



K34 Alcohol Exposure in Neonates

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Attendees will learn about the analysis of biomarkers in meconium for detection of fetal alcohol exposure.

Fetal alcohol syndrome (FAS) is a devastating disorder in the newborn, resulting from heavy maternal alcohol consumption during pregnancy and is the leading cause of non-hereditary mental retardation in the neonate. Estimates of the prevalence of FAS range from 0.5 to 3 per 1,000 live births in most populations. Children with significant prenatal alcohol exposure do not always exhibit the characteristic facial abnormalities associated with FAS, but still have mental impairments just as serious. Alcohol related neurodevelopmental disorder (ARND) and alcohol-related birth defects (ARBD) describe these conditions which are estimated to affect 3-4 times as many babies as FAS. The diagnoses of ARND and ARBD require confirmation of the mother's alcohol use during pregnancy in addition to psychological or neurological assessment of the child. Self-reported maternal history of alcohol use can be helpful in diagnosis, but a laboratory test may provide the physician with critical information, especially when an accurate maternal self-report is missing. Fetal exposure to alcohol can also cause CNS dysfunction, post-natal growth problems, cardiac defects and attention deficit disorders in the neonate. To date, diagnosis of fetal alcohol effect depends largely on maternal interview, although clinical tests are becoming more widely used.

Fatty acid ethyl esters (FAEE) are formed in the body, by esterification of ethanol with free fatty acids and trans-esterification of glycerides; and have been detected in the meconium of newborns.

This paper estimates the prevalence of fetal alcohol exposure in two populations by detecting fatty acid ethyl esters in meconium. The prevalence of FAEE's in the meconium from two separate groups of neonates using solid-phase extraction and analysis by gas chromatography-mass spectrometry in chemical ionization mode is presented.

Methods: *Extraction:* Fatty acid ethyl esters are sensitive to heat and light, and therefore, it is recommended that meconium specimens be immediately stored in amber or opaque containers upon collection, be shipped on ice and be stored frozen (-20°C). Meconium (0.5 - 1g) was allowed to thaw, and was homogenized in organic solvent. The extract was centrifuged and the supernatant passed through a solid-phase extraction cartridge. The fatty acid ethyl esters were eluted from the column, and evaporated to dryness under nitrogen at 37°C. The dried extract was reconstituted in hexane and analyzed using full scan chemical ionization GC/MS, with acetone as the reagent gas.

Analysis: A Varian Star 3400 bench top GC coupled to a Saturn II ion trap mass spectrometer was operated in the full scan positive chemical ionization mode. The GC column was a bonded phase fused silica (0.25 mm ID; 0.25 mm film thickness; 30 m length). The injector was operated at 250°C in splitless mode and the injection volume was 3 mL. The oven was programmed to 310°C and the reagent gas was acetone. Chemical ionization (CI) was chosen for this analysis, because electron impact ionization of these compounds yields identical fragments for the various FAEE's. In CI mode, a diagnostic ion for each compound is obtained.

Results: In the first study, seventy-three (16.7%) of the meconium specimens tested (n = 436) were considered to be positive for FAEE's. When broken down into quartiles, the mean values of total FAEE's measured were 1059 ng/g; 3133 ng/g; 6628 ng/g and 62115 ng/g. In the second study, thirty-five (11.9%) of the specimens (n = 292) were considered positive. When broken into quartiles, the mean values were 1139 ng/g; 3067 ng/g; 7674 ng/g and 50,143 ng/g. The overall FAEE profiles of the two study sets were remarkably similar.

Summary: When the total FAEE concentration is greater than 10,000 ng/g, in an adequate meconium specimen, it is likely that the newborn has been exposed to significant amounts of alcohol during pregnancy.

Meconium, Fatty Acid Ethyl Esters, Fetal Exposure to Alcohol