Metabolomics Research Group

Current Members:

Amrita K Cheema: Georgetown University
John M Asara: BIDMC/Harvard Medical School
Thomas Neubert: NYU (EB Liaison)
Chris Turck: Max Planck Institute (Chair)

Former Members

William Wikoff: UC Davis
Vladimir Tolstikov: Eli Lilly
Pavel Aronov: Stanford University

Future Members:

Andrew Patterson: Penn State University
Stephen Brown: University of Michigan
Design a study that resembles a typical metabolomics experiment

Participants asked:
- to identify quantitative differences between two groups of samples
- without (non-targeted) or with (targeted) spiked-in compound information
International representation of MRG study respondents

Participating Countries
- US
- Canada
- England
- Scotland
- Ireland
- Germany
- Spain
- Italy
- Netherlands
- Australia
- Japan
- South Korea
- China
- Singapore

Initial solicitation of interest from metabolomics labs, ABRF members, etc. by email.

~25% USA & Canada
~35% Europe
~25% Asia
Four principles of compound selection

1. Most of the spiked-in compounds should be endogenous with known concentrations in NIST plasma.

2. Compounds should be selected such that they are well distributed in terms of ability to analyze by a particular technique. For example, some compounds should be detectable with ESI+, whereas others should be detectable with ESI-, EI or APCI.

3. Compounds should be selected with a range of difficulty of identification, regardless of technique used.

4. High purity compounds should be chosen.
New NIST plasma standard is an ideal matrix for inter-laboratory studies

• Analyzed and validated by several groups on multiple analytical platforms.
• Can be used for comparisons over long periods of time.

NIST has generously donated the plasma that was used for the MRG study.
Lyophilization for sample preparation: Comparison to frozen sample

TIC (-) ESI

Total ion chromatogram of lyophilized sample superimposes with non-lyophilized sample.
Study Design

NIST plasma matrix
Pure compounds spiked into each tube

~100 µl per tube

Group A

Group B

Ratio of A and B = [0.68,0.81], with p < 0.01 after adjusting for endogenous plasma concentration

Taurine
n = 3, two concentration groups

Expected Concentration (µM)

0 50 100 150 200 250 300
A1 A2 A3 B1 B2 B3

Enough material to send to approximately 100 participants.
Limitation is the amount of NIST plasma available.
Expected Concentrations of 17 Spiked Metabolites (Adjusted Based on Endogenous Plasma Concentration)
## Expected Concentrations of 17 Spiked Metabolites in Plasma Study Samples

