Pilot experience of an External Quality Assurance for Acylcarnitines in plasma/serum

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ERNDIM (www.erndim.org) and CDC (www.cdc.gov) offer an external quality assurance (EQA) scheme for qualitative and quantitative acylcarnitines (ACC) in dried blood spots, respectively. However, ACC are usually determined in plasma/serum samples in some biochemistry genetics laboratories for diagnosis and/or treatment monitoring of organic acidurias or fatty acid oxidation (FAO) defects. A pilot interlaboratory comparison experience between eleven European laboratories was initiated in 2012. The aim of this experience was to improve the analytical performance and interpretation of acylcarnitines in plasma/serum samples.

**Samples**

Serum/plasma samples from the same individual with a confirmed diagnosis were pooled as received from diagnosis or treatment monitoring. Samples were kept frozen and were used after confirming the presence of marker acylcarnitines. Sixteen different samples (60 µL) with a short case report were circulated at room temperature by regular mail from Madrid or Copenhagen. Samples were received in 2-7 days.

**ACC analytical methods**

Participants were asked to precise the method used to quantify diagnostic ACC, to give reference values, possible diagnosis and advice for further investigations before three weeks. All labs used a M5/MS equipment. Five of eleven labs analyzed the ACC as butyalted derivatives and 6/11 undervetidated; 7/11 used the precursor ion acquisition mode and 4/11 multiple reaction monitoring (MRM); and only 3/11 separated the short-chain isomers.

**Results**

Reference values and pathological concentrations of ACC varied among laboratories. Figure 1 shows pathological and reference values for C16 and C18:1, marker ACC for CPTII or CACT deficiency, as an example of the variation of concentrations between the participants. The increases of C3; C5; C8; C10:1; C10: C16; C18:1; C16:0H; C18:1OH, C5-C8 were correctly identified by all labs, allowing the diagnosis of Propionic Acidemia; Isovaleric Acidemia; MCD and MADD by all participants (Table 1).

Despite a good identification of [C16OH; C18:1OH] and [C16; C18:1], markers for LCHAD/MTD defects and CPTII/CACT defects, respectively, some labs suggested only one disease.

However, the increases of dicarboxylic acylcarnitines (C3DC; C4DC, C5DC, C6DC) were not always identified. Diagnosis was not correct as exemplified in cases of malonic aciduria or HMG-CoA Lysase defect. Misinterpretation occurred in those labs that did not derivatize, separate isomers or use MRM acquisition. However some of these labs suggested further analyses to achieve the diagnosis.

Figure 2 shows an example of separation of isomers in an IBDH deficiency and a combined defect of SCAD and IVA.

### Table 2. External Quality Assurance for Acylcarnitines (ACC) in serum/serum plasma (pilot experience)

<table>
<thead>
<tr>
<th>Marker ACC</th>
<th>Identification</th>
<th>Correct Interpretation</th>
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<tbody>
<tr>
<td>C5OH/C4DC</td>
<td>2/9</td>
<td>SCAD+IVA</td>
</tr>
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**References**