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Find the Targets and Answers that Matter – Introducing the Absolute*IDQ* p400 HR Kit and Q Exactive MS for Targeted Metabolic and Lipid Profiling

Agenda

1. Background Information
2. Required Instrumentation
3. Easy and Streamlined Workflow
4. Components of the All-in-One Solution
5. Analytical Performance
6. Biological Relevance and Applications

Our Metabolic Phenotype Tells a Story of Who we Are

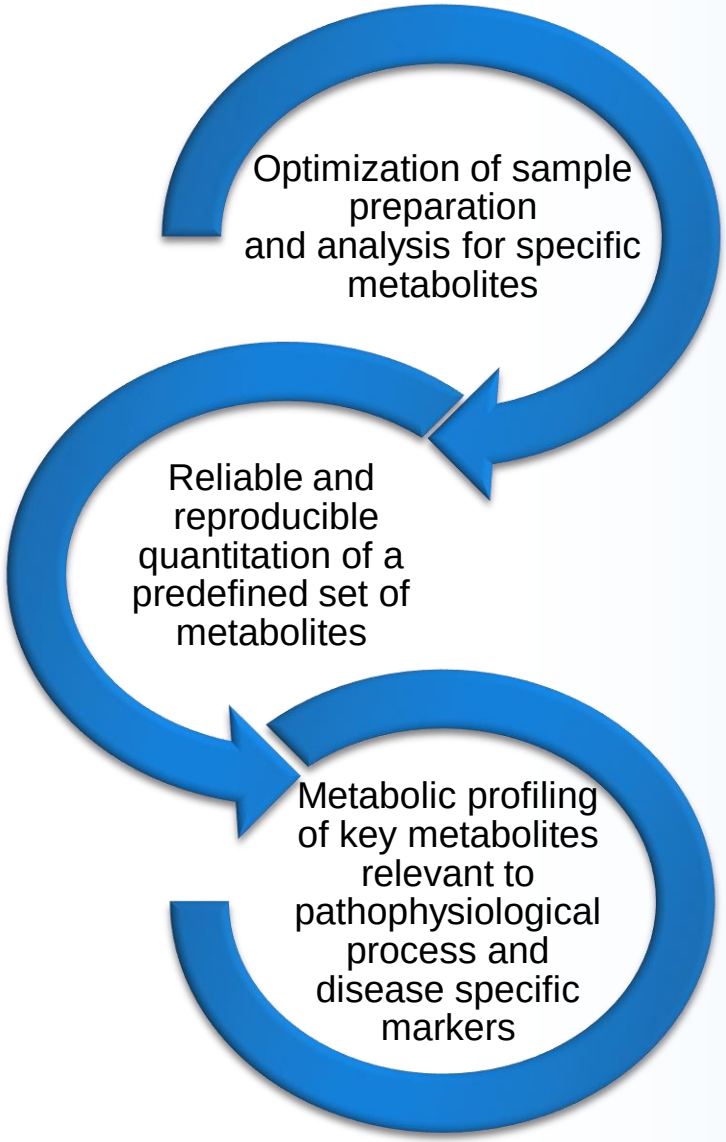
- Age
- Disease
- Environmental Factors
- Genetic Factors
- Lifestyle
- Nutrition
- Medication

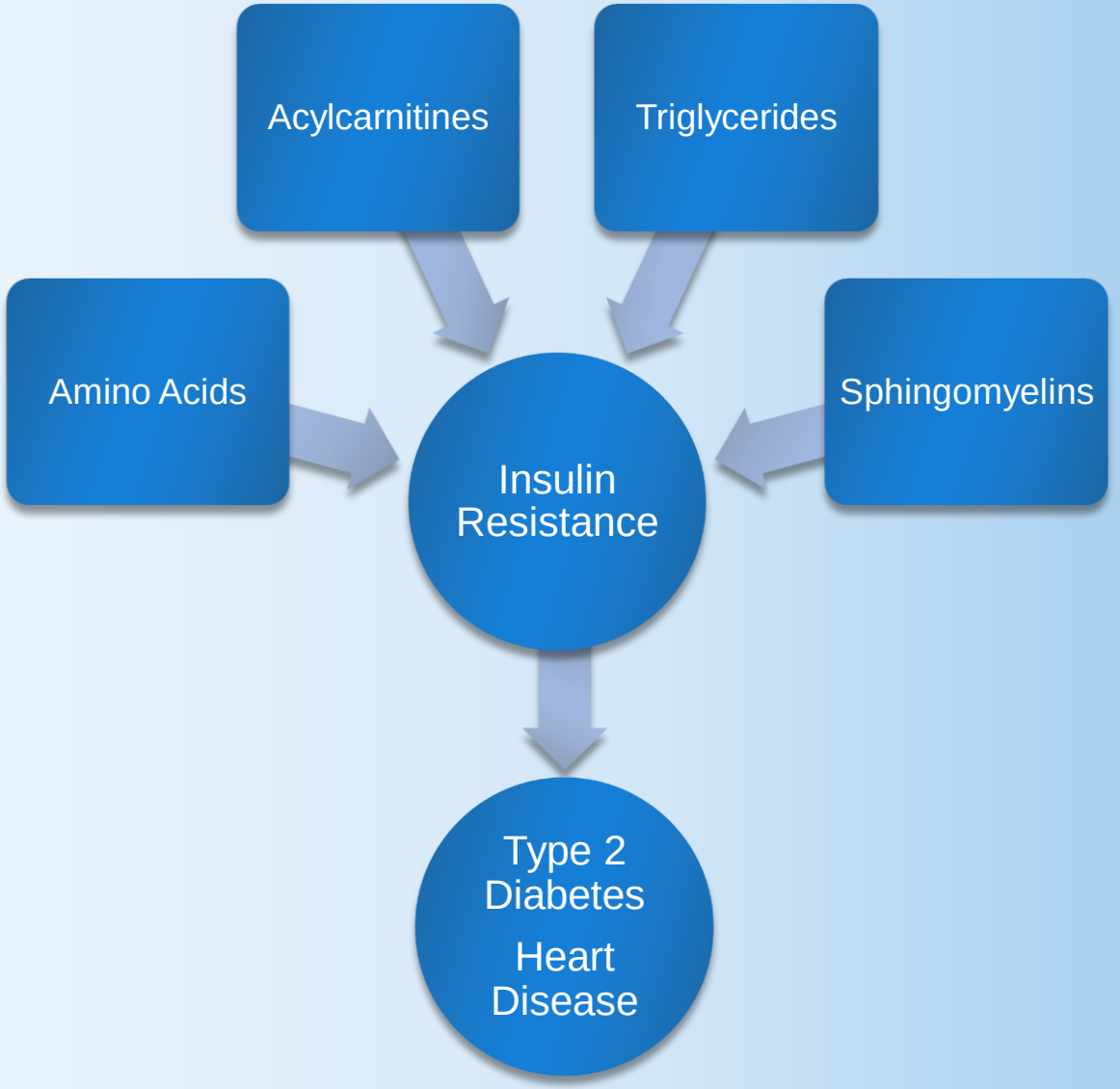
Metabolic Phenotyping

- Health Status
- Disease Risk Assessment
- Drug and Therapy Response Prediction
- Environmental Exposure

