



Unexpected interference of baby wash products with a cannabinoid (THC) immunoassay

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ABSTRACT

Objectives: The results of newborn drug screening have far-reaching impact not only in healthcare, but also in the legal domain. Therefore, the accuracy of these results cannot be undervalued. When false positive cannabinoid (THC) screening results for this population were suspected at our institution, a multidisciplinary approach was initiated to evaluate the screening process for any pre-analytical or analytical sources of error or interference.

Design and methods: Mixtures of drug-free urine with various commercial products and materials that commonly contact newborns in our nursery were prepared and tested using the immunoassay screening methods in our laboratory. Additional commercial products were similarly tested; and when available, individual surfactants common to the interfering products were also evaluated.

Results: Addition of Head-to-Toe Baby Wash to drug-free urine produced a dose dependent measurable response in the THC immunoassay. Addition of other commercially available baby soaps gave similar results, and subsequent testing identified specific chemical surfactants that reacted with the THC immunoassay.

Conclusion: We have identified commonly used soap and wash products used for newborn and infant care as potential causes of false positive THC screening results. Such results in this population can lead to involvement by social services or false child abuse allegations. Given these consequences, it is important for laboratories and providers to be aware of this potential source for false positive screening results and to consider confirmation before initiating interventions. Most importantly, we demonstrate the need for active involvement in the “total testing process,” as sources of error are not confined to the laboratory walls.

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Introduction

Identification of newborns exposed to drugs of abuse in utero is a challenging and even controversial process. Nevertheless, accurate assessment of drug exposure in this population is critical to ensure both protection of the child and support for the family. In studies designed to evaluate the effectiveness of assessment methods, interviews and questionnaires are found to identify a smaller proportion of women who self-report drug use in the previous month in contrast to the use of biological samples [1,2]. Testing of maternal or newborn

urine or meconium for the presence of drugs of abuse finds a higher prevalence, particularly among women who fail to receive prenatal care [1,2]. Because of growing concerns, a number of recommendations have been developed at local and national levels calling for more frequent assessment of high-risk women when factors such as limited prenatal care, high scores on self-assessment questionnaires, or placental abruption lead to suspicion for drug abuse [3–7]. To this end, healthcare providers often couple information gained through maternal interview and self-reporting with laboratory testing of biological samples such as maternal urine, newborn urine, or meconium to make decisions and recommendations [1–4].

In serving this population, most clinical laboratories perform urine drug testing (UDT) using immunoassay-based methods as a means to determine past drug use or exposure. These assays are designed to identify the presence of specific drugs or drug metabolites within a sample at a specific concentration, also known as the cut-off. For example, the presence of 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (THC acid) is used as an indicator of marijuana use or exposure since the compound is the major urinary metabolite of the primary

Abbreviations: UDT, urine drug of abuse testing; THC, tetrahydrocannabinol; Δ^9 -THC, Δ^9 -tetrahydrocannabinol; GC/MS, gas chromatography/mass spectrometry; EMIT, enzyme multiplied immunoassay technique; PEG 80, polyethyleneglycol 80.

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psychoactive component of the drug, Δ^9 -tetrahydrocannabinol (Δ^9 -THC). The concentration used to distinguish a positive result from negative, varies depending on the context in which testing is performed, the laboratory, and the immunoassay used, but typically ranges between 20 and 50 $\mu\text{g/L}$ (ng/mL). Marijuana is more complex than some of the other drugs of abuse in that it contains many more cannabinoids. This is important in that immunoassays for the drug class often cross-react with many of the other cannabinoids and/or their metabolites; and most importantly, some assays are reported to cross-react with unrelated, non-cannabinoid compounds. This issue is not unique to testing for cannabinoids and similar issues exist with the immunoassays used to detect other drugs of abuse. This limitation is highly dependent upon the antibody specificity, but is the reason UDT using immunoassay is considered a screening test. An additional limitation is the fact that few analytical platforms permit the laboratory to establish internal cut-offs and none are configured to permit the use of multiple cut-offs for different populations, that is, one cut-off for maternal or newborn testing with another for the pain clinics or emergency department. Finally, these methods are designed to be used only with urine specimens, thus alternate samples collected for drug testing, such as meconium, must either be forwarded to another laboratory for analysis or the assay modified by the laboratory. Despite these issues, clinical laboratories choose to use these methods because of their cost-effectiveness, ease of use, and short analysis time [8].

