

## Unresolved Discrepancies between Cannabinoid Test Results for Infant Urine

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**BACKGROUND:** False-positive drug screen results for tetrahydrocannabinol (THC) have been observed. This study investigated the rate of unconfirmed positive screen results in infant and noninfant urine samples and evaluated possible reasons for differences.

**METHODS:** The rate of unconfirmed positive THC screen results for urine samples was determined retrospectively in 2 independent data sets (n = 14 859, reference laboratory; n = 21 807, hospital laboratory) by comparing positive immunoassay-based drug screen results with the associated results of confirmation tests. We then assessed the rate of positive THC screens for samples with varying likelihoods of cannabinoid presence to evaluate the contribution of infant-specific urine constituents to positive results. Finally, a method to detect a THC metabolite (11-hydroxy- $\Delta^9$ -THC) that occurs in meconium was developed to determine its prevalence in infant urine.

**RESULTS:** Positive screen results failed to confirm more frequently in samples from infants (47%) than in noninfants (0.8%). The hospital laboratory observed a similar discrepancy with a different immunoassay. Infant samples with a high likelihood of containing cannabinoids despite negative confirmatory results had a similar rate of positive screening results (50%, n = 20), whereas all samples with a low likelihood of containing cannabinoids screened negative (n = 23). 11-Hydroxy- $\Delta^9$ -THC was not detected in any infant urine sample tested (n = 16).

**CONCLUSIONS:** Conventional confirmatory methods for THC may be inappropriate for urine samples from infants. Our results suggest that one or more currently unrecognized THC-associated compounds are responsible for positive THC screen results for infant urine, as opposed to an infant-associated interference.

Drug-of-abuse screening in neonates is used to determine whether an infant has been exposed to drugs or to confirm maternal drug use during pregnancy. Among the possible testing algorithms, the Substance Abuse and Mental Health Services Administration recommends confirmation of positive results obtained in an immunoassay screen by an independent method, which typically involves chromatographic separation coupled with mass spectrometric detection. Two positive results obtained with different analytical methods and different aliquots of the same sample confirm the presence of a drug. Many of these principles are applied in clinical testing. Owing to the specialized instrumentation commonly used, confirmation is often performed by reference laboratories that may not be aware of the results of the initial screen. Tetrahydrocannabinol (THC)<sup>4</sup> exposure is often assessed in urine by targeting the major urinary metabolite, 11-nor- $\Delta^9$ -carboxy-THC (9-COOH-THC), although upwards of 100 different metabolites are thought to occur (1). Although 9-COOH-THC is the most common target of confirmatory assays, immunoassay screens cross-react with other THC metabolites to varying degrees. At ARUP Laboratories, both immunoassay screening and a liquid chromatography–tandem mass spectrometry (LC-MS/MS) confirmatory method are available for urine THC testing. The screening assay (Syva Emit II Plus; Siemens Healthcare Diagnostics) has a cutoff of 20  $\mu\text{g/L}$  9-COOH-THC, but it shows >45% cross-reactivity with hydroxy metabolites of THC. In contrast, the LC-MS/MS confirmatory assay measures only 9-COOH-THC after alkaline hydrolysis of the glucuronide adducts (5- $\mu\text{g/L}$  cutoff).

Anecdotal evidence that urine samples from infants that show positive results in a urine THC drug screen often do not confirm positive when referred for quantitative testing by LC-MS/MS has surfaced over the past year. In response to these observations, we queried 9 consecutive months of laboratory test results (January through September 2011) to determine the confirmation rate of positive screens in urine samples from infants and noninfants. The data were collected by an individual not involved in the study, and sample identifiers were removed before data analysis. We included only samples for which both screening and confirmatory tests were performed at ARUP Laboratories (n = 14 859). The rate of unconfirmed positive THC

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<sup>4</sup> Nonstandard abbreviations: THC, tetrahydrocannabinol; 9-COOH-THC, 11-nor- $\Delta^9$ -carboxy-THC; LC-MS/MS, liquid chromatography–tandem mass spectrometry; 11-OH-THC, 11-hydroxy- $\Delta^9$ -THC.

