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Using Umbilical Cord Tissue to Identify Prenatal Exposure to Fentanyl and Other Commonly Abused Drugs

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Abstract

Background: Prenatal exposure to fentanyl may lead to Neonatal Abstinence Syndrome (NAS), a constellation of symptoms observed when newborns begin withdrawing from addictive substances such as opioids. The use of umbilical cord tissue segments (UC) for newborn toxicology has been increasing due to its apparent long detection window, sensitivity, and ease of collection. However, very little has been reported in the literature concerning the prevalence of *in utero* exposure to fentanyl and co-exposure with other commonly abused substances. **Specific aim**: The specific aims of this retrospective study are twofold. We will report prevalence of neonatal exposure to fentanyl for a nationwide high-risk population using UC submitted to a national reference laboratory for routine forensic toxicology analysis and the co-exposure patterns observed for these fentanyl-exposed neonates. **Methods:** A secondary analysis was performed using historical data for UC received between January 1, 2020 and December 31, 2020 for routine forensic toxicology analysis. **Results**: During the study period, our laboratory received 23,104 UC for analysis and 9667 (41.8%) of those UC were positive for at least one drug. The prevalence of fentanyl detection was 1.9% (n = 429). Of these 429 specimens there were 407 UC where both fentanyl and norfentanyl were detected. There were 14 UC where only fentanyl was detected and 8 UC where only norfentanyl was detected. When detected, the median concentrations of fentanyl and norfentanyl were 4029 pg/g (IQR: 1696, 9230 pg/g) and 10,756 pg/mg (IQR: 3925, 25,288 pg/g), respectively. Of the 429 positive fentanyl and/or norfentanyl UC, 33 (7.7%) were only positive for fentanyl and/or norfentanyl. Of the 396 polypositive UC, morphine was the highest co-exposure with 243 UC (56.6%) being positive for both fentanyls and morphine. The second most prevalent co-exposure observed was methamphetamine/amphetamine (n = 173; 40.3%) followed by cannabinoids (n = 113; 26.3%) and benzoylecgonine (cocaine metabolite; n = 106; 24.7%). **Conclusions:** Nonmedical use of fen-

tanyl is an alarming trend in this country including this maternal demographic reported here. Fentanyl was typically found with other commonly abused substances.

Keywords

Fentanyl, Norfentanyl, Umbilical Cord, Neonatal Abstinence Syndrome, NAS, Newborn Toxicology, Prenatal Drug Exposure, Polysubstance Abuse

1. Introduction

Fentanyl, a Schedule II narcotic analgesic, is a very useful tool in pain management but unfortunately has a high abuse potential [1] [2] [3]. Fentanyl's potency is 80 times higher than morphine which contributes to its usefulness for analgesia and anesthesia but can be problematic when used outside the supervision of a trained medical professional. Fentanyl has been used successfully for the management of pain during labor and delivery without negative implications for the neonate [4]. However, prolonged prenatal exposure may lead to a number of negative health consequences including Neonatal Abstinence Syndrome (NAS) [5] or Neonatal Opioid Withdrawal Syndrome (NOWS) [6], a more specific term to describe neonatal withdrawal symptoms associated with opioids.

Indicators of drug exposure can develop as soon as 1 to 3 days after delivery but in some cases, it may take up to a week after birth for a newborn to show any symptoms [7]. Short term symptoms can include excessive crying, fever, vomiting, tremors, seizures, and slow weight gain [7]. Hirai, Ko, Owens, Stocks, & Patrick [8] reported an 80% increase in the rate of NAS in the US between 2010 and 2017. Looking specifically at opioids, Hirai *et al.* [8] stated that Maternal Opioid-related diagnoses more than doubled from 3.5 (95% CI, 3.0 - 4.1) to 8.2 (95% CI, 7.7 - 8.7) per 1000 births.

Umbilical cord (UC) presents a unique opportunity for surveilling maternal substance use and potential confirmation of NAS or NOWS [9] [10] [11]. UC is available immediately following birth and, unlike other neonatal specimen types used for newborn toxicology, each birth produces enough specimen for analysis. UC analysis can identify maternal substance use during approximately the third trimester of pregnancy [11].

The specific aims of this retrospective study are twofold. First, we will report the prevalence of neonatal exposure to fentanyl for a nationwide high-risk population using UC tissue segments submitted to a national reference laboratory for routine forensic toxicology analysis. Second, we will report the prenatal drug exposure patterns observed for these fentanyl-exposed neonates.