As previously mentioned, immunoassays used in this context are considered screening methods. Samples which screen positive may be reanalyzed using more sensitive and specific techniques such as gas chromatography/mass spectrometry (GC/MS) or liquid chromatography tandem mass spectrometry (LC/MS/MS) to confirm the presence of a drug or metabolite, and while such is encouraged, it is not mandated in the clinical setting. Many clinical laboratories do not have the equipment or staff to perform confirmatory testing and must forward these samples to another laboratory. This practice may not be performed automatically for reasons of institutional compliance policies, provider preference, timeliness in reporting, and costs.

In our institution, UDT is performed using immunoassays with results reported as $<$ or \geq the respective cut-off for the given assay. In addition, a comment is appended noting that other compounds may be detected and that confirmation testing is available upon request. Our laboratory has initiated limited in-house confirmatory testing based upon test requests, but since the requests for cannabinoid confirmation are quite low, we send these samples to our reference laboratory. It is not routine practice to automatically confirm positive results for several reasons. First, rapid turnaround times are needed to serve several pain management clinics and a busy level one trauma center. Furthermore, we allow samples which screen below cut-offs to undergo confirmation testing. Laboratory scientists and staff provide consultations regarding these results and frequently assist providers in their interpretations and decisions to pursue confirmation, or not. Of the more than 50,000 urine drug screens performed annually, confirmation is requested on about 4%, most commonly for the opiate and benzodiazepine classes.

As part of on-going quality assurance, we routinely conduct studies to evaluate the cross-reactivity of commonly prescribed medications with the screening immunoassays used in our laboratory [9–11]. These studies are prompted by staff observations of increased rates of positive screening results, or by provider queries regarding screening results that do not confirm. Such a query was received in late July 2011 from our Newborn Nursery regarding the cannabinoids (THC) screen. Discussions with the staff revealed the department had increased the frequency of testing (Fig. 1) of their patients using both urine and meconium and observed an increase in the number of urine drug screens positive for THC without a concomitant positive meconium result. A review of the testing for this unit since the previous January did indeed show that 22 of the 120 urine drug screens

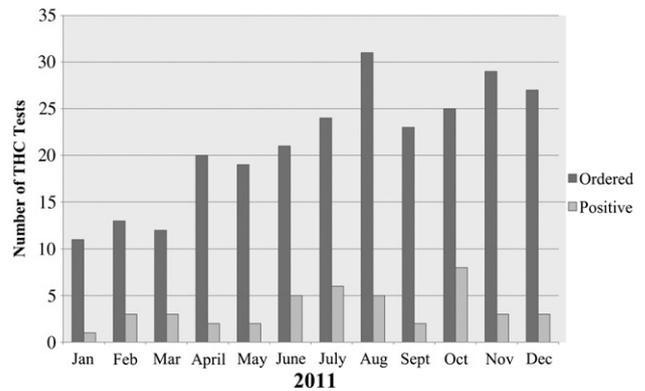


Fig. 1. In April 2011 an increase in the frequency of ordering urine drug screens for newborns is seen in response to adoption of recommendations for the use of biological testing of infants when the mother has had minimal prenatal care. Initially, the perceived concomitant increase in positive screening results is related to the overall increase in orders.

performed were positive for THC and that none of these had undergone confirmation testing. Interestingly, with the exception of one pair collected in April, there was concordance between the urine and meconium results for cannabinoids, i.e., positive urine screen with confirmed positive meconium, until July when three positive urine drug screens had corresponding negative meconium results. These three raised concern for potential false positives.

The immunoassay used to screen for this drug class in urine is designed to predominantly cross-react with 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid, although other metabolites are also detected [12]. As noted previously, samples can be forwarded to our reference laboratory for confirmation using a gas chromatography/mass spectrometry (GC/MS)-based assay which has a 3 $\mu\text{g/L}$ cut-off. Regular reviews conducted in our laboratory have consistently shown overall good agreement between screening and confirmation with less than 0.5% of samples disagreeing. These findings are predominantly from adults seen in pain management or addiction clinics, but are the reasons why we have very few requests for THC confirmation. All meconium samples are submitted to the same reference laboratory where testing includes preliminary immunoassay screening for THC acid (cut-off 10 ng/g) followed by LC/MS/MS confirmation (cut-off 10 ng/g).

After a review of the mothers' and newborns' drug histories did not reveal any known cross-reacting agents, we referred several THC screen-positive urine samples for confirmation and initiated additional discussions between key laboratory and clinical staff to assure all had a good understanding of the differences between the two sample types, detection windows, and expected agreement. These discussions identified nursery-specific factors that complicated sample collection and identified potential sources for pre-analytical variability. Specifically, there was considerable variation between nursing staff in cleansing the newborn prior to collection and in collecting the samples: Some placed cotton balls or gauze within the diaper, others used collection devices, while others turned diapers inside-out. These factors, in conjunction with a higher screen-to-confirmation discrepancy rate than typically seen in the adult population, implied an interference unique to the nursery. Therefore, we sought to examine all nursery-specific products that could potentially come in contact with newborn urine samples to determine any potential effect on THC screening.

