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A New Second Tier Screening for Congenital Adrenal Hyperplasia Utilizing One Dried Blood Spot

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Abstract : Newborn screening for Congenital Adrenal Hyperplasia (CAH) relies on quantification of 17α-hydroxyprogesterone using enzyme immunoassays. These assays, in spite of being rapid, readily available and easy to perform, its reliability was found questionable due to lack of selectivity and specificity resulting in large number of false-positives, consequently family anxiety and associated hospitalization costs. To improve specificity of conventional 17α-hydroxyprogesterone screening which may experience false transient elevation in preterm, low birth weight or acutely ill neonates, steroid profiling by LC-MS/MS as a second-tier test was implemented. Unlike the previously applied LC-MS/MS methods, with the disadvantage of requiring a relatively high number of blood drops. Since newborn screening tests are increasing, it is necessary to minimize the sample volume requirement to make the maximum use of blood samples collected on filter paper. The proposed new method requires just one 3.2 mm dried blood spot (DBS) punch. Extraction was done using methanol: water: formic acid (90:10:0.1, v/v/v) containing deuterium labelled internal standards. Extracts were evaporated and reconstituted in 10 % acetone in water. Column switching strategy for on-line sample clean-up was applied to improve the chromatographic run. The first separative step retained the investigated steroids and passed through the majority of high molecular weight impurities. After the valve switching, the investigated steroids are back flushed from the POROS® column onto the analytical column and separated using gradient elution. Found quantitation limits were 5, 10 and 50 nmol/L for 17α-hydroxyprogesterone, androstenedione and cortisol respectively with mean recoveries of between 98.31-103.24 % and intra-/ inter-assay CV% < 10 % except at LLOQ. The method was validated using standard addition calibration and isotope dilution strategies. Reference ranges were determined by analysing samples from 896 infants of various ages at the time of sample collection. The method was also applied on patients with confirmed CAH. Our method represents an attractive combination of low sample volume requirement, minimal sample preparation time without derivatization and quick chromatography (5 min). The three steroid profile and the concentration ratios (17OHP + androstenedione/cortisol) allowed better screening outcomes of CAH reducing false positives, associated costs and anxiety.

Keywords : congenital adrenal hyperplasia (CAH), 17α-hydroxyprogesterone, androstenedione, cortisol, LC-MS/MS
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