2. Experimental Section

Materials and Methods

Reagent water utilized for this procedure used an in-line de-ionization system

manufactured by EVOQUA (Broadview, IL, USA). All reagents and solvents used were purchased from Fisher Scientific (Pittsburg, PA, USA) ACS grade, HPLC grade or equivalent. Fentanyl, fentanyl-d_5, norfentanyl, and norfentanyl-d_5 were purchased from Cerilliant (Round Rock, TX, USA). All stock solutions for standards were prepared in-house with the addition of methanol. Fentanyl Direct Elisa Kits were purchased from Immunalysis Corporation (Pomona, CA, USA). Certified Negative UC (CNC) were selected from negative specimen pools.

Initial testing was performed using enzyme-linked immunosorbent assay (ELISA) and confirmation of presumptive positives were analyzed using a previously published method [12]. Briefly, UC aliquots (0.5 g) were homogenized in a 50 mL screw topped tube using a Bullet Blender® (Next Advance, Averill Park, NY, USA) in 3 mL of acetone and 3 stainless steel wood screws. Homogenates were decanted into a 10 mL reservoir fitted with a 10-micron frit and the filtrate was evaporated under a stream of nitrogen at 60˚C. The residues were reconstituted in 700 mL of buffer that was provided by the immunoassay manufacturer and analyzed following manufacture instructions (Fentanyl ELISA kit, Immunalysis Corporation, Pomona, CA). Specimens that demonstrated a response equal to or greater than a 500 pg/g cutoff calibrator were considered presumptive positive.

Presumptive positive UC specimens were confirmed using a second aliquot (1.0 g) of UC and analyzed using a previously published LCMSMS assay [12]. Aliquots were homogenized as described above. Extracts were purified using solid phase extraction (SPE; Clean Screen Extraction Columns, CSDAU020, 10 mL reservoir, 200 mg bed, United Chemical Technologies, Bristol, PA). The cartridges were conditioned with 3 mL Methanol, 3 mL DI H_2O and 3 mL 0.1 Phosphate Buffer (pH 6.0) prior to loading the extracts. The SPE cartridges were rinsed with 3 mL DI Water, 3 mL 0.1 M HCl and 1 mL Hexane followed by elution with freshly prepared Methylene Chloride/Isopropanol/Ammonium Hydroxide (80:20:2). Eluates were evaporated under a stream of nitrogen at 60˚C and reconstituted with mobile phase A (DI Water with 6% Acetonitrile/0.1% Formic Acid).

Separation was accomplished on an Agilent 1200 HPLC system using 50 × 2.0 mm Synergi Polar-RP column with 2.0 mm particle size (Phenomenex, Torrance, CA, USA). Mobile phase B (acetonitrile with 0.1% formic acid) was set to 15% for 1 minute and was then increased to 60% at 6.5 minutes. At 6.6 minutes, mobile phase B was returned to 15% at 11 minutes. The column compartment was held at 50˚C.

Detection of analytes was achieved using a Sciex 5500 Tandem Mass Spectrometer with an electrospray ionization source in the positive ionization mode. Select mass spectrometer settings are listed in **Table 1**. Specimens that demonstrated a response for fentanyl and/or norfentanyl greater than the response of a 500 pg/g cutoff calibrator were considered positive.

Table 1. Selected mass spectrometer settings.

Mass Spectrometer Parameters					
Analyte	**Precursor Ion (*m/z*)**	**Product Ion (*m/z*)**	**Declustering Potential (V)**	**Collision Energy (V)**	**Cell Exit Potential (V)**
Norfentanyl-d_5	238.1	84.0*	41	25	8
Norfentanyl	233.1	84.0*	16	23	8
		150.1	16	25	14
Fentanyl-d_5	342.2	188.2*	16	29	18
Fentanyl	337.15	187.9*	1	29	19
		104.9	1	51	16

*Quantitation transition.

This secondary analysis was performed using de-identified historical data for UC received between January 1st 2020 and December 31st 2020 for routine forensic analysis at a national reference laboratory (United States Drug Testing Laboratories, Des Plaines, IL, USA). UC were obtained for cases that fit local hospital testing criteria and collected following established procedures (https://www.usdtl.com/). Specimens were refrigerated following collection and shipped ambient overnight to the laboratory. Once received at the laboratory, specimens were tested same day, and stored refrigerated until testing was complete.