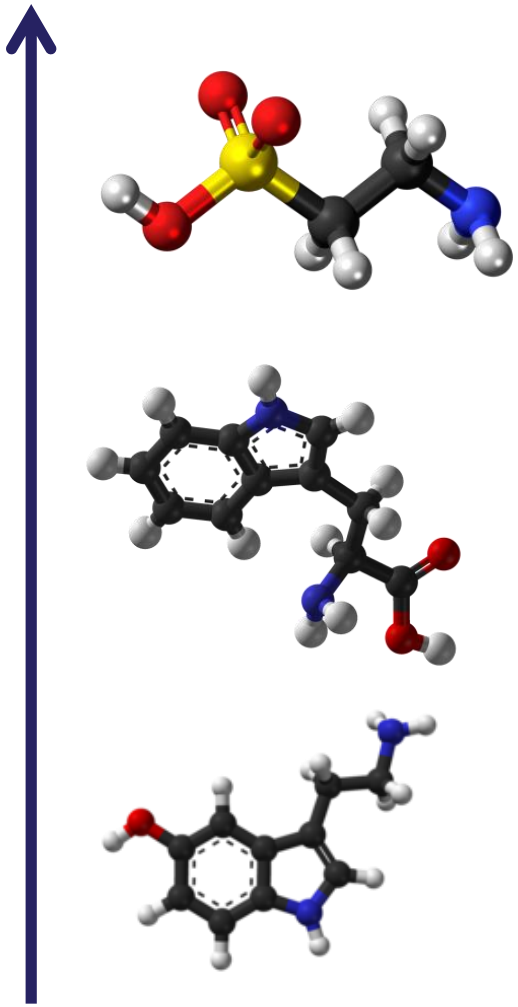
Precision Medicine Public Healthcare

Targeted Metabolomics - Quantitation of Key Metabolites in Biological Processes





Targeted Metabolomics - Quantitation of Key Metabolites During Sleep Deprivation



Taurine

Tryptophan

Serotonin

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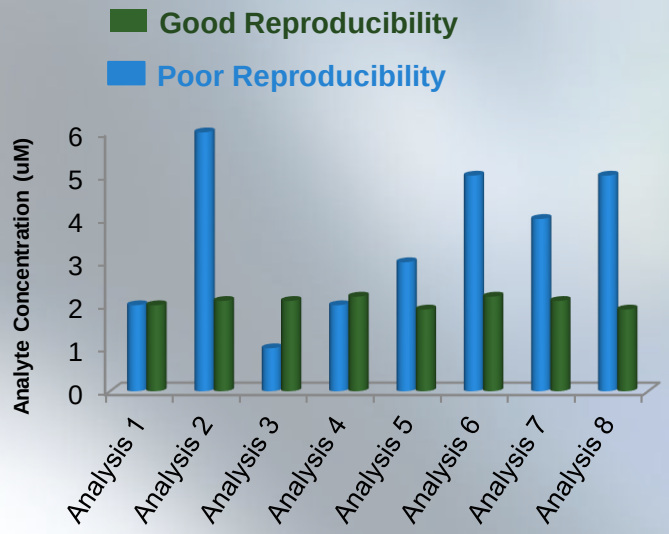
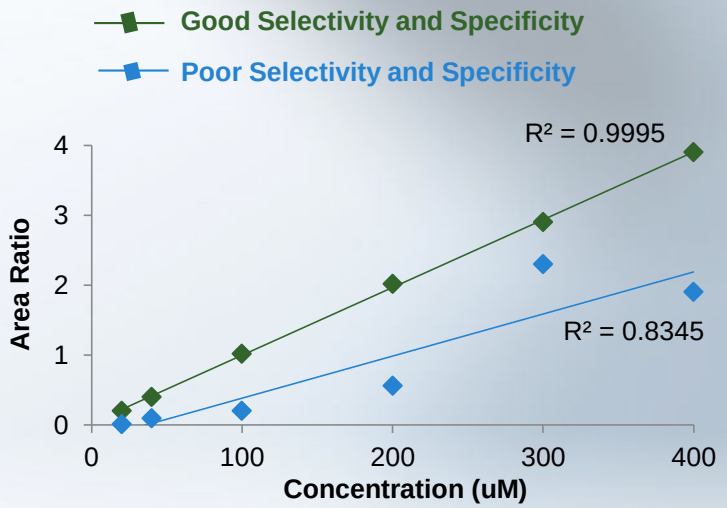
Challenges in Targeted Metabolomics Analysis





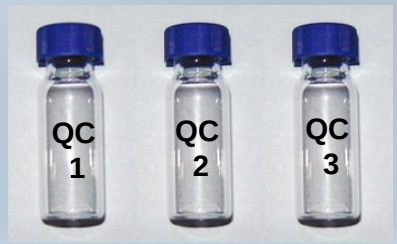
Standardization

Reproducibility

Large Sample Size

Lack of selectivity and specificity

Poor or No Quality-Control



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Absolute*IDQ*® p400 HR Kit and Q Exactive Orbitrap MS

The Absolute*IDQ*® p400 HR Kit Provides a:

- Standardized
- Quality-controlled and
- Reproducible quantitative assay

The HRAM unique capabilities of the Thermo Scientific™ Q Exactive™ Orbitrap MS complements the analysis by providing:

- Resolution and accurate quantitation of isobaric lipid species
- Increased selectivity and specificity for lipid analysis in FIA
- Accurate quantitation with less interferences

Quantitative, Standardized and Reproducible Metabolomics

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Metabolite and Lipid Coverage

408 Endogenous Metabolites

- 11 metabolite classes
- 365 lipids and 43 small molecules

- Amino acids (21)
- Biogenic amines (21)
- Hexoses (1)

Polar lipids
- Phosphatidylcholines (172)
- Lysophosphatidylcholines (24)
- Sphingomyelins (31)
- Ceramides (9)

Neutral lipids
- Acylcarnitines (55)
- Diglycerides (18)
- Triglycerides (42)
- Cholesteryl esters (14)

Key Benefits at a Glance

- Ready-to-use quantitative kit for Q Exactive MS platform
- Simple and efficient automated workflow
- High-throughput and minimal sample volume
- Robust platform for longitudinal & inter-lab comparability

Broad Metabolic and Lipid Profiling

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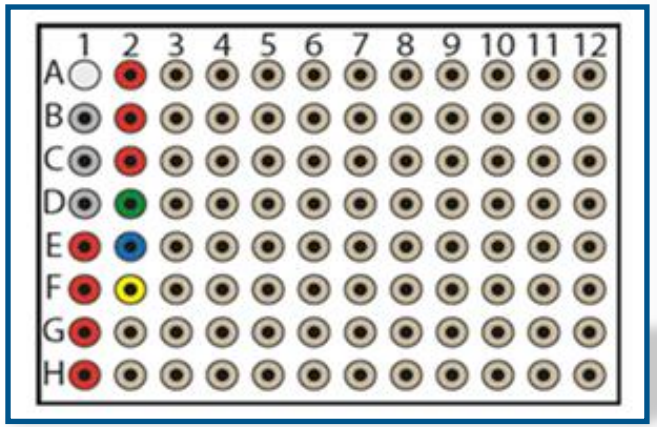
Instrumentation and Plate Layout

Thermo Scientific™ Q Exactive™ Orbitrap™ Family of HRAM Mass Spectrometers

- Thermo Scientific™ Q Exactive™ Focus MS
- Thermo Scientific™ Q Exactive™ MS
- Thermo Scientific™ Q Exactive™ Plus MS
- Thermo Scientific™ Q Exactive™ HF MS