**Table 1. Confirmation rate of samples with positive results in the THC screen.**

	Reference laboratory <sup>a</sup>		Hospital laboratory <sup>b</sup>	
	Infants	Noninfants	Infants	Noninfants
No. of screened samples	146	14 713	2294	19 513
Age, years <sup>c</sup>	0.00 (0.00–1.86)	36.30 (2.30–99.43)	0.00 (0.00–0.99)	34.90 (1.00–91.30)
Positive screen results				
No. of samples	38	3024	14	2788
Positives, % (95% CI)	26.0% (18.9%–33.1%)	20.6% (19.9%–21.3%)	0.6% (0.3%–0.9%)	14.3% (13.8%–14.8%)
Positive screen results not confirming				
No. of samples	18 of 38	24 of 3024	6 of 8 <sup>d</sup>	12 of 239 <sup>e</sup>
False positives, % (95% CI)	47.4% (31.5%–63.3%)	0.8% (0.5%–1.1%)	75.0% (45.0%–105%)	5.0% (2.2%–7.8%)
Difference in confirm rates	$\chi^2(1) = 601; P < 0.0001$		$P < 0.0001$ , Fisher exact test	

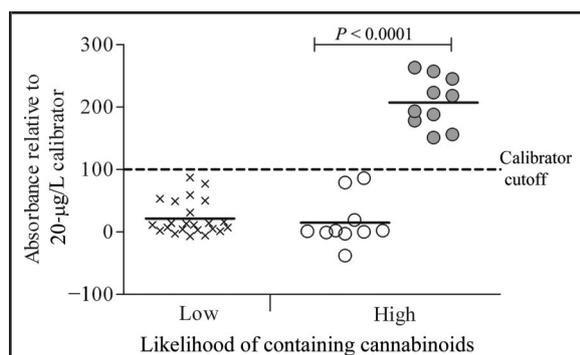
<sup>a</sup> ARUP Laboratories, Salt Lake City, UT.  
<sup>b</sup> University of Iowa Hospital Laboratory, Iowa City, IA.  
<sup>c</sup> Data are presented as the median (range).  
<sup>d</sup> Only 8 of 14 samples testing positively in the urine screen had confirmatory testing performed.  
<sup>e</sup> Only 239 of 2788 samples testing positively in the urine screen were sent for confirmatory testing.

screens (positive result in the THC immunoassay screen, negative result in the confirmatory test) was significantly higher in infants than in noninfants (Table 1). To determine if this situation was unique to the reference laboratory, we also analyzed 52 months of data from an independent hospital laboratory that used a different immunoassay platform (THC II; Roche Diagnostics; 50- $\mu\text{g/L}$  9-COOH-THC cutoff) and reached a similar conclusion (Table 1). In this data set, 14 infant samples screened positive for THC, and 8 were sent for confirmation. Thirteen of these 14 infants had also undergone meconium testing, and 11 of these meconium samples tested positive for 9-COOH-THC. One sample tested negative. The last sample tested negative for THC but positive for a number of other drugs of abuse, and the clinical history indicated that THC exposure within 1 to 2 days of delivery was likely. Thus, of the 14 newborns with positive results in THC screens, 11 had congruent meconium 9-COOH-THC findings, including 4 of 6 infants who had negative urine test results for 9-COOH-THC by confirmation methods and 5 of 6 infants who had positive results in the urine screen that were not followed up. Methods performed at both institutions followed institutional ethics review board–approved protocols. All samples used in this study were deidentified before use, in accordance with these procedures.

The positive results in the THC urine screen in infants appear to be attributable to a compound other than 9-COOH-THC. Several possibilities could explain the discrepancy between the screen and confirmation results. First, it is possible that a compound specific to infant urine (whether an endogenous con-

stituent or an exogenous contaminant) could produce false-positive screening results. Second, neonatal THC screening may not be the standard of care at all hospitals and is often ordered in children who are at a high risk of having been exposed to illicit substances. Positive THC screening results may thus reflect compounds other than cannabinoids that are present in the urine of infants at high risk of drug exposure, or they may reflect environmental or socioeconomic factors associated with their high-risk status. Third, it is possible that THC metabolites other than 9-COOH-THC predominate in neonatal urine. Indeed, liver microsomal enzymes purportedly involved in THC metabolism are differentially produced during development, with the production of adult-type enzymes occurring only several months after birth for some enzymes and with differences in activity between children and adults noted for others (2–5).

