## Services Offered

1) Quantitative Acylcarnitines by HPLC-MS including Total Carnitine, Free Carnitine, and Butyrobetaine

2) Carnitine Alone (Total, Free, Butyrobetaine)

Sample Types (minimum amounts):
- Plasma (50 µl)
- Skeletal Muscle (5 mg)
- Urine (350 µl)

## Acylcarnitine Analysis

<table>
<thead>
<tr>
<th>Separation of Isomers</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesized Standards</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Accurate Quantitation</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>

## Team Members
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- Maria Stoll
- Paul Minkler
- Steve Ingalls

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### Carnitine and Acylcarnitine Analysis using HPLC-MS/MS

**Plasma and Urine from Patient containing Pivaloylcarnitine**

The concentration in urine is 5.63 µmol/g creatinine.
The concentration in plasma is 0.05 µmol/L
Follow-up to Newborn Screening by analogy to Amino Acid Analysis

Newborn Screening of Amino Acids
- Tandem MS
- Follow-up to Positive Samples
  - Reanalyze using chromatographic method
  - Accurate quantification
  - Resolution of isomers (e.g. leucine and isoleucine)
  - False positives identified

Newborn Screening of Acylcarnitines
- Tandem MS
- Follow-up to Positive Samples
  - Reanalyze using HPLC-MS/MS
  - Accurate quantification
  - Resolution of isomers (e.g. butyryl- and isobutyrylcarnitine)
  - False positives identified

Carnitine and Acylcarnitine Analysis by HPLC-MS/MS

We developed a validated HPLC-MS/MS method for the quantitative determination of carnitine and acylcarnitines
- Chromatographically removes isobaric contaminants
- Chromatographically resolves isomeric compounds
- MRM triple quadrupole detection
- Rigorously quantitative
  - Standardized compounds
  - Internal standards
  - Multiple-point calibration curves

When should you use acylcarnitine analysis by HPLC-MS/MS?
- Follow-up to positive newborn screening results
- Patients with disease
  - False-negative newborn screening results
  - Not tested by newborn screening
  - Adolescent- or adult-onset disease
- Protocols for treatment and metabolism research
  - Accurate quantification of carnitine and acylcarnitine biomarkers
  - Selective, accurate, and precise to meet FDA standards

HPLC-MS/MS chromatogram of a calibration curve high point. Overlaid XICs of 77 transitions monitored for 65 compounds and 12 internal standards (internal standards are colored red).


Skeletal Muscle from Patient with Elevated Long-Chains
All in nmol/g wet weight: cis,cis-5,8-tetradecadienoyl- (2.76), lauroyl- (2.20), cis-5-tetradecenoyl- (2.74), S-3-hydroxy-palmitoyl- (1.19), myristoyl- (8.84), palmitoleoyl- (4.85), linoleoyl- (34.3), palmitoyl- (46.2), oleoyl- (75.2), stearoyl- (17.8)

Plasma from Patient with LCHAD Deficiency
All in μmol/l: S-3-hydroxy-myristoyl- (0.10), R-3-hydroxy-myristoyl- (<0.05), cis,cis-5,8-tetradecadienoyl- (0.26), lauroyl- (0.13), cis-5-tetradecenoyl- (0.25), S-3-hydroxy-palmitoyl- (0.25), R-3-hydroxy-palmitoyl- (0.06), myristoyl- (0.08), linoleoyl- (0.12), S-3-hydroxy-stearoyl- (0.15), R-3-hydroxy-stearoyl- (0.12), palmitoyl- (0.13), oleoyl- (0.12), stearoyl- (<0.05)