UHPLC

- Thermo Scientific™ Vanquish™ UHPLC, Thermo Scientific™ UltiMate™ 3000 RSLC



96 well plate format

7 calibration standards

3 quality controls samples

Blank and zero samples

Internal standards

Up to 82 samples per plate

Developed for Q Exactive Orbitrap MS

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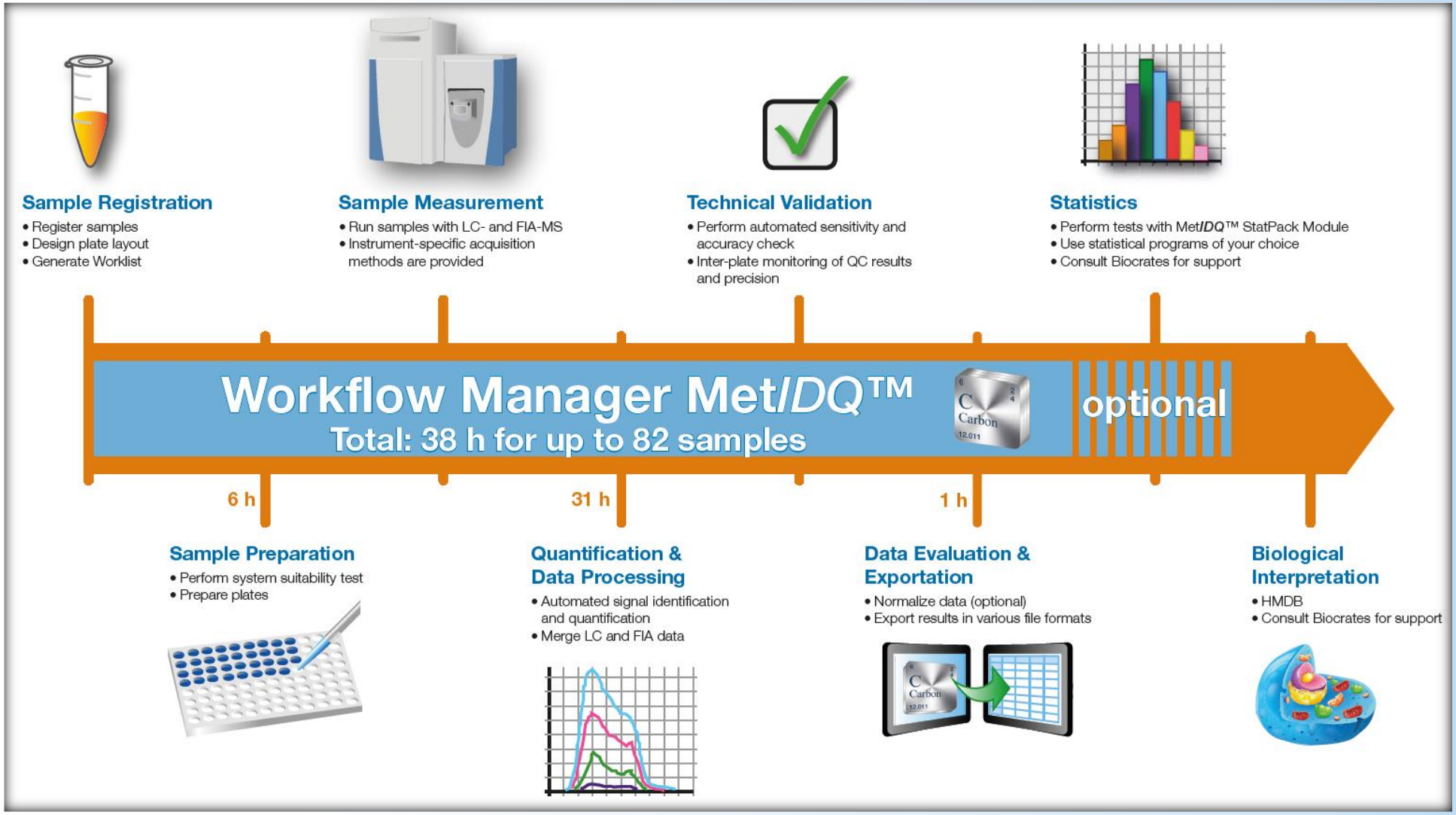
Workflow Overview



Easy and Streamlined Workflow from Sample Preparation to Data Analysis

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Kit Overview

Kit Components

- **Reagents and Consumables**
  - Patented 96-well filter plates
  - Internal standards
  - Calibration standards
  - Quality controls
  - Instrument suitability test mix
- **Methods and Protocols**
  - Sample preparation protocol
  - Instrument-specific acquisition & quantitation methods
- **Workflow Manager Met*IDQ*™**
  - Process guidance
  - Automated technical validation

Benefits

- All in one box
- Standardized & quality controlled
- Only 10 µL sample required
- Validated for human plasma
- Ready-to-use and user-friendly
- Longitudinal and inter-lab comparability
- Save precious sample material

Standardized and Quality Controlled Assay

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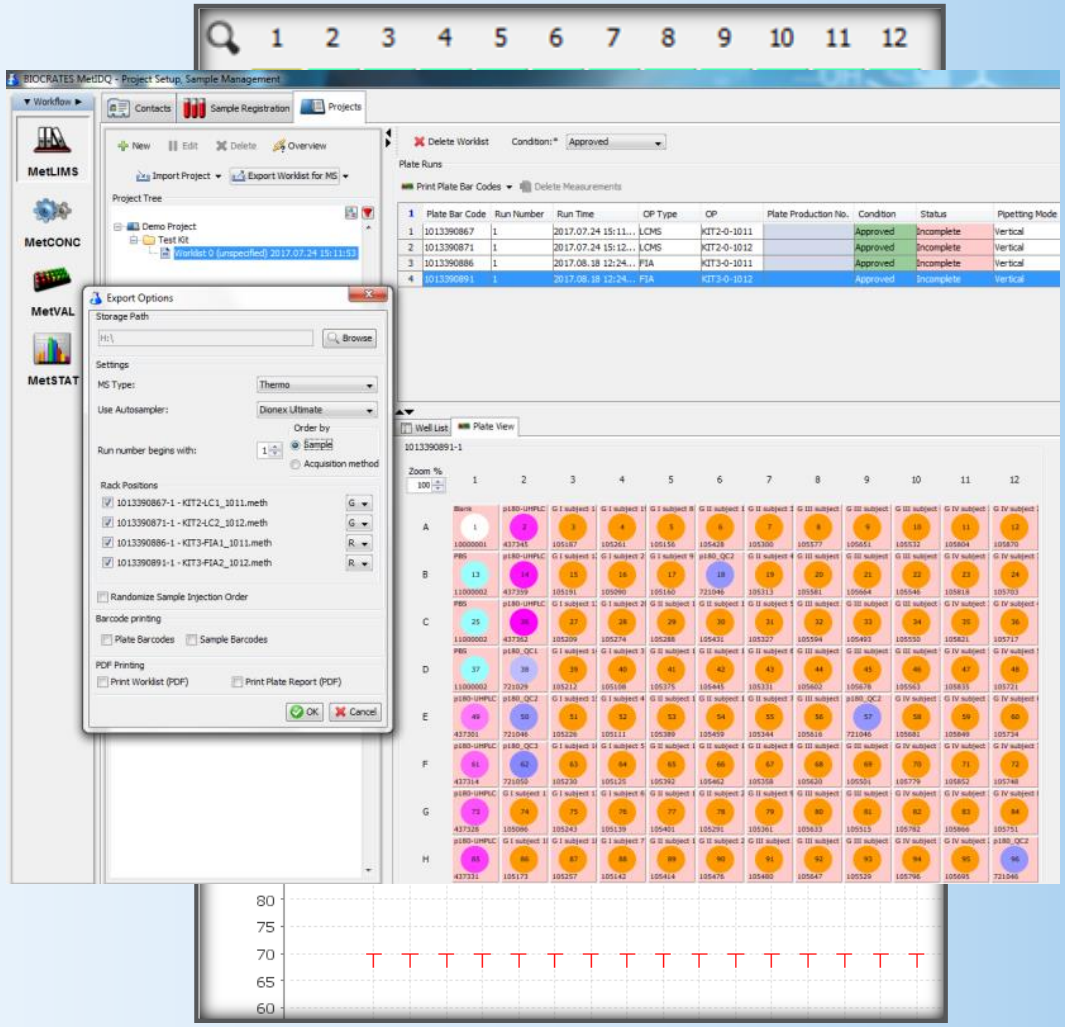
MetIDQ™ Software for Workflow Management