To address the first possibility, we obtained infant urine samples ( $n = 23$ ) submitted for analyses other than for drug or toxicologic testing and analyzed them with the THC immunoassay screen. Most often, these samples had been submitted for urine organic acid or amino acid analysis, probably owing to newborn screening efforts, and thus they were considered less likely to contain THC metabolites/cannabinoids. Recently, contamination of urine with bathing products has been suggested to cause false-positive THC results in immunoassays (6). Cotten et al. found that adding some baby wash soaps to pooled urine interfered with several THC immunoassays, including the Emit assay. If an infant-specific contaminant contributes to screen positivity, the rate of positive screen results in this low-



**Fig. 1. THC immunoassay screen results.**

Signal intensity is expressed relative to the cutoff calibrator (20  $\mu\text{g/L}$  9-COOH-THC). Samples with absorbance values above the cutoff are reported as positive. Infant urine samples submitted for testing other than for drug testing are assumed to have a low likelihood of containing cannabinoids (crosses). Samples submitted for confirmatory testing only, with negative results, constitute the high-likelihood group (circles). The signal intensities of the positively testing samples (gray circles) and the negatively testing samples (white circles) within the high-likelihood group were significantly different (Mann–Whitney test). All samples were from infants  $\leq 7$  days old.

likelihood set of infant samples should be similar to that observed in the retrospective data set (approximately 50%). None of these samples screened positive, however.

To address the second possibility, we screened (Emit THC assay) 20 infant urine samples that had been sent to ARUP Laboratories for confirmatory testing only (and thus with a higher likelihood of containing THC metabolites or cannabinoids) and generated negative results in the confirmatory test. Half of the samples had screened positive. As illustrated in Fig. 1, samples that screened positive demonstrated absorbance values well above the cutoff value. Absorbance values were significantly higher in positive samples, compared with those that had screened negative ( $P < 0.0001$ , 2-tailed Mann–Whitney test; Fig. 1). These results, together with the negative results for the samples less likely to contain THC metabolites/cannabinoids, argue against an infant-specific urine constituent or an external contaminant—including baby wash products—having contributed to the positive screen results. We cannot rule out the possibility that some samples were too dilute for detecting THC metabolites. Because the conventional means of assessing urine concentration involves creatinine measurement, which is not appropriate for infant samples, this fact

remains a potential limitation of this study. Given that the high-likelihood sample group showed a screen/confirm discrepancy similar to that observed in the retrospective data analysis, results of these experiments are likely generalizable to the infant population, if we can assume that the degree of urine dilution is random and varies little among infants.

Finally, to address the possibility that a different THC metabolite predominates in infant urine, we developed a method to detect and quantify 11-hydroxy- $\Delta^9$ -THC (11-OH-THC). This metabolite is present in meconium samples, is pharmacologically active, cross-reacts in cannabinoid immunoassays, and is the precursor to the 9-COOH-THC metabolite (1, 7–9). In brief, we added deuterated 11-OH-THC internal standard (Cerilliant) to 1 mL of infant urine and hydrolyzed the sample enzymatically overnight (16 h) at 37  $^{\circ}\text{C}$  in 0.1 mol/L sodium acetate, pH 5, containing 20 000 U of  $\beta$ -glucuronidase (Campbell Science). This enzyme preparation exhibits some sulfatase activity, and we did not separate out the efficiencies of the glucuronide and sulfate hydrolyses. After adding sodium hydroxide and centrifugation to remove precipitates, we applied the hydrolyzed urine to a preconditioned solid-phase extraction column (Strata-X-C; Phenomenex), washed the column with 0.1 mol/L HCl, and eluted with 20 mL/L glacial acetic acid in acetonitrile. The eluant was dried, resuspended in acetonitrile, and analyzed by LC-MS/MS on a Waters Acquity TQD system with an Acquity UPLC (HSS C18 UPLC column, 2.1  $\times$  50 mm, 1.8  $\mu\text{m}$ ) and an electrospray ionization source, which was used in positive ion mode. Multiple reaction monitoring was used to detect 2 transitions for both the analyte and the internal standard (11-OH-THC:  $m/z$  331.2 $\rightarrow$ 193.1 and  $m/z$  331.2 $\rightarrow$ 201.2; 11-OH-THC- $d_3$ :  $m/z$  334.3 $\rightarrow$ 196.2 and  $m/z$  334.3 $\rightarrow$ 201.4). We prepared a calibration curve (2.5, 5, 10, 50, 100, and 500  $\mu\text{g/L}$ ) in pooled adult urine that had previously been confirmed to be negative for THC and processed the calibration samples along with test samples. Because the glucuronide of 11-OH-THC is not readily available for purchase, we used pooled samples of adult urine known to contain THC to monitor hydrolysis. Hydrolysis was confirmed by detecting 11-OH-THC in the adult positive-control sample (mean, 59  $\mu\text{g/L}$  11-OH-THC) after incubation with enzyme and comparing this result with an incubation in the absence of enzyme (mean,  $< 2.5$   $\mu\text{g/L}$ ). Assay sensitivity was estimated by diluting the pooled positive control and measuring the concentration in triplicate over 3 days. This experiment yielded a mean concentration of approximately 6.9  $\mu\text{g/L}$  with a CV of  $< 10\%$ , and we defined this value as our limit of quantification. The analyte signal, however, was linear over a 200-fold interval (2.5–500  $\mu\text{g/L}$ ,  $r^2 > 0.999$ ; calibrators quantified

within 15% of target), and no ion suppression was apparent in any of the adult or infant samples tested. Of the 20 samples with a high likelihood of containing THC and previously analyzed by the Emit assay, 16 had sufficient volume to be assayed for 11-OH-THC. 11-OH-THC was not detected in any of these samples (all had signals less than that of the lowest calibrator).

