K56 Detection of *In Utero* Ethanol Exposure Via Ethyl Glucuronide and Ethyl Sulfate Analysis in Placenta

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Learning Overview: This presentation will demonstrate to attendees the value of utilizing placenta as an alternative matrix to meconium for the determination of *in utero* ethanol exposure, and the extent to which the two matrices can be compared.

Impact on the Forensic Science Community: This research will impact the forensic science community by providing a sensitive and specific method employing an alternative matrix to improve the interpretation of *in utero* ethanol exposure that will help toxicologists, legal personnel, and clinicians understand the newborns' outcomes and the medical and legal consequences of this exposure.

According to the 2016 National Survey on Drug Use and Health, ethanol was the second most prevalent substance (8.3%) after tobacco (10%) among pregnant women in the United States, with the ethanol prevalence being higher among 26- to 44-year-old pregnant women than in the 18- to 25-year-old group (9.1% vs. 6.5%). Alcohol consumption during pregnancy may happen due to preconceived notions among the population that a minor amount of alcohol during pregnancy can be harmless. However, there is no amount of ethanol deemed safe for consumption during pregnancy. Alcohol exposure during pregnancy constitutes one of the leading preventable causes of birth defects, mental retardation, and neurodevelopmental disorders in exposed children. Objective analytical methods are necessary to monitor this exposure.

A method for the determination of *in utero* ethanol exposure utilizing the direct minor metabolites of ethanol, Ethyl Glucuronide (EtG), and Ethyl Sulfate (EtS), has been developed, validated, and applied to authentic placenta samples from newborns whose meconium tested positive for EtG (>5ng/g). A 0.1g of placenta was mechanically homogenized in methanol and extracted using weak anion exchange solid phase cartridges and analyzed via Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Chromatographic separation was performed by Kinetex® Polar C18 column using a gradient elution of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Each compound was quantified and confirmed using its respective internal standard and two transitions in Multiple Reaction Monitoring (MRM) mode. Validation results demonstrated a linear range of 10ng/g–500ng/g with a limit of quantification of 10ng/g for both EtG and EtS, with residuals within ±20% and a coefficient of determination greater than 0.99. Imprecision was maintained below 12%, and accuracy was within 83.1%–112.4%. Matrix effects (-69.9%– -15.2%), extraction efficiency (75%–92.7%), process efficiency (22.5%–76.3%), and interferences and autosampler stability at 10˚C were also assessed during validation.

The validated method was applied to 59 authentic placenta samples. EtG and/or EtS was detected in 8 out of 59 samples with ranges of 26.5ng/g–266.5ng/g and 11ng/g–24.3ng/g, respectively. Results from placenta were compared to different EtG meconium cutoffs from the literature in paired samples to establish relative rates of agreement between the two matrices. The highest agreement of 50% was achieved at EtG cutoff of 444ng/g in meconium, and the lowest agreement of 13.6% at EtG analytical cutoff of 5ng/g in meconium.

A sensitive and specific method for the determination of EtG and EtS in placenta was developed. Comparing meconium and placenta, meconium showed a higher sensitivity, but placenta could be employed as alternative sample if meconium is not available. To date, this is the first study to compare placenta and meconium to detect ethanol exposure during pregnancy.

Ethanol, Ethyl Glucuronide, Ethyl Sulfate