<table>
<thead>
<tr>
<th>Substance Name</th>
<th>MW</th>
<th>Spiked Concentration (µM)</th>
<th>Endogenous Concentration (µM)</th>
<th>POS Mode</th>
<th>NEG Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sample A</td>
<td>Sample B</td>
<td>Ratio A/B</td>
<td>Ratio A/B</td>
</tr>
<tr>
<td>Sarcosine</td>
<td>89.10</td>
<td>10</td>
<td>20</td>
<td>Probably Negligible</td>
<td>0.50 ↘</td>
</tr>
<tr>
<td>Betaine</td>
<td>117.15</td>
<td>50</td>
<td>100</td>
<td>33-88</td>
<td>[0.62,0.73] ↘</td>
</tr>
<tr>
<td>Urea</td>
<td>60.06</td>
<td>4000</td>
<td>8000</td>
<td>0.50</td>
<td>0.63 ↘</td>
</tr>
<tr>
<td>Taurine</td>
<td>125.15</td>
<td>50</td>
<td>100</td>
<td>55-162</td>
<td>[0.68,0.81] ↘</td>
</tr>
<tr>
<td>Nicotinic acid (niacin)</td>
<td>123.11</td>
<td>50</td>
<td>100</td>
<td>49-53</td>
<td>[0.66,0.67] ↘</td>
</tr>
<tr>
<td>Creatine</td>
<td>131.14</td>
<td>50</td>
<td>100</td>
<td>30-55</td>
<td>[0.62,0.68] ↘</td>
</tr>
<tr>
<td>Suberic acid</td>
<td>174.20</td>
<td>5</td>
<td>10</td>
<td>3.6</td>
<td>0.63 ↘</td>
</tr>
<tr>
<td>Quinolinic acid</td>
<td>167.12</td>
<td>3</td>
<td>6</td>
<td>0.47</td>
<td>0.54 ↘</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>151.06</td>
<td>5</td>
<td>20</td>
<td>Dose Dependent</td>
<td>0.25 ↘</td>
</tr>
<tr>
<td>Acetylcarnitine</td>
<td>203.12</td>
<td>16</td>
<td>8</td>
<td>6</td>
<td>1.57 ↗</td>
</tr>
<tr>
<td>Caffeine</td>
<td>194.08</td>
<td>8.50</td>
<td>48.50</td>
<td>Dose Dependent 2-10mg/L</td>
<td>0.18 ↘</td>
</tr>
<tr>
<td>Creatinine</td>
<td>113.06</td>
<td>69.98</td>
<td>9.98</td>
<td>70</td>
<td>1.75 ↗</td>
</tr>
<tr>
<td>DL-indole-3-lactic acid</td>
<td>205.07</td>
<td>4.2</td>
<td>1.2</td>
<td>2.8</td>
<td>1.75 ↗</td>
</tr>
<tr>
<td>Indoxyl sulfate</td>
<td>213.01</td>
<td>2</td>
<td>18</td>
<td></td>
<td>0.11 ↘</td>
</tr>
<tr>
<td>L-arginine</td>
<td>174.11</td>
<td>3.7</td>
<td>48.7</td>
<td>80</td>
<td>0.65 ↘</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>131.09</td>
<td>54.5</td>
<td>4.5</td>
<td>60-80</td>
<td>[1.59,1.78] ↗</td>
</tr>
<tr>
<td>Xanthosine</td>
<td>284.08</td>
<td>7.00</td>
<td>2</td>
<td>5</td>
<td>1.71 ↗</td>
</tr>
</tbody>
</table>

*Urea and Indoxyl sulfate were not detected by any of the participating laboratories.*
## MRG Member Results

<table>
<thead>
<tr>
<th>Substance</th>
<th>Expected Ratio A/B</th>
<th>MRG M1 Observed Ratio A/B</th>
<th>MRG M2 Observed Ratio A/B</th>
<th>MRG M3 Observed Ratio A/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcosine</td>
<td>0.5</td>
<td>1.08</td>
<td>0.97</td>
<td>1.38</td>
</tr>
<tr>
<td>Betaine</td>
<td>[0.62,0.73]</td>
<td>3.53</td>
<td>0.81</td>
<td>2.92</td>
</tr>
<tr>
<td>Urea</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taurine</td>
<td>[0.68,0.81]</td>
<td>0.84</td>
<td>0.28</td>
<td>0.35</td>
</tr>
<tr>
<td>Nicotinic acid (niacin)</td>
<td>[0.66,0.67]</td>
<td>5.11</td>
<td>0.28</td>
<td>5.52</td>
</tr>
<tr>
<td>Creatine</td>
<td>[0.62,0.68]</td>
<td>0.79</td>
<td>0.50</td>
<td>1.54</td>
</tr>
<tr>
<td>Suberic acid</td>
<td>0.63</td>
<td>0.17</td>
<td>1.19</td>
<td>0.19</td>
</tr>
<tr>
<td>Quinolinic acid</td>
<td>0.54</td>
<td>0.37</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>0.25</td>
<td>8.78</td>
<td>8.06</td>
<td>8.68</td>
</tr>
<tr>
<td>Acetylcarnitine</td>
<td>1.57</td>
<td>0.72</td>
<td>0.43</td>
<td>0.62</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.18</td>
<td>0.78</td>
<td>0.15</td>
<td>0.29</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.75</td>
<td>1.61</td>
<td>1.78</td>
<td>1.55</td>
</tr>
<tr>
<td>DL-indole-3-lactic acid</td>
<td>1.75</td>
<td>0.42</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Indoxyl sulfate</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-arginine</td>
<td>0.65</td>
<td>0.17</td>
<td>2.10</td>
<td>1.71</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>[1.59,1.78]</td>
<td>0.86</td>
<td>0.59</td>
<td>0.75</td>
</tr>
<tr>
<td>Xanthosine</td>
<td>1.71</td>
<td>0.16</td>
<td>0.60</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Urea and Indoxyl sulfate were not detected by any of the participating laboratories.
Results Reporting Format