Secondary analysis of de-identified results did not require institutional review board approval. The positivity rates, medians, and interquartile ranges were calculated using Excel. Additionally, polysubstance use patterns were evaluated.

3. Results

During the study period, our laboratory received 23,104 UC for analysis that included fentanyl and 9667 (41.8%) of those UC were positive for at least one drug. The prevalence of fentanyl for this group of UC specimens was 1.9% (n = 429). Of these 429 specimens, there were 407 UC where both fentanyl and norfentanyl were detected. There were 14 UC where only fentanyl was positive and 8 UC where only norfentanyl was positive. When positive, the median concentrations of fentanyl and norfentanyl were 4029 pg/g (IQR: 1696, 9230 pg/g) and 10,756 pg/mg (IQR: 3925, 25,288 pg/g), respectively (**Figure 1**). The measured concentrations of fentanyl and norfentanyl were strongly associated (r = 0.758, **Figure 2**).

Of the 429 positive fentanyl and/or norfentanyl UC, 33 (7.7%) were only positive for fentanyl and/or norfentanyl. Of the 396 polypositive UC, morphine was the highest co-exposure with 243 UC (56.6%) being positive for both fentanyls and morphine. The second most prevalent co-exposure observed was methamphetamine/amphetamine (n = 173; 40.3%) followed by cannabinoids (n = 113; 26.3%) and benzoylecgonine (cocaine metabolite; n = 106; 24.7%). We did not

observe any specimens that were positive for fentanyls and amphetamine, while negative for methamphetamine. These and the remainder of the observed co-exposure rates are depicted in Figure 3.

4. Discussion

This study has reported the prevalence of prenatal fentanyl exposure using UC from a nationwide high-risk population. While this was a convenience sampling and not a rigorously designed epidemiological study, a 1.9% positive rate for prenatal fentanyl exposure in this population is alarming and warrants further study. While not as prevalent as prenatal marijuana exposure during the same time period (26%; unpublished USDTL data), a 1.9% positivity rate parallels cocaine (1.9%), methadone (1.5%) and oxycodone (0.9%).

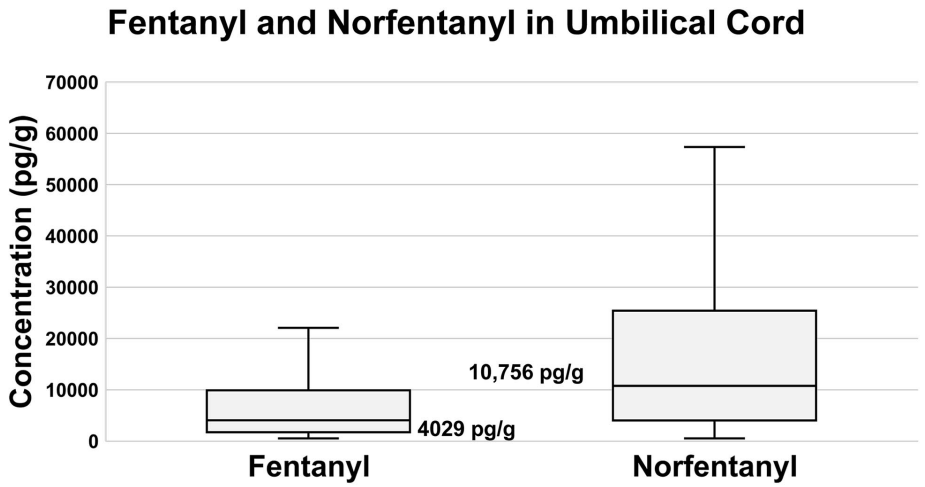

Figure 1. Box and whisker plot of the observed concentrations of fentanyl and norfentanyl in UC between January 01, 2021 and December 31, 2021.