Automated Workflow

- **Information Management**
  - Sample registration, plate layout design
  - Worklist generation
- **Quantitation and Data Processing**
  - Automated signal identification & quantification
  - Data merging
- **Automated Technical Validation**
  - Transparent display of generated data
  - Analytical approval of the plate
  - Inter-plate monitoring of QC results and precision
- **Data Evaluation and Export**
  - Data normalization (optional)
  - Export of results in various file formats
- **Statistical Analysis (optional)**
  - Basic statistics & data visualization



Streamlined Workflow Management and Data Processing Automation

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MetIDQ™ Software for Workflow Management

Automated Workflow

- **Information Management**
  - Sample registration, plate layout design
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- **Automated Technical Validation**
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- **Data Evaluation and Export**
  - Data normalization (optional)
  - Export of results in various file formats
- **Statistical Analysis (optional)**
  - Basic statistics & data visualization

Benefits

- Transparent, automated and guided process
- Quantitative results for all analytes
- Reduced complexity
- Efficient and time saving
- High confidence in results

Streamlined Workflow Management and Data Processing Automation

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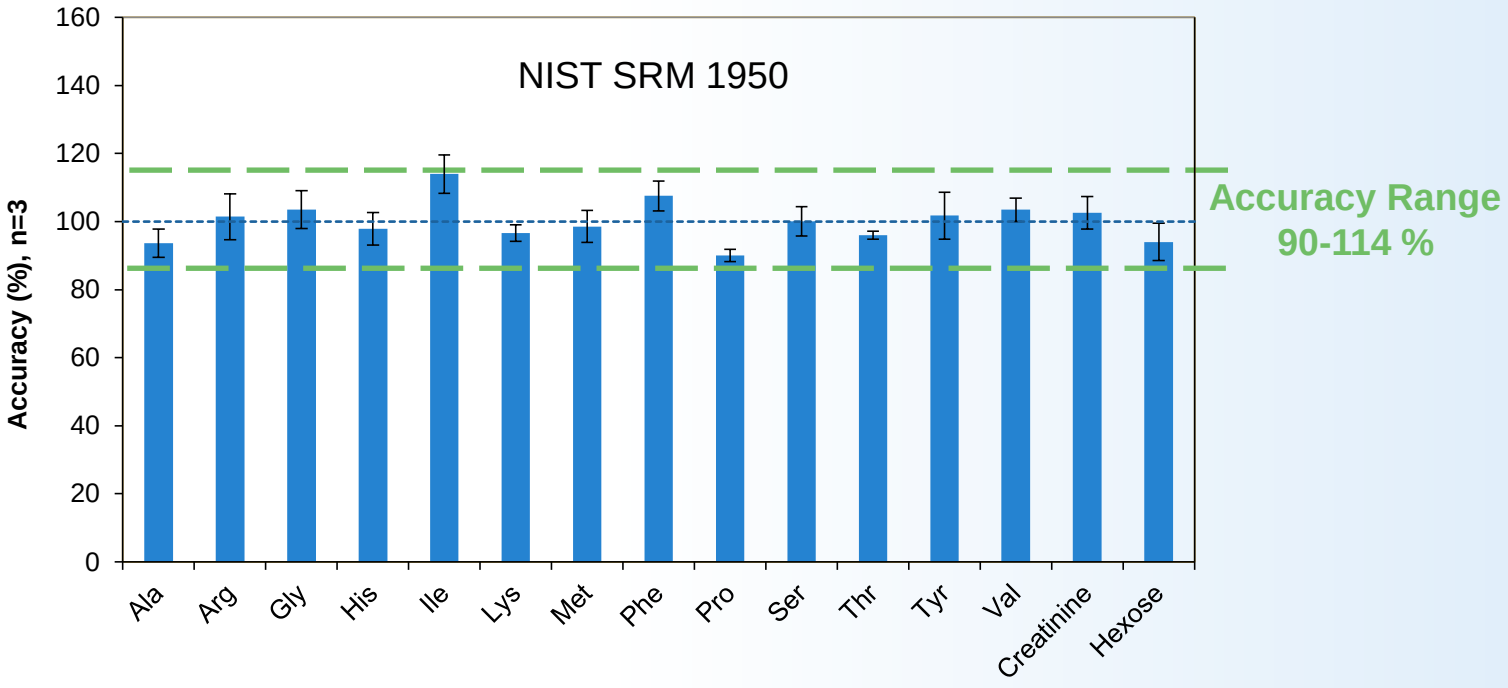
Accuracy Assessed with NIST Reference Material



The field MUST become more quantitative if findings are to be translated to practical, clinical applications."

