MS confirmation of drugs in meconium have been reported (7, 8) with differing cutoffs.

Results and Discussion

Experiment 1

All specimens were first analyzed by GC-MS before they were screened by immunoassay to validate the reliability of the screening procedure. The 26 negative specimens were negative by GC-MS and immunoanalytically. However, the three screening procedures were substantially different in their ability to detect drugs of abuse in meconium (Table 1). Method 1 detected only 19.6% of positive samples; all were cannabinoid positive as extracted by method 1b.

In method 1a, the inorganic acid failed to extract cocaine or opiate metabolites from meconium. Method 2 was an improvement on method 1, probably because of the incorporation of an organic solvent vs an inor-}

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* Number of samples, 100; overall number of positives, 111.

** n/a, not available.

To verify that the differences in sensitivity were a result of the superior extraction efficiency of method 3 and not because of inherent differences in the immunoassay technique chosen (EMIT vs FPIA), an additional comparative study was carried out. From the original 126 samples, 10 samples were blindly selected and extracted according to methods 1 and 2. The extracts were analyzed by FPIA instead of EMIT. Only two of 10 samples analyzed by method 1b gave a readable result by FPIA. The other eight samples yielded error messages on the instrument, indicating excessive background noise in the specimen. Overall, FPIA yielded only one more positive result with method 1 and the same number of positive rates with method 2. The samples extracted by method 3 were analyzed by FPIA only because of sample turbidity, which prevented the use of EMIT.

We conclude that the immunoassay does not substantially affect the outcome of the analysis but the extraction procedure does.

Experiment 2

A total of 535 samples (57%) screened positively for one or more drugs. No samples screened positively for phencyclidine, and of these, 285 (53.3%) were confirmed by GC-MS. The breakdown of each drug class is shown in Table 2. The overall false-positive rate was 46.7%.

Our immunoassay cutoffs were intentionally low because the sensitivity required for meconium testing is higher than the requirement for urinalysis. The amount of meconium available for testing is usually very small, especially in premature babies; thus analytical procedures for meconium drug testing must be...
designed to accommodate the limited sample size. Failure to address this issue would render the procedure unavailable to the very infants who are in most need of correct diagnoses. Raising the immunoassay cutoff concentrations would result in a decreased number of false-positive screens but would also increase the number of false-negative rates. False-negative rates are also increased by the use of inefficient extraction procedures described above. False-positive screens are not a problem if confirmed by a different technique.

Amphetamines. The greatest discrepancy between screen and confirmatory data occurred with the amphetamine drug class. Various nonprescription medications contain cross-reacting substances such as phenylpropanolamine or pseudoephedrine. Phenylethylamine, an endogenous compound, also interferes with the polyclonal assay and with GC-MS confirmations. Of the positive amphetamine screens, 74.3% contained pseudoephedrine or phenylethylamine but not amphetamine or methamphetamine.

THC metabolite. Nonsteroidal anti-inflammatory drugs, particularly ibuprofen, cross-react in immuno-reactive systems with the THC metabolite assay and produce false-positive screens.

Opiates. Positive results for opiates may derive from prescription medications containing, e.g., hydrocodone or hydromorphone (5).

Cocaine metabolite(s). Possibly, not all unconfirmed screen-positive rates for cocaine are falsely positive. The recent discovery of m-OH-BZE, a minor urinary cocaine metabolite, as a contributor to meconium immunoassay screens shows that fetal metabolism does not reflect adult metabolism (9). In almost one-fourth of meconium samples, this compound is the only cocaine metabolite present (10). Other drugs may not metabolize in the fetus as predicted by the adult model, and unidentified metabolites may contribute to immunoreactive responses.

The goal of the meconium test is to provide the clinician with an accurate fetal drug exposure history; therefore, sensitivity is of primary importance. A sensitive screen assay sacrifices specificity; therefore, a specific confirmatory procedure is necessary. Such an approach does not lend itself to reporting screen-only results. Healthcare professionals should be aware of the possible consequences that may arise from such diagnoses.

References