Newsletter for the TDM and Toxicology Division of AACC

Fall 2013

Therapeutics & Toxins News

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Detection of in utero drug exposure using umbilical cord tissue

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Neonates exposed to drugs *in utero* can experience pre-term delivery, neonatal withdrawal syndrome and other long and short term health problems. 5.9% of pregnant women used illicit drugs during their pregnancies in 2011-2012 (1). This is an increase from 4.4% in 2009-2010. Timely and accurate detection of drug exposure helps clinicians to treat acute complications and develop a comprehensive treatment plan to maximize outcomes for these children. Meconium has become the specimen of choice for detection of *in utero* drug exposure, but has several limitations. Meconium can be expelled *in utero* and be unavailable for testing. It is also a very complex matrix, difficult to collect and often there is insufficient specimen to complete testing (2, 3). Recently, umbilical cord tissue has been used for detection of pre-natal drug exposure (4-9). Umbilical cord offers several advantages over meconium. It is available immediately after birth, every child has one, and there is ample specimen (a typical cord is about 22 inches long). Testing umbilical cord tissue also eliminates the possibility of detecting drugs administered to treat the baby after birth.

Logo for Therapeutic and Toxin Newsletter

"5.9% of pregnant women used illicit drugs during their pregnancies in 2011-2012 "

Analysis

Specimens are usually analyzed using a traditional two-step "screen with reflex" approach where an immunoassay screen identifies the drug class, followed by identification and quantitation of specific drugs and metabolites by gas chromatography mass spectrometry or liquid chromatography tandem mass spectrometry (GC-MS or LC-MS/MS). While this approach is the standard in most toxicology laboratories, it has some limitations. Differences in cross-reactivity for drugs in an immunoassay panel compared to the chosen calibrator can result in reduced sensitivity for some compounds. Differences in cutoffs between the immunoassay and the confirmation assays may affect detection, and the drugs analytes detected in the screen and (continued on page 2)

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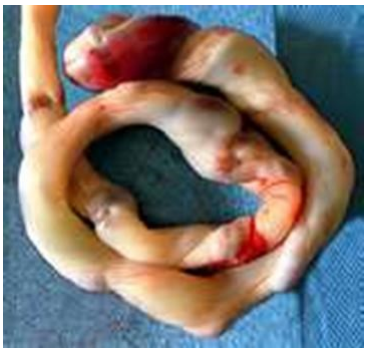
confirmation panels may not be aligned. These issues can lead to apparent false positives or false negatives and discrepant results. Confirmation of multiple drug classes requires multiple additional tests and additional specimen. Drug concentrations do not provide information that correlates to duration or amount of pre-natal drug exposure, so quantitation may not be needed to develop a treatment plan. Recently, liquid chromatography coupled to high resolution accurate mass spectrometry using time-of-flight instrumentation (TOF) has been used for qualitative detection of drugs and metabolites in urine, serum, meconium, and more recently, umbilical cord tissue (10-14). This one-step testing approach provides information on specific drugs and metabolites, eliminates the occurrence of false negatives and false positives resulting from discrepancies between screen and confirmation methods, and provides a faster time to result with cutoffs that are comparable to other mass spectrometry methods (14). Detection of *in utero* marijuana exposure was less sensitive (1 ng/g) by TOF when compared to GC-MS (0.05 ng/g) or ultra-sensitive ELISA (0.10 ng/g) (15).

"Drug concentrations do not provide information that correlates to duration or amount of pre-natal drug exposure"



Considerations

Details regarding the specific formulation, the amount/dose or time and length of exposure cannot be established from umbilical cord tissue results. The actual distribution of drug metabolites in umbilical cord tissue is not well-understood at this time. The window of detection for drugs in umbilical cord tissue is not conclusively known, but is thought to be similar to that of meconium. Concentrations of drugs and metabolites in cord tissue are generally lower than those found in meconium. Drugs administered during labor and delivery and prescribed drugs can be detected in umbilical cord tissue, therefore, a careful chart review and history should be used to distinguish administered or prescribed drugs from illicit use. (continued on page 3)