For each compound:

- m/z, ion mode (mass spectrometry)
- Molecular formula (or multiple formulas if ambiguous)
- Fold-change between groups
- Statistical metric for observed difference
- Compound identity
Techniques Used

- **GC-MS**: 3.13%
- **LC-MS**: 16.70%
- **NMR**: 4.17%

  - QTOF: 5
  - QqQ: 3
  - Orbitrap: 1
  - Unspecified: 7

- **Amine-HPLC**: 1
- **UPLC, LC**: 4
- **LC**: 2
- **UPLC**: 1

- Total Participants (including MRG members) = 17
- Total Platforms Used = 23
- Quantitative Data Returned = 11 (73.3%)
Accuracy of Metabolite Identification = 88.2%

Accuracy of Metabolite Quantification = 38.1%
Detection of Spiked Metabolites

Right Trend for Quantitation

Opposite Trend for Quantitation

sarcosine 7 1 1
betaine 8 1 1
taurine 8 1 1
nicotinic acid (niacin) 5 0 0
creatine 7 1 1
sulberic acid 7 1 1
quinolinic acid 7 1 1
acetaminophen 7 0 0
Acetylcarnitine 5 1 1
caffeine 13 1 1
creatinine 6 1 1
DL-indole-3-lactic acid 8 1 1
L-arginine 14 1 1
L-isoleucine 0 1 1
Xanthosine 2 0 0
Quantitative Accuracy

creatinine

m/z 114.0667 (ES+)

A = 69.98µM
B = 9.98µM
C = 70µM

Expected Ratio A/B (Dashed Line): 1.75

Method
- GC-MS
- LC-MS
- NMR

Reported results:
- GC-MS: 2.5, 3.0
- LC-MS: 1.5, 2.0, 3.0
- NMR: 1.5, 1.0, 0.5
Quantitative Accuracy

L-arginine

m/z 175.1195 (ES+)

A= 3.7µM
B= 48.7µM
C= 80µM

Expected Ratio A/B (Dashed Line): 0.65

Method
- GC-MS
- LC-MS
- NMR
Quantitative Accuracy
L-isoleucine
m/z 130.0868 (ES-)

A = 54.5µM
B = 4.5µM
C = 60-80µM
Expected Ratio A/B (Range): [1.59, 1.78]
Quantitative Accuracy
acetaminophen
m/z 152.0712 (ES+)

A = 5µM
B = 20µM
C = Dose Dependent
Expected Ratio A/B (Dashed Line): 0.25

Method
- GC-MS
- LC-MS
- NMR
Quantitative Accuracy

acetylcarnitine

m/z 204.1236 (ES+)

A= 16µM
B= 8µM
C= 6µM

Expected Ratio A/B (Dashed Line): 1.57

Method
- GC-MS
- LC-MS
- NMR
Conclusions

- LC-MS was the most commonly used platform to analyze study samples.
- For the LC-MS platforms, the metabolite detection accuracy was dependent on the protocol used for sample processing as well as the analytical conditions (column chemistry, mobile phase, etc.).
- The quantification trends were quite consistent for the laboratories that used LC-MS platforms.
- Quantitative data for Taurine, Suberic acid, Caffeine, and Creatinine were most consistent across laboratories and analytical platforms.
- Quantification of metabolites with high endogenous plasma concentrations turned out to be the most challenging.
- A combination of platforms increased the accuracy and overall rate of detection.

- Average Detection Rate is 31.76%.
  - Average Detection Rate is 22.59% for untargeted and 39.71% for targeted methods, a 75% increase in detection rate.
- Using 2 different platforms, the detection rates were 52.9% and 64.7%, respectively.
- Using different separation systems in conjunction with MS-based platforms resulted in the highest detection rate (88.2%).