Materials and methods

Reagents and materials

All immunoassays were performed in the William W. McLendon Clinical Laboratories Core Laboratory using the THC (Cannabinoid)

Table 1
Measured cannabinoid concentrations with Head-to-Toe Foaming Wash.

Soap added (mL)	Apparent cannabinoids (µg/L)	Interference ^a	Fold change
0	<5		
0.02	8.4	+ 3.4	1.68
0.04	15.6	+ 10.6	3.12
0.06	19.3	+ 14.3	3.86
0.08	22.0	+ 17.0	4.40

^a Calculated as apparent cannabinoids concentration measured-response of drug-free urine.

reagent on the Vitros 5600 (Ortho Clinical Diagnostics, Inc, Rochester, NY). The assay uses 20 µg/L as the cutoff for a positive result and has a limit of detection of 5 µg/L. Testing is performed in the semi-quantitative mode although patient samples are reported as ≥20 µg/L and <20 µg/L. We have found the assay to have excellent precision near and above the cut-off: 25 µg/L ± 3.9% and 49.7 µg/L ± 5.8%. The drug free control has never given a result other than <5 µg/L. Additional immunoassays used included those for opiates, benzodiazepines, amphetamines, barbiturates, benzoylecgonine (cocaine metabolite), and methadone (all Ortho Clinical Diagnostics, Inc.). Selected samples were tested using Siemens EMIT and Beckman Olympus immunoassays, as noted, courtesy of the clinical laboratories of Rex Healthcare (Raleigh, NC) and Duke University Hospital (Durham, NC). Pooled, volunteer provided, drug-free urine was used for interference testing.

We examined products used routinely in our newborn nursery, as well as similar products. These included Head-to-Toe Foaming Wash (Johnson & Johnson, New Brunswick, NJ), Johnson's Bedtime Bath (Johnson & Johnson, New Brunswick, NJ), Baby Magic: Hair and Body Wash (Nattera Inc., Flower Mound, TX), Night-Time Baby Bath (CVS Caremark Corp., Woonsocket, RI), CVS Baby Shampoo (CVS Woonsocket, RI), Aveeno Soothing Relief Creamy Wash (Johnson & Johnson, New Brunswick, NJ), Aveeno Wash Shampoo (Johnson & Johnson, New Brunswick, NJ), Medichoice Baby Wipes (Owens & Minor Mechanicsville, VA), Kendall Curly Gauze Sponge (Covidien, Mansfield, MS), Cotton Ball X-large (6'S) (Custom Hospital Products, Portland OR), Huggies Newborn Diapers (Kimberly-Clark Corporation, Neenah, WI), Huggies Preemie Diapers (Kimberly-Clark Corporation, Neenah, WI), U-Bag Urine Collection Preemie (Briggs Healthcare Waukegan, IL), U-Bag Urine Collectors Newborn (Briggs Healthcare Waukegan, IL), and a hygienic cleansing towelette (PDI Inc., Orangeburg, NY) containing *Hamamelis virginiana* (Witch Hazel), glycerin, benzalkonium chloride, and methylparaben. Active ingredients in these baby washes, cocamidopropyl betaine, polyquaternium 11, sodium lauryl sulfate (29% w/v), and the PEG 80 sorbitan laurate-cocamidopropyl betaine-sodium lauryl sulfate mixture (PEG 80 mix) were purchased from The Conservatorie (Rancho Cucamonga, CA). The PEG 80 mixture consisted of 15–20% PEG-80 sorbitan laurate, 12–16% cocamidopropyl betaine, 10–14%

Table 2
Interference of various commercial soaps with the cannabinoid immunoassay.

Commercial soaps	Apparent cannabinoids (µg/L)	Interference ^a	Fold change
Drug-free urine	<5.0		
J&J Head to Toe	16.2	+ 11.2	3.24
J&J Bedtime Bath	25.2	+ 20.2	5.04
CVS Night-Time Baby	27.3	+ 25.3	5.46
CVS Baby Wash	15.8	+ 10.8	3.16
Aveeno Soothing Relief Creamy Wash	23.6	+ 18.6	4.72
Aveeno Wash Shampoo	23.4	+ 18.4	4.68
Baby Magic	18.2	+ 13.2	3.64
Hospital hand soap (Gel)	12.8	+ 7.8	2.56
Hospital hand soap (Foam)	<5.0		

^a Calculated as apparent cannabinoids concentration measured-response of drug-free urine.

sodium laureth sulfate, 3–6% sodium chloride, 2–5% disodium cocamidopropylacetate, <3% PEG-150 distearate, and <3% sodium laureth-13 carboxylate. BioRad S2E (low opiate) urine toxicology control, containing 65 µg/L 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid was purchased from BioRad Laboratories (Hercules, CA).