Drugs/Drug Classes	Range of Cutoff Concentrations
Opioids: buprenorphine, codeine, fentanyl, heroin (6-acetylmorphine), dihydrocodeine, hydrocodone, hydromorphone, meperidine, methadone, morphine, naloxone, naltrexone, oxycodone, oxymorphone, propoxyphene, tapentadol, tramadol	1–10 ng/g
Stimulants: amphetamine, cocaine, methamphetamine, MDMA ("Ecstasy"), MDEA ("Eve"), MDA, phentermine	8 ng/g
Sedative-hypnotics: alprazolam, amobarbital, butalbital, clonazepam, diazepam, flunitrazepam, flurazepam,lorazepam, midazolam, nitrazepam, nordiazepam, oxazepam, phenobarbital, secobarbital, temazepam, triazolam, zolpidem	5–40 ng/g
Phencyclidine (PCP)	4 ng/g

Detection of in utero drug exposure using umbilical cord tissue

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Conclusions

Umbilical cord tissue is a viable option for detection of *in utero* drug exposure with several advantages over meconium, and is a good choice for testing, especially if meconium is not available. Liquid chromatography with high resolution mass spectrometry can provide accurate, qualitative results with a faster turn-around-time than the traditional "screen with reflex" approach. Confirmation testing by GC-MS or LC-MS/MS is required if quantitative results are desired, but may not be necessary for successful treatment of drug exposed neonates.

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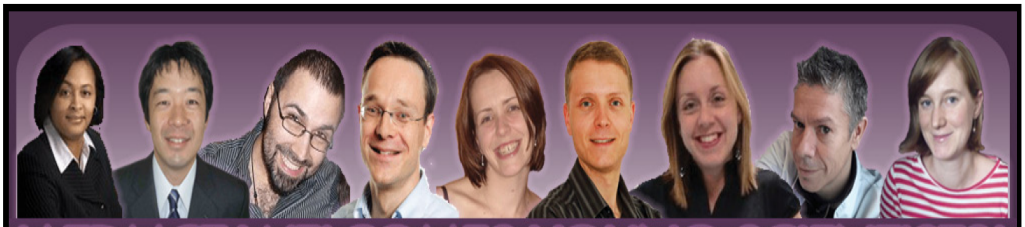
"Umbilical cord tissue is a viable option for detection of in utero drug exposure with several advantages over meconium, and is a good choice for testing, especially if meconium is not available."

"IATDMCT Welcomes Young Scientists for Membership"



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Urinary ethanol markers for use and abuse

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Ethanol has been consumed by man for at least as long as history has been recorded. In the middle ages when distillation was introduced to Europeans from the Arabs, many believed the elixir of life had been discovered and a remedy for all diseases was now available, hence the etymology of the word "whiskey" from the Gaelic word "usquebaugh" for "water of life". In truth, ethanol is of very little therapeutic value (one exception being as an intravenous antidote to methanol or ethylene glycol poisoning) and excessive consumption can lead to a host of social and medical problems. Ethanol is primarily a CNS depressant, and although some stimulatory effects can be described, these are primarily due to depressing inhibitory brain mechanisms[1].

Absorption

Rapid absorption occurs in the stomach, small and large intestine. Maximal blood concentrations can be measured approximately 30-90 minutes after the last drink. Ethanol vapor can also be absorbed through the lungs. Delayed gastric emptying (as with the presence of food) can delay absorption through the small intestine. Once in the small intestine however, ethanol absorption is complete, rapid and generally independent of the presence or absence of food in the GI tract. These variables often result in very different absorption profiles among individuals or within the same individual under different circumstances[1, 2].

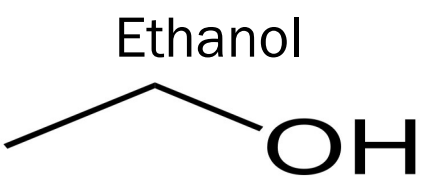
"Ethanol is primarily a CNS depressant, and although some stimulatory effects can be described, these are primarily due to depressing inhibitory brain mechanisms."

Distribution

Once absorbed, ethanol is evenly distributed in the body water to the point that a blood concentration can be estimated given a known dose, body weight, gender and % body fat [2]. The placenta is permeable to ethanol and thus accesses the fetal circulation during pregnancy[1]. Females have been shown to have a smaller volume of distribution compared to males[2].

Metabolism

Ethanol metabolism occurs independent of dose (zero-order kinetics), although first order kinetics have been described at low blood concentrations (<0.02g/dL) or very high concentrations. Females have also been shown to metabolize ethanol at a faster rate than males [1, 2]. Approximately 90-98% of an ingested dose of ethanol is metabolized by oxidation. This occurs primarily in the liver by alcohol dehydrogenase to produce acetaldehyde. Cytochrome P450 enzymes also convert ethanol to acetaldehyde. Acetaldehyde is converted to acetyl CoA (via acetate) for fatty acid synthesis through the citric acid cycle or elimination. The dehydrogenase enzymes responsible for alcohol metabolism exhibit genetic polymorphisms that are expressed with different frequencies in different racial populations. (continued on page 6)



Urinary ethanol markers for use and abuse
(continued from page 5)

This polymorphism can also contribute to variable rates of metabolism seen in different individuals. A very small percentage of an ethanol dose (<0.1%) is conjugated to either glucuronic acid or sulfonic acid [1-3].

