Multiplexed 2nd-tier strategies in newborn screening for propionic- and methylmalonic acidurias, homocystinuria and neonatal vitamin B_{12} deficiency

Joachim Janda, Jana Hauke, Friederike Hörster, Patrik Feyh, and Jürgen G. Okun

1 Introduction

To reduce false positive results, second-tier (2ndt) methods for homocystinuria, remethylation disorders or defects in the propionate-pathway have been introduced in newborn screening (NBS) laboratories for nearly two decades [1]. The respective physiological conditions are biochemically linked by the potential involvement of vitamin B_{12}, hence analytical results of such methods often are interpreted jointly in differential diagnostics. Therefore, methods capable to determine multiple specific biomarkers are favoured, although this can pose challenges in terms of analytical specificity and robustness.

2 Methods

A 2nd-tier method to analyse total homocysteine (HCY), propionylglycine (ProGly), methylmalonic (MMA) and methylcitric acids (MCA) in dried blood spots (DBS) based on LC-MS/MS has been developed and validated. Analytes are derivatised to butyl-esters after extraction in presence of corresponding isotopically labelled standards and separated in LC using a Kinetex EVO-C18 column and a 9-min-gradient before being detected with specific mass transitions. Each sample batch includes seven calibrators and two quality controls (QCs, high and low). Calibrations are controlled using external QCs.

3 Results

Factors identified in method development, that strongly influence reproducibility were the composition of extraction and injection solutions, pH during extraction, and the mode of derivatization. In instrumental analysis, aside from the LC gradient, the selection of mass transitions can improve specificity. Intra- and interday precision values ranged from -10.3% to 3.1% and from 4.0% to 11.1% respectively, with trueness ranging from -7.2% to 2.5% and from 4.7% to 11.0%, respectively. Preliminary cutoffs based on 205 normal control samples for HCY, MMA, MCA, and ProGly were 6.22, 1.4, 1.0, and 16.4 µmol/L, respectively. During five months routine application, 39,897 newborns were screened, of which 2,690 underwent 2nd-tier analysis. Of these, 90.8% were found to be without abnormalities, corresponding to a decrease of 96% of the false-positive rate.

4 Conclusion

2nd-tier methods to determine HCY, MMA, MCA in DBS have been shown to be useful in NBS to reduce false-positive numbers of suspected homocystinuria, remethylation disorders or defects in the propionate-pathway, hence reducing the recall-rate [2,3]. A corresponding method that additionally covers ProGly has been successfully developed, validated and applied in a high-throughput newborn screening setting. During a five-month period, potential false-positives were reduced by 96% compared to an absence of the 2nd-tier analysis.

5 References

[1] G. La Marca, S. Malvagia, E. Pasquini, M. Innocenti, M.A. Donati, E. Zammarchi, Rapid 2nd-tier test for measurement of 3-OH-propionic and methylmalonic acids on dried blood spots: reducing the false-positive rate for propionylcarnitine during expanded newborn screening by liquid chromatography-tandem mass spectrometry, Clin Chem 53 (2007) 1364–1369. https://doi.org/10.1373/clinchem.2007.087775.

[2] J.A. Dubland, B. Rakić, H. Vallance, G. Sinclair, Analysis of 2-methylcitric acid, methylmalonic acid, and total homocysteine in dried blood spots by LC-MS/MS for application in the newborn screening laboratory: A dual derivatization approach, J Mass Spectrom Adv Clin Lab 20 (2021) 1–10. https://doi.org/10.1016/j.jmsacl.2021.03.001.

[3] C.T. Turgeon, M.J. Magera, C.D. Cuthbert, P.R. Loken, D.K. Gavrilov, S. Tortorelli, K.M. Raymond, D. Oglesbee, P. Rinaldo, D. Matern, Determination of Total Homocysteine, Methylmalonic Acid, and 2-Methylcitric Acid in Dried Blood Spots by Tandem Mass Spectrometry, Clin Chem 56 (2010) 1686–1695. https://doi.org/10.1373/clinchem.2010.148957.