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OVERVIEW OF METHODS FOR DETERMINATION OF ACYLCARNITINES IN SERUM OR PLASMA

This document summarizes a number of diagnostic tests commonly used to analyse acylcarnitines (AC) in serum or plasma to the diagnosis of inborn errors of metabolism.

Detailed protocols are not included here; these can be found in the literature references provided.

Acylcarnitine analysis has proven a useful tool in the evaluation of patients at risk for inborn errors of fatty acid oxidation and for organic acidemias that are primarily due to defects in branched-chain amino acid metabolism (Matern 2014). Given the diverse clinical presentation of these conditions, AC analysis has become an integral part of the biochemical genetic laboratory investigation of a large number of patients (Carpenter and Wiley 2002), (Millington, Norwood et al. 1989), (Vreken, van Lint et al. 1999).

Analysis in serum or plasma allows for using the same sample taken for other determinations that contribute to the diagnosis or monitoring of metabolic diseases (Van Hove, Kahler et al. 2000), (Delolme, Vianey-Saban et al. 1997).

AC analysis is almost exclusively performed by tandem mass spectrometry (MS/MS) using stable isotope labeled internal standards that allow quantitation of AC species (Matern 2008). This technique has been displacing others in the last years, like GC/MS (Lowes, Rose et al. 1992), (Jones and Chalmers 1995), (Costa, Struys et al. 1997).

Basically, all the methods include an extraction from the sample by mixing with methanol or acetonitrile to precipitate and separate the proteins (Liu and Pasquali 2005), and the addition of isotopically labeled AC to be used as internal standards. Following steps vary depending on the possibility of derivatization, chromatographic separation and methods to quantify (Ruiz-Sala, Ruijter et al. 2016).

The improvement of sensitivity of the newest mass spectrometers has allowed the analysis of the free AC without derivatization (Ghoshal, Guo et al. 2005).

Derivatization as butyl esters was used to gain sensitivity in the first mass spectrometers, to ensure the positive charge of the AC. Also the use of methyl esters has been reported; however, the identification of the analytes could be more difficult.

Flow injection analysis (FIA) is the more commonly use procedure to start the analysis, since the run times are shortened and also simplify the quantification of the AC. However, this kind of injection does not consider the separation of isomers, that sometimes is essential for diagnosis, avoiding more diagnostic tests (Abdenur, Chamoles et al. 1998), (Silva, Selhorst et al. 2001). The insertion of a liquid chromatographic column solves this problem (Ferrer, Ruiz-Sala et al. 2007), (Gaskell, Guenat et al. 1986).

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Tandem mass spectrometry acquisition modes are mainly two: Precursor ion scanning of *m/z* 85, which detects all compounds with this fragment, or MRM (multiple reaction monitoring) to improve sensitivity of a selected number of AC. Also precursor ion of *m/z* 99 is used (Millington and Stevens).

Finally, different quantification methods can be employed. Software used in newborn screening is also applicable in FIA, it is fast and simple (Rashed, Ozand et al. 1995). The introduction of calibration curves improves quantitative parameters as accuracy or linearity of response (Minkler, Stoll et al.).

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