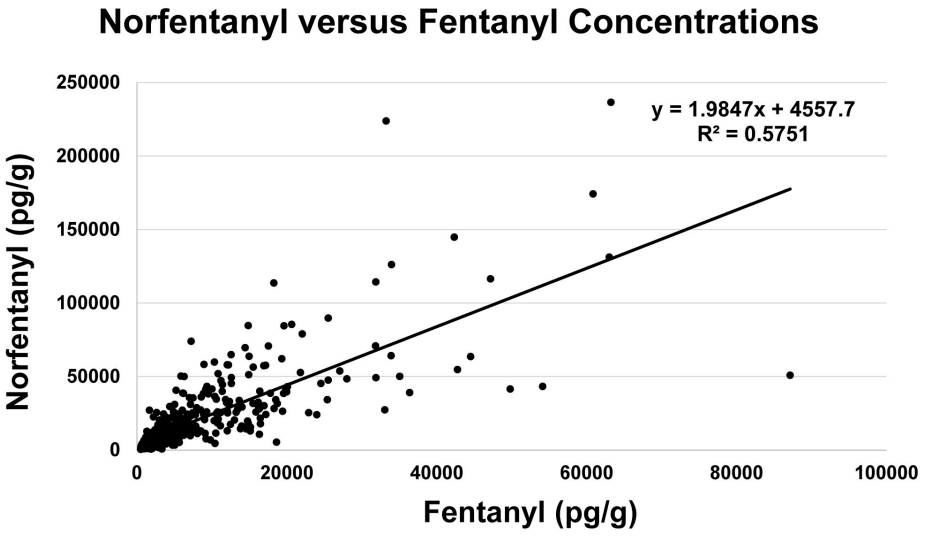

Figure 2. Comparison of fentanyl and norfentanyl concentrations in UC between January 01, 2021 and December 31, 2021.

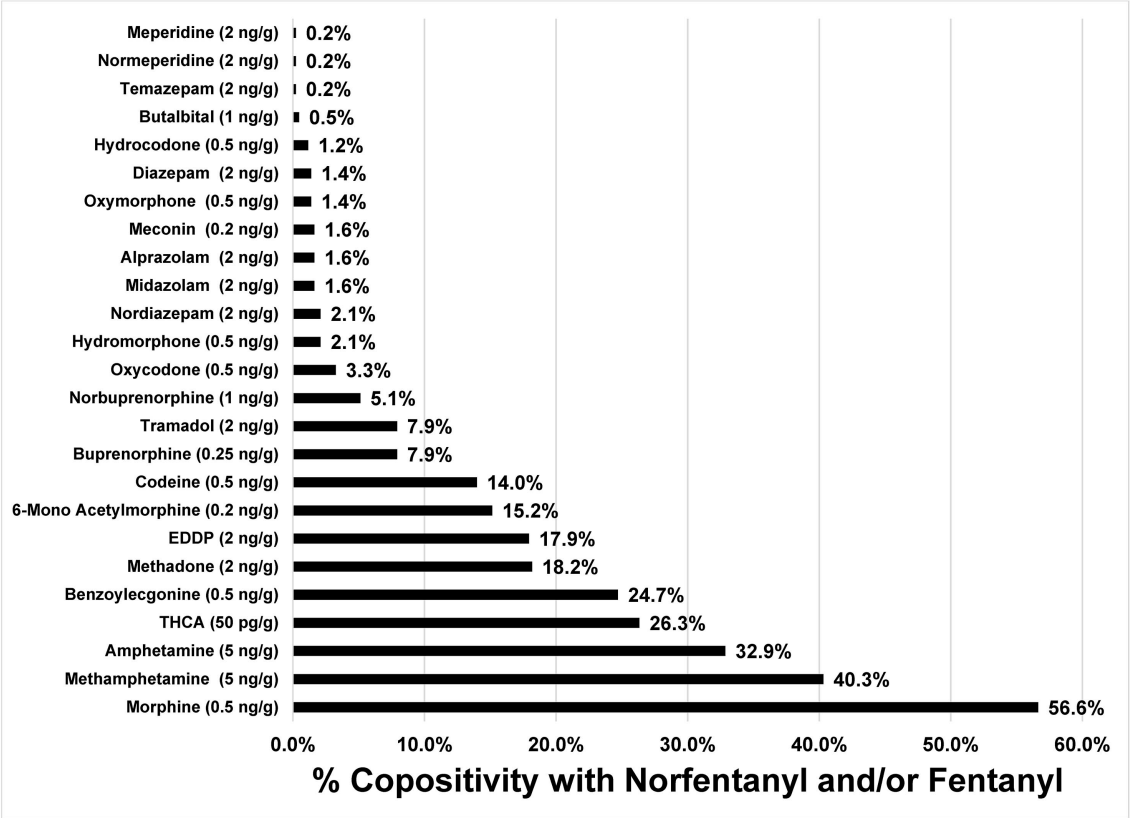

Figure 3. Co-positivity rates with fentanyl in UC between January 01, 2021 and December 31, 2021.