David Wishart, 2015

Excellent Accuracies in Measured Concentrations *vs* the NIST Reference Material

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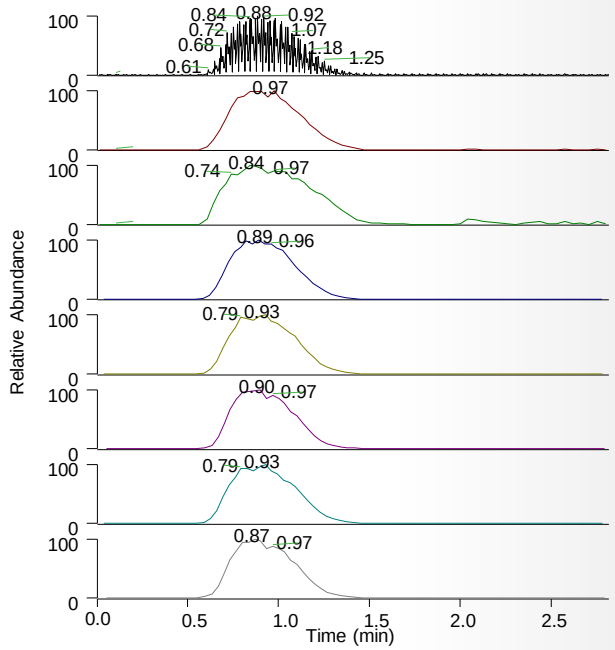
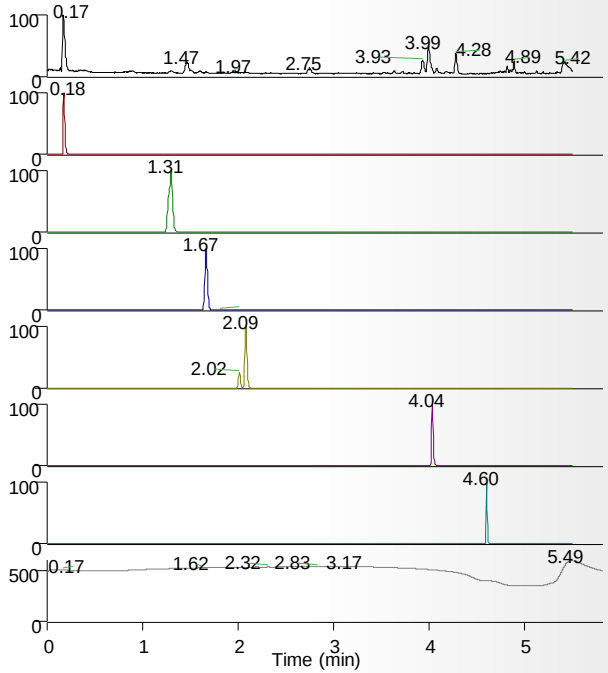
Analytical Performance: Inter-day Comparability

FIA-MS



Accuracy
81-110%,
Precision (CV)
10-25%

LC-MS



Accuracy
92-104%,
Precision (CV)
4-15%

Excellent Accuracy, Precision and Inter-day Comparability

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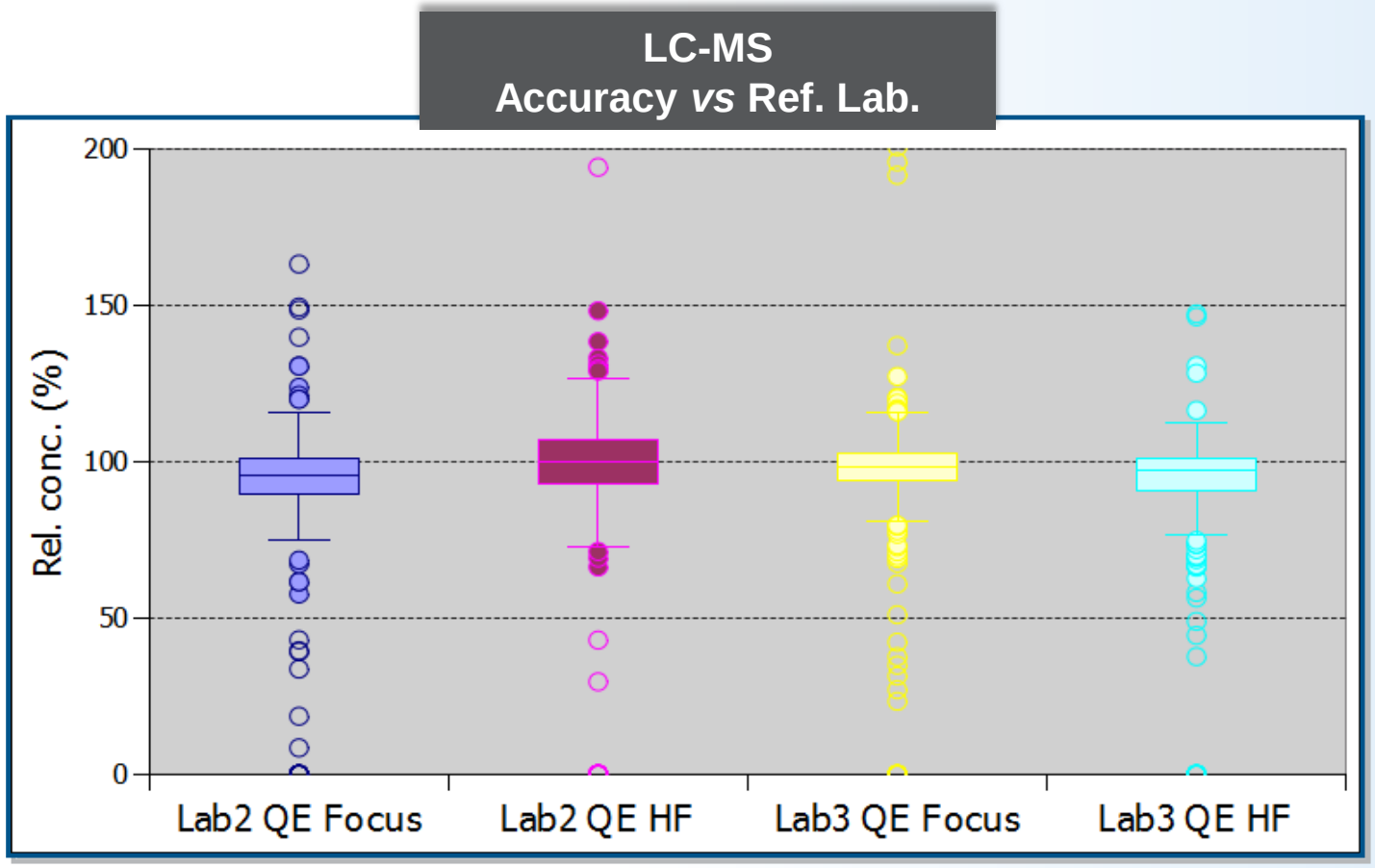
Inter-laboratory Comparability



- 3 laboratories
- 5 instruments (Q Exactive Focus MS and Q Exactive HF MS)
- Reference lab 1 (Q Exactive Focus MS)
- 6 individual human plasma samples
- Resolution for LC-MS:

70k (Q Exactive Focus MS)
60k (Q Exactive HF MS)