Samples preparation and studies

Aliquots of urine (1 mL) were incubated with the baby wipes, gauze, cotton balls, Head-to-Toe Foaming Wash (0.04 mL), diapers (preemie and newborn), and U-Bag Urine Collection (preemie and newborn) overnight followed by extraction of the remaining liquid and subsequent testing using the Vitros THC immunoassay.

Dose dependent studies were conducted by adding increasing amounts of Head-to-Toe Foaming Wash (0.02, 0.04, 0.06, and 0.08 mL) to 1 mL of drug-free urine, vortexing of the sample for 30 s, and testing using the Vitros THC immunoassay. The amounts of materials added reflect an attempt to balance accurate pipetting in the simulation with the amount of material that may have contaminated patient samples. Similar studies were conducted with the additional washes and soaps purchased by adding 0.08 mL of each to 1 mL of drug-free urine and testing as described above. The composition of the products yielding a positive interference were compared and an assessment of the cross-reactivity of the primary surfactant components (determined by order of listing on each product) was performed by adding 0.08 mL of each compound into a 1 mL aliquot of drug-free urine, vortexing for 30 s, and testing using the Vitros THC immunoassay.

To assess the potential for the collection devices used in the newborn nursery to bind THC thereby imparting a negative bias on UDS results, 3 mL of urine previously screened positive for benzoylecgonine and benzodiazepines was mixed with 2 mL of the low opiate urine toxicology control fluid and incubated overnight with various materials and retested using all drug of abuse screening immunoassays.

Results

Initial screening of drug-free urine incubated with baby wipes, gauze, cotton balls, diapers (preemie and newborn), and U-Bag Urine Collection (preemie and newborn) showed no reactivity with the THC assay. However, the addition of the Head-to-Toe Baby Wash yielded a response above the limit of detection, but below the cut-off, at 10.2 µg/L. Subsequent evaluation of increasing amounts (0.02–0.08 mL) of Head-to-Toe Baby Wash revealed a dose dependent increase with results exceeding the threshold for a positive screening result (Table 1) when less than 0.1 mL of wash was added to the samples. Similar dose dependent results were obtained using the Siemens EMIT assay but not using the Beckman Olympus THC assay (data not shown).

All additional soap products tested were found to cause some level of reactivity with the THC assay except for the hospital foaming hand soap. Four out of seven commercial baby soaps caused assay

Table 3
Interference of individual surfactants with the cannabinoid immunoassay.

Compound (0.08 mL)	Apparent cannabinoids (µg/L)	Interference ^a	Fold change
Drug-free urine	<5		
Cocamidopropyl betaine	42.6	+ 37.6	8.52
PEG 80, sorbitan laurate, cocamidopropyl betaine	40.8	+ 35.8	8.16
Sodium lauryl sulfate	<5		
Polyquaternium 11	46.1	+ 41.1	9.22

^a Calculated as apparent cannabinoids concentration measured-response of drug-free urine.

Table 4
Effects of various textile matrices on cannabinoid positive samples.

Original apparent cannabinoid concentration ($\mu\text{g/L}$)	Concentration after incubation ($\mu\text{g/L}$)			
	Baby wipes	Gauze	Cotton balls	Diaper
19	15.2	13.6	15.1	14.2
54	39.8	41.4	40	44.3
73.3	63.4	63.9	64.8	64.1

interference sufficient to yield a positive screen result (Table 2). The other three caused positive interference, but not to an extent that resulted in a positive screening result. Specific components of the baby washes were associated with the interference (Table 3). Evaluation of polyquaternium 11, cocamidopropyl betaine, and a mixture of PEG 80, sorbitan laurate, and cocamidopropyl betaine all showed strong reactivity in the THC assay, while sodium lauryl sulfate showed no reactivity.