Only 7.7% of the positive fentanyl specimens were positive for only fentanyl. Considering that fentanyl is a popular additive to many street drugs this is not entirely unexpected. The co-exposure rates determined by the analysis of UC do not mirror the findings of the National Forensic Laboratory Information System (NFLIS). The NFLIS gathers drug seizure and laboratory data around the country on behalf of the DEA. The data presented by NFLIS show that between 2014 and 2019, fentanyl is primarily reported by itself followed by co-reporting with heroin, cocaine, and methamphetamine, respectively.

The literature contains many examples of fentanyl measurements in UC blood, plasma, and serum [13] [14] [15]. Limited examples exist where fentanyl in UC was reported in the literature. Marin *et al.* [11] reported the detection of fentanyl in 3 UC specimens with a qualitative liquid chromatography time-of-flight method using a 1000 pg/g cutoff. They reported 3 positive UC and at least one of the positives was most likely due to administration of fentanyl for a procedure on the prior day. Due to the qualitative nature of the assay, concentrations were not reported. A subsequent paper [16] using the same method and laboratory, reported 66 (6.4%) fentanyl positive results (qualitative) where all 66 results were explained by hospital administration of fentanyl during labor and delivery.

The most recent report of the detection of fentanyl in UC compared maternal medical records (n = 62) to the UC results obtained from a laboratory that used a screen (ELISA; cutoff 500 pg/g) and reflex to quantitative confirmation

(LCMSMS; cutoff 500 pg/g) approach [12]. This study reported that none of the mothers with a record of receiving fentanyl during labor and delivery (n = 37) were positive at the 500 pg/g cutoff. This was not consistent with the findings of the previous two reports. Jones *et al.* [12] did report a case that was positive fentanyl and norfentanyl with observed concentrations of 6469 pg/g and 5241 pg/g, respectively. This single case was also positive for methamphetamine, heroin, and nicotine metabolite.

There were a few strengths of this study that warrant pointing out. One strength for this study was the use of a carefully selected screen and confirm strategy using a highly sensitive immunoassay initial test with confirmation of presumptive positives using a highly specific LCMSMS method. Another strength of this study was the large sample volume from a nationwide high-risk population. This population was considered high risk because the selection criteria at the hospitals are designed to initiate testing when there is reasonable suspicion of prenatal drug exposure.

There were several limitations of the study that restrict the generalizability of our findings. One limitation was the lack of access to the medical records as these historical results were de-identified. The specific selection criteria used by the hospitals were blind to the laboratory, as well. Another limitation is the inability to determine if the co-exposures were from simultaneous use. Lastly, the window of detection for drugs in UC has not been definitively determined primarily due to the ethical conflict of executing a prospective random controlled study of prenatal drug exposure. A future direction of study could couple toxicological finding with a detailed analysis of maternal medical records, maternal survey of substance abuse, and prospectively monitoring these children to evaluate additional long-term negative health consequences related to drug co-exposure.

5. Conclusion

Fentanyl misuse and abuse is an ongoing public health concern in this country and our data demonstrates that the maternal-child health environment is not immune to this concern. Fentanyl is observed as frequently in newborn toxicology specimens as other problematic substances of abuse and when fentanyl is detected other substances are generally detected as well. Further study is warranted to investigate potential long term negative health consequences for unsupervised prenatal fentanyl exposure.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] United States Drug Enforcement Agency DEA (2022) Facts about Fentanyl. https://www.dea.gov/resources/facts-about-fentanyl#:~:text=Fentanyl%20is%20a%20Schedule%20II,for%20potential%20misuse%20or%20abuse

[2] European Monitoring Centre for Drugs and Drug Addiction EMCDDA (2011, September). https://www.emcdda.europa.eu/publications/drug-profiles/fentanyl_en#pharmacology

[3] National Institute of Drug Abuse NIDA (2016) Drug Facts: What Is Fentanyl? https://www.drugabuse.gov/publications/drugfacts/fentanyl#ref

[4] Shum, S., Shen, D.D. and Isoherranen, N. (2021) Predicting Maternal-Fetal Disposition of Fentanyl Following Intravenous and Epidural Administration Using Physiologically Based Pharmacokinetic Modeling. *Drug Metabolism and Disposition*, **49**, 1003-1015. https://doi.org/10.1124/dmd.121.000612

[5] Finnegan, L, Connaughton, J., Kron, R. and Emich, J. (1975) Neonatal Abstinence Syndrome: Assessment and Management. *Addictive Diseases*, **2**, 141-158.

