

Title: Automated 2nd-Tier Testing for Amino Acids and Acylcarnitines in Newborn Screening

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Introduction: Newborn Screening for amino acids and acyl carnitines includes several analytes that are not specific with the flow injection method that is used as 1st-tier testing. In order to eliminate the interference of isobaric substances a 2nd-tier method with a repeat punching and extraction is necessary. We report an automated method, where the 2nd-tier method can be performed using the primary extraction.

Methods: 1st-Tier measurement of amino acids and acyl carnitines was performed with the Chromsystems Masschrom Kit and the Labsystems NeoMass AAAC Plus with flow injection. After the evaluation of each microtiter plate, positive samples for isovalerylcarnitine and glutarylcarnitine remeasured from the same plate, after a switch to an LC-MS/MS protocol. An aliquot of the samples were taken with the injector needle, mixed with flow solvent A, from a separate vial, mixed within the needle and then supplied to the respective column.

Instrumentation: UPLC: Waters Acquity I-Class Premier* equipped with Column Manager and Sample Manager FTN (Flow through needle). Mass Spectrometer: Waters Xevo TQ-S Micro. Column: Waters Cortecs T3 C18 2.7um

Results: Isovalerylcarnitine, 2-methyl-butyrylcarnitine, and pivaloylcarnitine could be separated from each other, as well as glutarylcarnitine and hydroxy-hexanoylcarnitine. Since we use additional control samples from recipe, the kit-controls and a blank are used as calibrators for the 2nd-tier method, and the recipe controls as controls, we are able to also quantify the concentrations without any additional punching and extraction. The total runtime of this method is 3 minutes.

Conclusions: We could prove that with this configuration 2nd-tier methods can be applied without any additional hands-on time. The only manual procedure at the moment is to copy the positive samples from the Ionlynx output into the target lynx sample list. However, the programming of an automation for this procedure is already in progress.