

# Reduction of false positive results in newborn screening by second-tier testing using tandem Mass Spectrometry

K. Schöngruber, S. Baur, W. Röschinger, R. Arnecke, E. Schuhmann

Labor Becker, München

## Background-Aim

Newborn screening (NBS) is used for the early detection of certain genetic, endocrine, and metabolic disorders that lead to mental or physical disabilities and in some instances to death if treatment is initiated too late. The paediatric guidelines list 19 target diseases that must be included in the NBS, as so-called sensitive searching tests. Another 15 diseases are identified in a pilot project. Furthermore, second (2<sup>nd</sup>)-tier tests, as specific confirmatory tests, are added to the NBS to reduce false positive results. These are implemented to differentiate isobaric metabolites, which cannot be separated by Flow injection analysis-mass spectrometry (FIA-MS) and to verify elevated analyte levels.

## Method

The NBS by FIA-MS allows to rapidly analyze acylcarnitines and amino acids among others based on their specific mass to charge ratio and fragmentation pattern. Nevertheless, isobaric compounds such as leucine and isoleucine for example cannot be separated by FIA-MS. In case of a positive screening result a follow up test for confirmation or differentiation is carried out. Therefore, chromatographic separation via a HPLC column is performed to separate the key metabolites prior to analysis by tandem MS.

## Result

The 2<sup>nd</sup>-tier tests for MSUD (maple syrup urine disease), VLCAD (very long-chain acyl-CoA dehydrogenase)- / LCHAD (long-chain 3-hydroxyacyl-CoA dehydrogenase)-deficiency, and glutaric aciduria type 1 are carried out to confirm or rule out pathologic values. In addition, 2<sup>nd</sup>-tier tests are used to distinguish among two diseases e.g. between methylmalonic and propionic acidemia. Another reason for false positive screening results is the intake of the antibiotic pivmecillinam by the mother. Therefore, the 2<sup>nd</sup>-tier test separates pivaloylcarnitine (PC, derived from the antibiotic) and the metabolic product isovalerylcarnitine (IC), which both consist of five C atoms. In the case of elevated PC- and normal IC-levels, no action is required. However, if IC is increased, action must be taken quickly and the child must be treated at a metabolic clinic. Since November 2020, we are pleased to report that only about three percent of children with suspected IVA have actually been confirmed and for the other 2<sup>nd</sup>-tier tests the results look roughly similar. Encouragingly, our recall rate is just below 0.30% according to the screening report of 2019. In summary, all of these 2<sup>nd</sup>-tier tests are necessary to reduce false positive results and thereby to decrease our recall rates.

## Conclusion

If there is an inborn disorder the affected newborn must be treated as soon as possible in order to prevent mental and physical disabilities or even to avoid imminent death. The development of 2<sup>nd</sup>-tier tests helps to detect false indications of disorders and thus avoids an unnecessary burden for parents and their infants.