A Rapid and Simple 2nd-Tier LC-MS/MS Method for IVA and GA-I Newborn Screening

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Background: Newborn screening (NBS) for glutaric aciduria type I (GA-I) and isovaleric acid (IVA) have a high number of false positive results, due to different reasons. The marker metabolite for GA-I in NBS is glutarylcarnitine (C5DC), which cannot be distinguished from the isobaric hydroxyhexanoylcarnitine (C6-OH) with flow injection methods used. The same is true for IVA NBS with isovalerylcarnitine (C5) being the marker metabolite, which also cannot be distinguished from methylbutyrylcarnitine, valerylcarnitine, and pivaloylcarnitine.

Methods: Amino acids and acylcarnitines are extracted from the dried, and measured using the Chromsystems NBS test kit. In case of an elevated concentration for C5DC or C5, the residual extract from the NBS sample, is diluted 1:1 with flowsolvent A (acetonitrile with 0.1% formic acid). A blank, Chromsystems dried blood control samples, and recipe dried blood control samples are also diluted with flow solvent A (1:1). The analytes are then separated on reversed phase column (Phenomenex KINETEX 2,6 µm; C18; 100 Å; 100*3 mm), with a binary gradient (flow solvent B: water with 0.1% formic acid), using an Agilent 1260 Infinity binary HPLC pump, and AbSCIEX Triple Quad 5500.

Results: C5DC, C6OH, isovalerylcarnitine, methylbutyrylcarnitine, valerylcarnitine, and pivaloylcarnitine can be separated from each other, with a total runtime of 25 minutes. For the calculation of concentrations, the blank and the Chromsystems dried blood controls are used for the calibration curve, and the Recipe dried blood controls samples are used as controls.

Conclusion: We report a rapid and easy 2nd-tier method for GA-I and IVA newborn screening. Implementation of this 2nd-tier method in our laboratory has dramatically reduced the false positive rate.