Excellent Performance for Amino Acids and Biogenic Amines

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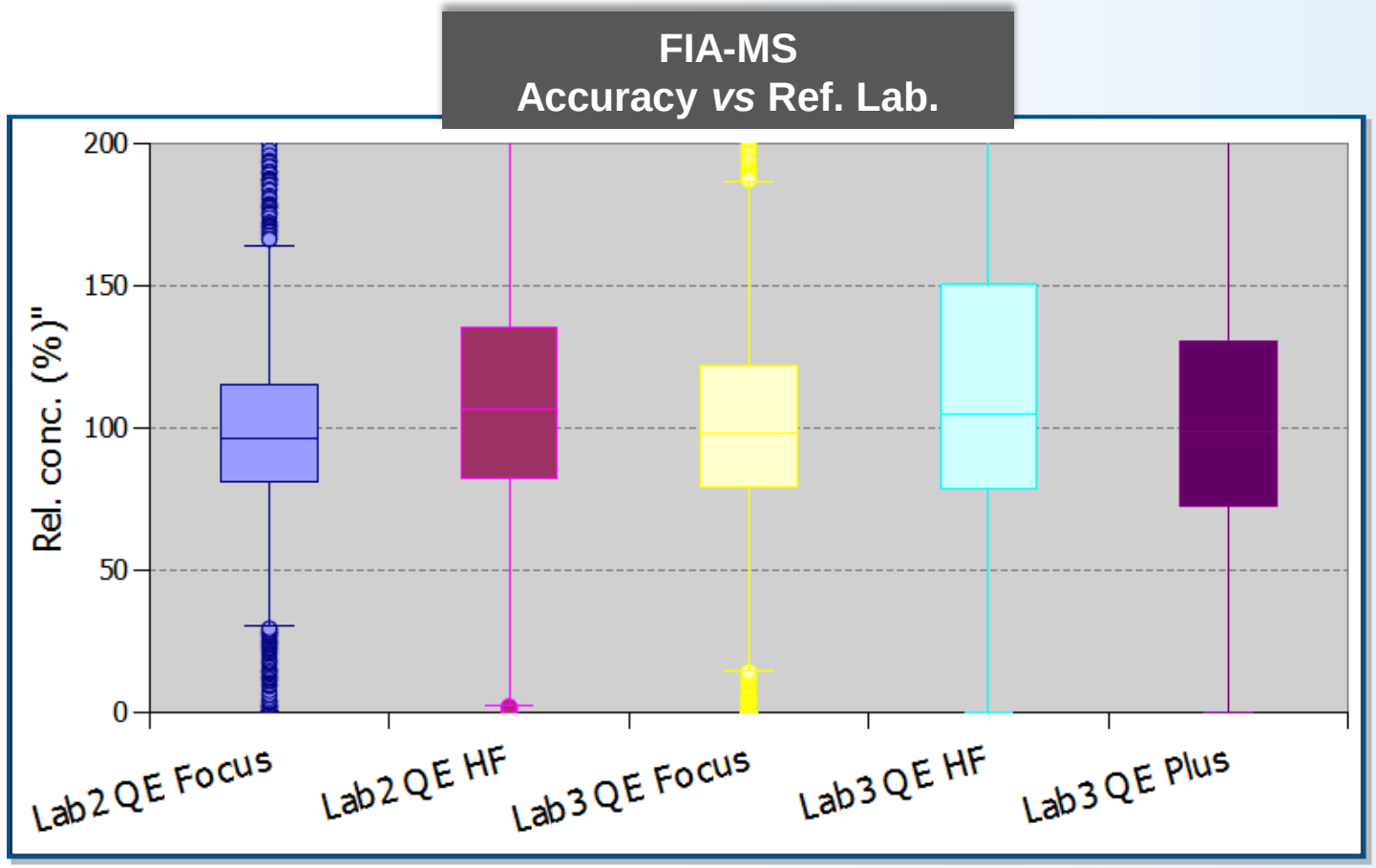
Inter-laboratory Comparability



- 3 laboratories
- 6 instruments (Q Exactive Focus MS, Q Exactive Plus MS and Q Exactive HF MS)
- Reference lab 1 (Q Exactive Focus MS)
- 6 individual human plasma samples
- Resolution for FIA-MS:

70k (Q Exactive Focus MS)
70k (Q Exactive Plus MS)
120k (Q Exactive HF MS)

High Inter-laboratory Comparability for Hexoses, Acylcarnitines and Lipids

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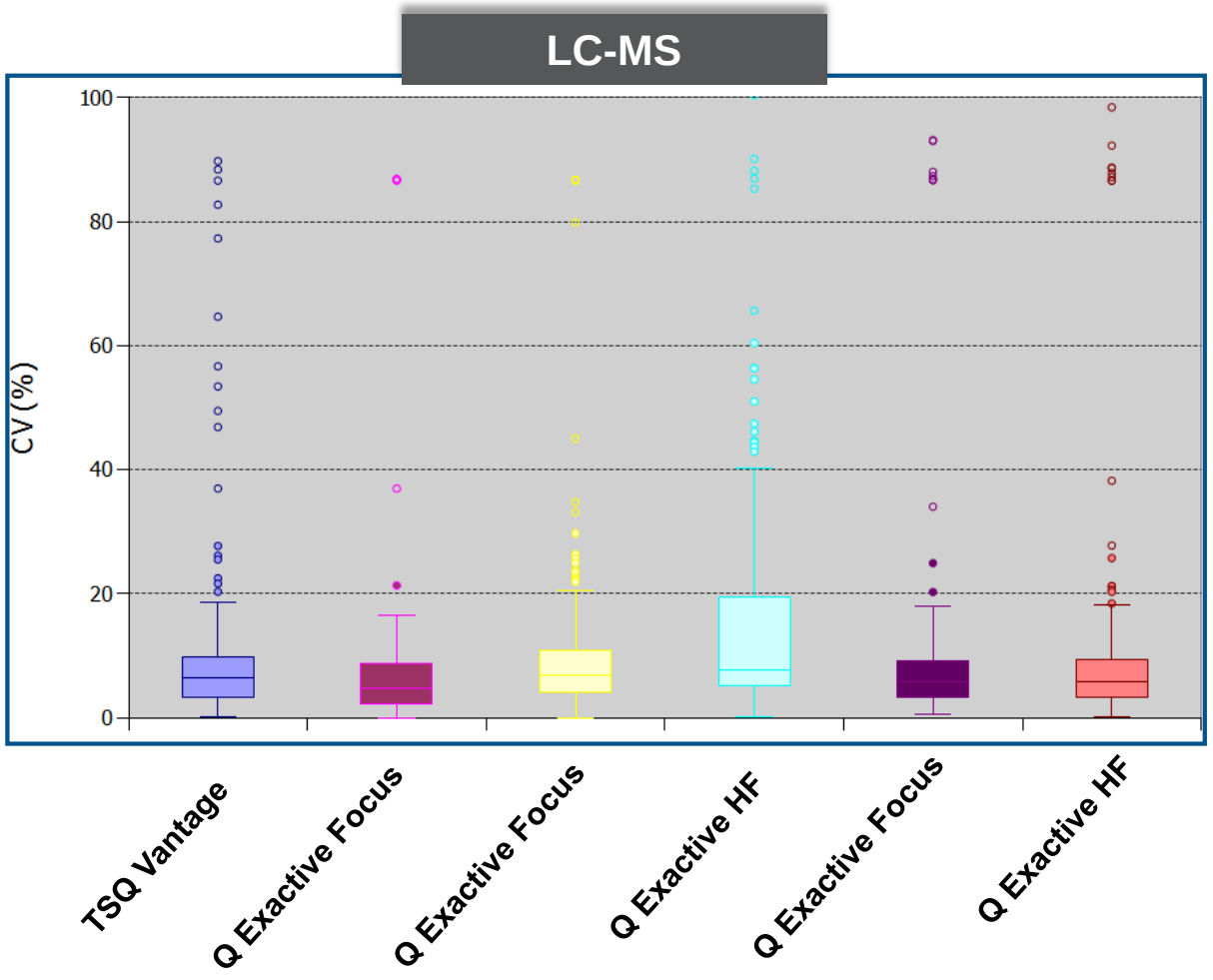
Analytical Precision



- 3 laboratories
- 6 instruments (Q Exactive Focus MS and Q Exactive HF MS, Thermo Scientific™ TSQ Vantage™ MS)
- Reference lab 1 (Q Exactive Focus MS)
- 6 individual human plasma samples
- Resolution for LC-MS:

70k (Q Exactive Focus MS)
60k (Q Exactive HF MS)