[6] Sutter, M.B., Leeman, L. and Hsi, A. (2014) Neonatal Opioid Withdrawal Syndrome. *Obstetrics and Gynecology Clinics*, **41**, 317-334. https://doi.org/10.1016/j.ogc.2014.02.010

[7] United States National Library of Medicine (2021, October 8) Neonatal Abstinence Syndrome: Medlineplus Medical Encyclopedia. MedlinePlus. https://medlineplus.gov/ency/article/007313.htm

[8] Hirai, A.H., Ko, J.Y., Owens, P.L., Stocks, C. and Patrick, S.W. (2021) Neonatal Abstinence Syndrome and Maternal Opioid-Related Diagnoses in the US, 2010-2017. *Journal of the American Medical Association*, **325**, 146-155. https://doi.org/10.1001/jama.2020.24991

[9] Montgomery, D., Plate, C., Alder, S.C., Jones, M., Jones, J. and Christensen, R.D. (2006) Testing for Fetal Exposure to Illicit Drugs Using Umbilical Cord Tissue vs Meconium. *Journal of Perinatology*, **26**, 11-14. https://doi.org/10.1038/sj.jp.7211416

[10] Jones, J., Jones, M., Jones, B., Sulaiman, K., Plate, C. and Lewis, D. (2015) Detection of Codeine, Morphine, 6-Monoacetylmorphine, and Meconin in Human Umbilical Cord Tissue: Method Validation and Evidence of *in Utero* Heroin Exposure. *Therapeutic Drug Monitoring*, **37**, 45-52. https://doi.org/10.1097/FTD.0000000000000104

[11] Marin, S., Metcalf, A., Krasowski, M., Linert, B., Clark, C., Strathmann, F. and McMillin, G. (2014) Detection of Neonatal Drug Exposure Using Umbilical Cord Tissue and Liquid Chromatography Time-of-Flight Mass Spectrometry. *Therapeutic Drug Monitoring*, **36**, 119-124. https://doi.org/10.1097/FTD.0b013e3182a0d18c

[12] Jones, J., Coy, D., Mitacek, R., Thompson, S. and Maxwell, S. (2020) Neonatal Cord Tox Panel and Maternal Perinatal Fentanyl Exposure: A Retrospective Chart Review. *American Journal of Analytical Chemistry*, **12**, 324-331. https://doi.org/10.4236/ajac.2021.129020

[13] Desprats, R., Dumas, J.C., Giroux, M., Campistron, G., Faure, F., Teixeira, M.G., Pontonnier, G., *et al.* (1991) Maternal and Umbilical Cord Concentrations of Fentanyl after Epidural Analgesia for Cesarean Section. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, **42**, 89-94. https://doi.org/10.1016/0028-2243(91)90167-J

[14] Fleet, J.A., Belan, I., Gordon, A.L. and Cyna, A.M. (2020) Fentanyl Concentration in

Maternal and Umbilical Cord Plasma Following Intranasal or Subcutaneous Administration in Labour. *International Journal of Obstetric Anesthesia*, **42**, 34-38. https://doi.org/10.1016/j.ijoa.2020.01.001

[15] Tran, K., Maxwell, L., Cohen, D., Adamson, P., Moll, V., Kurth, C. and Galinkin, J. (2012) Quantification of Serum Fentanyl Concentrations from Umbilical Cord Blood during *ex Utero* Intrapartum Therapy. *Anesthesia & Analgesia*, **114**, 1265-1267. https://doi.org/10.1213/ANE.0b013e3182378d21

[16] Palmer, K.L., Wood, K.E. and Krasowski, M.D. (2017) Evaluating a Switch from Meconium to Umbilical Cord Tissue for Newborn Drug Testing: A Retrospective Study at an Academic Medical Center. *Clinical Biochemistry*, **50**, 255-261. https://doi.org/10.1016/j.clinbiochem.2016.11.026