Attempts to illicit false negative results from drug-spiked urine using a variety of textile matrices showed no appreciable change in assay reactivity although slight decreases in measured THC concentrations were observed and may be attributed to THC adsorption to the fiber matrix. The change in response was small, $<10 \mu\text{g/L}$ as seen in Table 4, but could potentially affect results that are $\pm 5 \mu\text{g/L}$ from the $20 \mu\text{g/L}$ cut-off. Importantly, there was no decrease in the measured analyte for any of the other drugs of abuse immunoassays after incubation with these materials. Nor did any of the wash products or surfactants introduce a positive bias with the other drug immunoassays.

Discussion

We have identified washes and soaps used within our Newborn Nursery as a reagent dependent and population specific interference leading to false positive urine drug screening results for cannabinoids. The investigation was complicated by the realization that there was no standard newborn urine collection protocol in place at our institution. Application of the urine collection bag, gauze, or diaper could occur before or after cleansing and washing. Furthermore, when the baby was washed prior to collection, no reproducible rinsing technique was utilized. The amounts of washes and soaps used within these studies were quite small, less than 0.1 mL, and easily represent the amounts that could remain on the skin after use. For samples that screened positive but confirmed negative, no aberrant pH values were noted, reducing suspicion of external contamination of the urine sample. Identification of the cause of this pre-analytical derived error was through the combined efforts and communication between the laboratory and clinical staff. In addition to the product used within our institution, we also identified other soap-based products that could potentially cause a false positive result if only screening is performed.

These findings were interesting because Mikkelsen, et al. and Uebel, et al. independently found hand soaps to be an effective means of thwarting detection of illicit drug by producing false negative screening results when small amounts were added to urine in the setting of workplace drug testing [13,14]. This approach to circumvent detection has prevailed within the workplace setting and is one of the reasons urine pH continues to be measured. Interestingly, Warner reported the potential for Joy liquid detergent to cause both false negative as well as false positive cannabinoid results depending on the immunoassay used [15]. There is no mention of detergents or soaps as interferents in the product information in the Vitros 5600 Cannabinoid reagent [12]. Unfortunately, despite the identification of several surfactant components which contribute to the interference, the exact mechanism of interference and its selectivity for the cannabinoid immunoassay remains unclear.

Most importantly, these findings raise questions about UDT confirmatory testing in the clinical setting. Unlike forensic settings where confirmation using either GC/MS or LC tandem MS is expected, even

mandated, before a result is released, the clinical setting is challenging and many situations warrant action using a screening result. A result held for confirmation in such cases could delay the implementation of needed care. However, one can easily argue that either option (releasing versus holding non-confirmed results) is a potential patient safety issue.

This exercise demonstrates that good communication between the clinical laboratory and the medical team can balance the limitations of testing results with clinical needs. In response to the observations made by the Newborn Nursery staff and the resulting investigations, the decision was made to reflex all positive urine drug screening results from this unit for confirmation. Screening results continue to be reported as performed, but the clinical staff acts on positive results more cautiously. Education on the limits of drug screening has prompted clinical staff to use other methods of assessment, in conjunction with screening and confirmation results, to assess and act on patient needs.

A review of the 136 results in the five months since implementation shows 21 urine drug screens positive for cannabinoids: all but five were submitted for confirmation. Unfortunately, this has led to a new discovery of an additional barrier: nine of the specimens that were submitted for confirmation had insufficient volume to perform the confirmation. Of those with sufficient volume, five did not confirm and two were reported as having an interfering substance. Sixteen of the samples had a corresponding meconium confirmed to be positive for THC acid. The difficulty in acquiring sufficient urine for testing from the newborn population cannot be underestimated and clearly there is a need for confirmation methods which require lower volumes.

Conclusions

Identification of intrauterine drug exposure is an important aspect of newborn care, especially when the mother has had little documented prenatal care. Biological testing of meconium and urine is considered a better means of identifying drug-exposed neonates than relying on maternal self-reporting. While clinical laboratories rely on immunoassays to provide rapid results, we show that some commonly encountered materials can lead to unexpected and erroneous measurements. Prenatal exposure to illicit drugs is considered child abuse in some regions of these United States so urine drug screen fidelity is imperative [16]. False positives from surfactant contamination may lead to unnecessary involvement by social services. Thus, it is important the laboratory is aware of this potential source of error, as results have great economic and legal impacts on patient care. Therefore this finding underscores the importance of diligent monitoring by the laboratory of the “total testing process” within the greater healthcare system.

Given these potential consequences, we recommend that all positive newborn urine and meconium screening results undergo reflex confirmatory testing using a more sensitive and specific method such as GC/MS or LC tandem MS. We also suggest that this is an area in which improved assays, both screening and confirmatory, are needed that can help clinical laboratories meet the challenges unique to the application of drug testing within this population.

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