Very Good Analytical Precision in LC-MS for all Q Exactive Orbitrap MS tested

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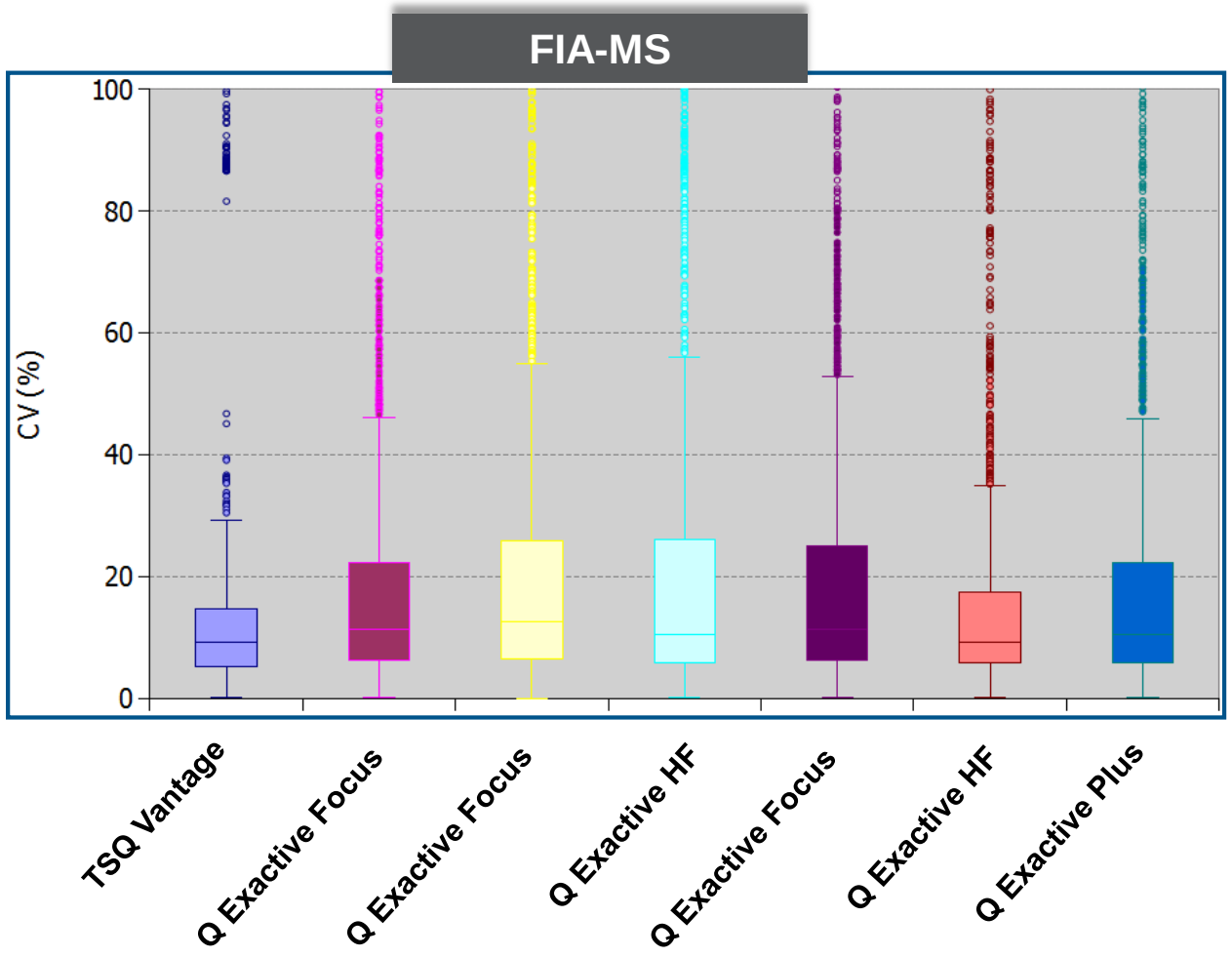
Analytical Precision



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Importance of Inter-laboratory Reproducibility

Metabolomics (2016)12:149
DOI 10.1007/s11306-016-1094-6

ORIGINAL ARTICLE

Metabolomics enables precision medicine: "A White Paper, Community Perspective"

Beger et al., Metabolomics (2016) 12: 149

"[…] Biocrates commercially supplies kits for analysis of targeted areas of metabolism. […]
Each kit provides advantages in interlaboratory use […]."

Ring Trials

- 2 worldwide first ring trials (2016)

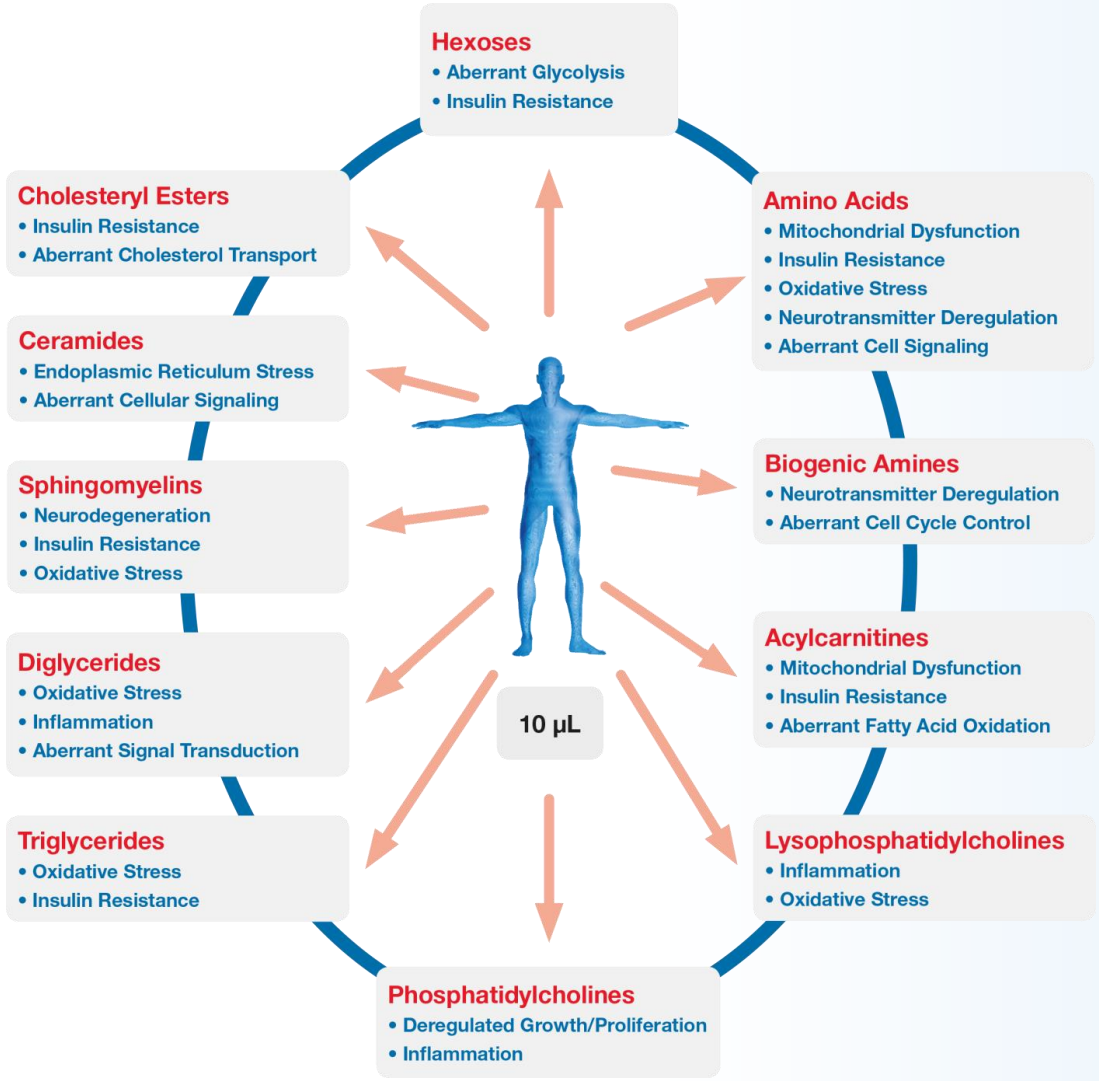
Readiness of kit technology

- First ring trial scheduled for Absolute*IDQ* p400 HR kit

Absolute*IDQ* p400 HR kit

- Quantitative results with high accuracy
- Inter-day comparability
- Multi-center and multi-instrument evaluated
- Robust platform
- Confident results