Although 9-COOH-THC and 11-OH-THC do not appear to be the metabolites responsible for the positive THC screen results, the screen results cannot be explained by nonspecific interferences, because none of the samples with a low likelihood of containing cannabinoids screened positive. Furthermore, the majority of the screen-positive urine samples in the hospital data set showed congruent meconium 9-COOH-THC results. It is possible that other THC metabolites or different forms of 9-COOH-THC and/or 11-OH-THC (such as sulfate conjugates or conjugation at different sites on the molecule) predominate in infant urine because of differences in THC metabolism and/or stability during the perinatal period. Indeed, fetal liver and adult liver differ with respect to the expression of cytochrome P450 genes (2). Several metabolic enzymes are thought to be involved in THC metabolism in humans (1). Developmental changes in enzyme production could yield a different metabolite profile in infants.

Our results suggest that current confirmatory THC assays designed to detect the 9-COOH and/or 11-OH metabolites of THC may be inappropriate for confirming THC screen results for infant urine samples. It would be helpful if future studies identified THC metabolites with prospectively collected infant urine samples matched for hospital location, used the same sample-collection protocols, and stratified the infants according to risk of drug exposure. Further investigations are also required to determine whether similar mechanisms account for

the unconfirmed screen results in adult samples, or if the rate of unconfirmed noninfant screen results represents the false-positive rate of the screening assays themselves. In the meantime, infant urine samples with positive THC screen results that fail to confirm may need to be interpreted in conjunction with maternal urine drug-testing results, secondary immunoassay results, and clinical/social findings. Until the compounds contributing to positive urine screen results in infants are identified, we encourage the use of alternative specimens for the detection and investigation of neonatal exposure to cannabinoids. Screen-positive cannabinoid results from infant samples should not be reported without confirmation or appropriate consultation, because they cannot currently be interpreted.

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## References

- Huestis MA. Human cannabinoid pharmacokinetics. *Chem Biodivers* 2007;4:1770–804.
- Nagata T, Takahashi Y, Ishii Y, Asai S, Sugahara M, Nishida Y, et al. Profiling of genes differentially expressed between fetal liver and postnatal liver using high-density oligonucleotide DNA array. *Int J Mol Med* 2003;11:713–21.
- de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Cytochrome P450 3A: ontogeny and drug disposition. *Clin Pharmacokinet* 1999;37:485–505.
- Leeder JS. Pharmacogenetics, pharmacogenomics, and pharmacoproteomics. In: Yaffe SJ, Aranda JV, eds. *Neonatal and pediatric pharmacology*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p 44–59.
- Rane A. Drug metabolism and disposition in infants and children. In: Yaffe SJ, Aranda JV, eds. *Neonatal and pediatric pharmacology*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p 32–43.
- Cotten SW, Duncan DL, Burch EA, Seashore CJ, Hammett-Stabler CA. Unexpected interference of baby wash products with a cannabinoid (THC) immunoassay. *Clin Biochem* 2012;45:605–9.
- ElSohly MA, Feng S.  $\Delta^9$ -THC metabolites in meconium: identification of 11-OH- $\Delta^9$ -THC, 8 $\beta$ ,11-diOH- $\Delta^9$ -THC, and 11-nor- $\Delta^9$ -THC-9-COOH as major metabolites of  $\Delta^9$ -THC. *J Anal Toxicol* 1998;22:329–35.
- Gray TR, Barnes AJ, Huestis MA. Effect of hydrolysis on identifying prenatal cannabis exposure. *Anal Bioanal Chem* 2010;397:2335–47.
- Coles R, Clements TT, Nelson GJ, McMillin GA, Urry FM. Simultaneous analysis of the  $\Delta^9$ -THC metabolites 11-nor-9-carboxy- $\Delta^9$ -THC and 11-hydroxy- $\Delta^9$ -THC in meconium by GC-MS. *J Anal Toxicol* 2005;29:522–7.

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