Elimination

As mentioned above acetaldehyde generated from ethanol oxidation can be used in the citric acid cycle leading to increased fatty acid synthesis (i.e. fatty liver) or eliminated [3]. Oxidation products and ethanol conjugates are excreted in the urine; the remaining dose can be eliminated via the lungs (expired air) or feces[1, 2].

Toxicology

Ethanol toxicity is largely related to its mechanism of action and its metabolism. Increased membrane fluidity in the brain is a major component of its CNS effects, can result in toxic effects. Increased fatty acid synthesis can lead to fatty liver. Protein and nucleic acid adduct formation due to the reactive nature of acetaldehyde is also possible. Chronic abuse of alcohol leads to a large spectrum of symptoms including liver damage due to a buildup of acetaldehyde, neurologic disorders (e.g. seizures, etc.), nutritional disorders, etc. and death. Concurrent use of ethanol with other CNS depressants can exacerbate the depressant effects of both leading to severe adverse drug reactions and death[1, 2].

"Ethanol toxicity is largely related to its mechanism of action and its metabolism. Increased membrane fluidity in the brain is a major component of its CNS effects, can result in toxic effects."

Analysis

Ethanol use can be determined in a variety of biological matrices. Analysis of breath is commonly used by law enforcement to determine recent alcohol use associated with driving. This involves the oxidation of expired ethanol to acetic acid and water in the flow cell of the analyzer. An electrical current is measured in proportion to the amount of acetic acid produced and an alcohol concentration is extrapolated. Ethanol is also commonly measured in blood[2]. This is performed by extracting vaporized ethanol from the headspace of a sealed vessel containing the blood sample. This vapor is injected onto a gas chromatograph and a detector response is measured proportional to the concentration of ethanol in the sample. This method is also useful in distinguishing specific alcohols in the system (e.g. ethanol, methanol, isopropanol, etc.). A major disadvantage to measuring ethanol directly is its short half-life (2-14 hr).
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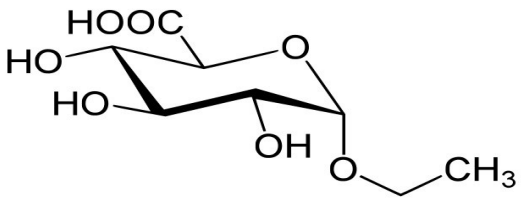
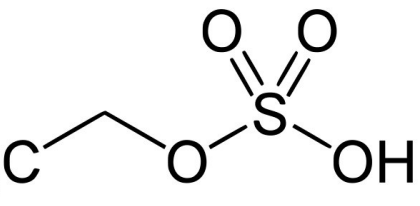
Ethyl glucuronide



Ethyl Sulfate



Urinary ethanol markers for use and abuse
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Recently the measurement of conjugated metabolites of ethanol (ethyl glucuronide and ethyl sulfate) in urine has shown to provide a much longer window of detection for alcohol use (up to 80 hrs for ethyl glucuronide) [4]. This can be extremely useful in determining alcohol use days after ingestion in forensic investigations and also in abstinence programs where blood collection may be less desirable or impractical. These conjugates can be readily analyzed in diluted or extracted urine using liquid chromatography and mass spectrometry [5-8].

Interpretive issues

It is important to note that urine concentrations of alcohol or its metabolites cannot be used to extrapolate an exact dose of alcohol or degree of impairment[2]. Direct analysis of ethanol in urine can be complicated by the fact that fermentation in the urine can produce ethanol and thus lead to a false result[9, 10]. Also, recent studies have investigated the occurrence of false positive ethyl glucuronide results due to exposure to hand sanitizers, mouthwash or other "incidental" exposures to ethanol. Results suggest that to concomitant presence of ethyl sulfate in the urine can help distinguish actual ethanol consumption from dermal exposures associated with these alcohol containing products[10].

"Direct analysis of ethanol in urine can be complicated by the fact that fermentation in the urine can produce ethanol and thus lead to a false result."

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Annual Meeting
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October 17—21, 2014, Sheraton New Orleans Hotel, New Orleans, LA.
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