Broad Analyte Coverage



Membrane composition

Cellular signaling

Bionergetic processes (mitochondrial beta oxidation of fatty acids, glutaminolyis, glycolysis)

Oncology
Cardiology
Neurology
Aging
Diabetes
…….

Unique Analyte Coverage Provides Important Biological Information

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Metabolic Signatures-Newborn Screening

Metabolomics (2016)12:149
DOI 10.1007/s11306-016-1094-6

ORIGINAL ARTICLE

Metabolomics enables precision medicine: "A White Paper, Community Perspective"

Beger et al., Metabolomics (2016) 12: 149

„Today, clinicians capture only a very small part of the information contained in the metabolome, as they routinely measure only a narrow set of blood chemistry analytes … will be replaced by … a far more comprehensive metabolic signature."

Newborn Screening

- Phenylketonuria
  - Phe ↑, Phe/Tyr > 2.5
- Medium Chain Acyl-Coa-dehydrogenase (MCAD) deficiency
  - C6> 0.21, C8> 0.32, C10> 0.48, c10:1> 0.28
  - C8/C10 > 1.6, C8/C12> 1.6

Metabolic Signatures-Precision Medicine

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Fischer Ratio

- Important in assessing:
- Liver metabolism
- Liver dysfunction

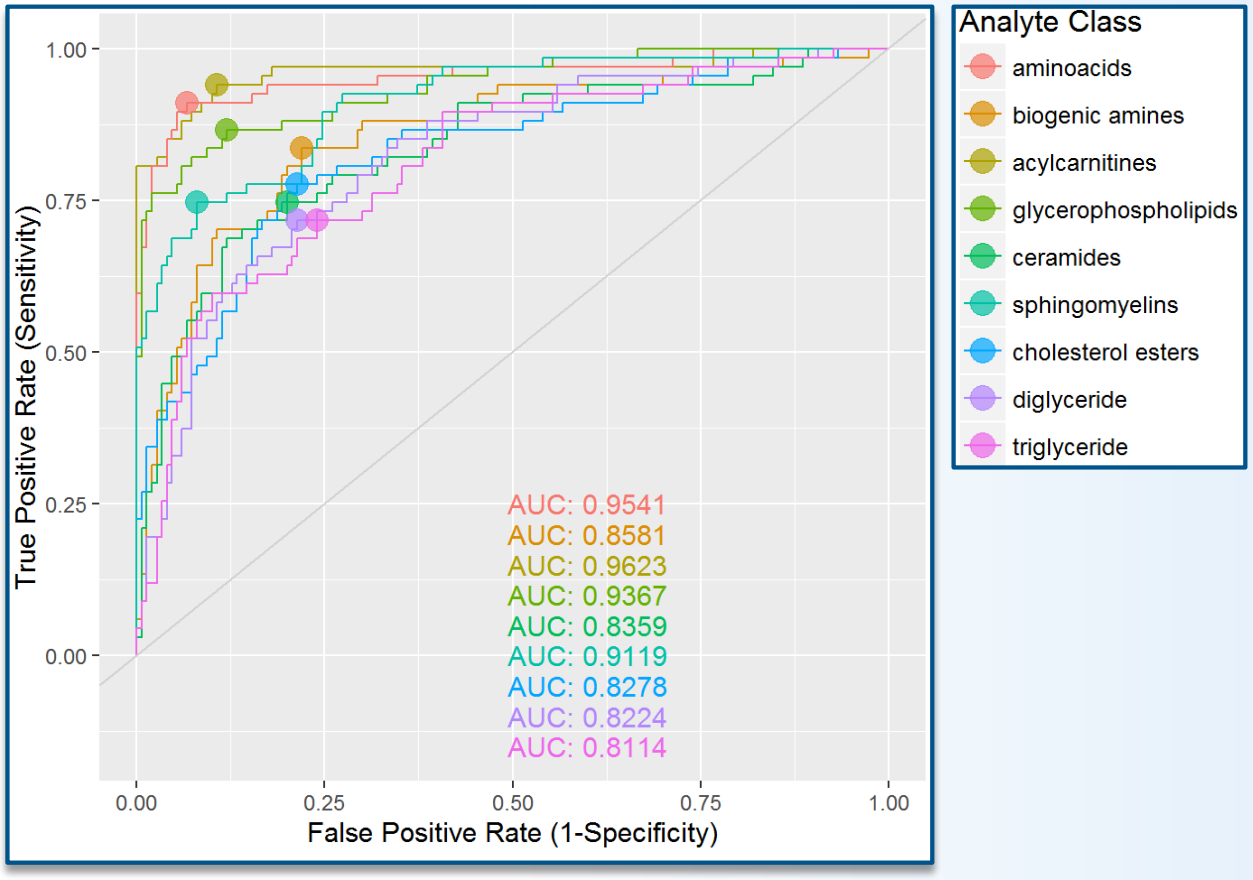
i.e. BCAA/AAA ratio increases with decrease severity of liver failure

$$\frac{\text{Val + Leu + Ile}}{\text{Phe + Tyr + Trp}} \rightarrow \frac{\textbf{BCAA}}{\textbf{AAA}}$$

Fischer et al., Am J Surg (1974) 127: 40

Case Study-Advantage of Broad Metabolite Coverage

Sample Description	Dataset
Healthy Controls	150
Ovarian Cancer Study Subjects	69



- Identification of key metabolites for ovarian cancer
- AA and AC are the top markers
- Importance of affected pathways in tumor pathophysiology and other disease
- Unique metabolite portfolio of the Absolute*IDQ*® p400 HR Kit provides added value

Biocrates Life Sciences in collaboration with Prof. Burwinkel (Heidelberg University, Germany)

Identification of Key Metabolites that are Significantly Altered Between the two Groups

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Conclusions

- The Absolute*IDQ*® p400 HR Kit is a ready-to-use solution designed for targeted metabolomics on Q Exactive Orbitrap HRAM MS.
  - Ready to implement within a day, without method development
- The kit provides quantitative results for up to 408 metabolites covering a broad range of biological functions.
- The kit delivers a standardized, quality-control, and targeted quantitative assay for accurate and confident metabolic profiling
- The simple, efficient, and automated workflow ensures high throughput and use of minimal sample volume.
- The robust platform with standardized procedures assure longitudinal and inter-lab comparability.

Standardized and Reproducible Quantitation of Hundreds of Lipids and Small Molecules for Broad Lipid and Metabolic Profiling

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