

Find the Targets and Answers that Matter

Introducing the *AbsoluteIDQ[®] p400 HR Kit* and *Q Exactive[™] Orbitrap[™] MS* for Targeted Lipid and Metabolic Profiling



MetIDQ[™] Software

1. Background Information

2. Absolute/DQ® p400 HR Kit

- Metabolite Coverage
- Requirements
- Kit Components
- Kit Workflow

3. Analytical Performance

4. Biological Relevance & Applications



Absolute/DQ® p400 HR Kit

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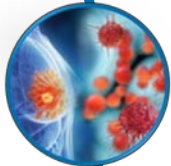
Absolute/DQ® p400 HR Kit



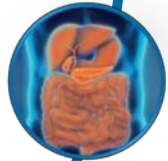
Globally operating biotech company based in Innsbruck, Austria



> 15 years of experience, with **> 800 publications**



Fully integrated mass spectrometry based solutions for **targeted metabolic phenotyping**



Services & kits: quantitative analysis of **> 800 endogenous metabolites**, statistical analysis, and biological interpretation



Hypothesis driven biomarker research **in pre-clinical, clinical, and epidemiological research**

Metabolites

188



Absolute/DQ® p180 Kit

- amino acids, biogenic amines
- acylcarnitines, hexoses
- glycerophospho- and sphingolipids

20



Biocrates® Bile Acids Kit

- primary bile acids
- secondary bile acids
- human and rodent specific bile acids

17



Absolute/DQ® Stero17 Kit*

- mineralocorticoids
 - glucocorticoids
 - sex steroids
- *For US/Canada: License pending

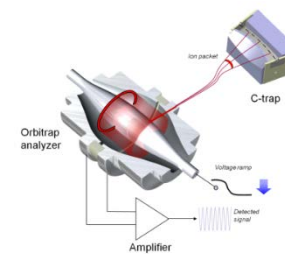
QQQ
MS

408



Absolute/DQ® p400 HR Kit

+ 220 lipids!



HRAM
MS

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Thermo Fisher Scientific, CCL2012

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Absolute/DQ® p400 HR Kit

Some interesting metrics

- ✓ **HMDB metabolites: 390,000**
- ✓ **Chemicals with ^{15}N , ^{13}C , ^2H labeled standards: 400**
- ✓ **Typical number of features in discovery untargeted platforms: 10,000**
 - ✓ **Compounds Identified: 200 – 1000?**

Source: David Wishart, presentation at MSACL 2017 EU (Salzburg, Austria) September 2017

- ✓ **Biocrates p400HR – identified and quantifiable metabolites: 408**

408 Endogenous Metabolites

- ✓ **11 metabolite classes**
- ✓ **365 lipids & 43 small molecules**

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)

Standard Thermo Scientific™ Orbitrap™ HRAM Mass Spectrometer (+ UHPLC)

- ✓ Q Exactive™ Focus
- ✓ Q Exactive™
- ✓ Q Exactive™ Plus
- ✓ Q Exactive™ HF



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Key Benefits at a Glance

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



Absolute/DQ® p400 HR Kit

Easy & Streamlined Workflow

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Sample Registration

- Register samples
- Design plate layout
- Generate Worklist



Sample Measurement

- Run samples with LC- and FIA-MS
- Instrument-specific acquisition methods are provided



Technical Validation

- Perform automated sensitivity and accuracy check
- Inter-plate monitoring of QC results and precision



Statistics

- Perform tests with Met/DQ™ StatPack Module
- Use statistical programs of your choice
- Consult Biocrates for support

Workflow Manager Met/DQ™
Total: 38 h for up to 82 samples



optional

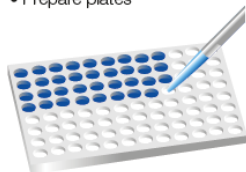
6 h

31 h

1 h

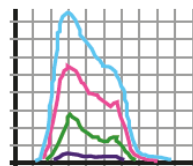
Sample Preparation

- Perform system suitability test
- Prepare plates



Quantification & Data Processing

- Automated signal identification and quantification
- Merge LC and FIA data



Data Evaluation & Exportation

- Normalize data (optional)
- Export results in various file formats



Biological Interpretation

- HMDB
- Consult Biocrates for support



Kit Components

✓ Reagents & Consumables:

- Patented 96-well filter plates
- Internal standards
- Calibration standards
- Quality controls
- Instrument suitability test mix

✓ Methods & Protocols:

- Sample preparation protocol
- Instrument-specific acquisition & quantitation methods

✓ Workflow Manager Met/DQ™

- Process guidance
- Automated technical validation

- All in one box
- Ready-to-use & user-friendly
- Standardized & quality controlled
- Only 10 µL sample required
- Validated for human plasma



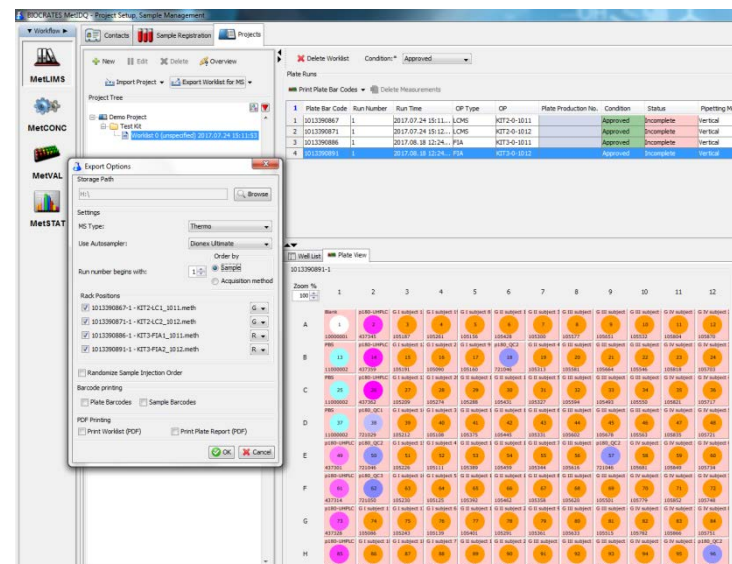
Absolute/DQ® p400 HR Kit

Workflow Manager Met/DQ™: Automated & Guided Process

Transparent, Automated Workflow

1. Information Management

- Sample registration, plate layout design
- Worklist generation



Transparent, Automated Workflow

1. Information Management

- Sample registration, plate layout design
- Worklist generation

2. Quantification & Data Processing

- Automated signal identification & quantification
- Data merging

3. Automated Technical Validation

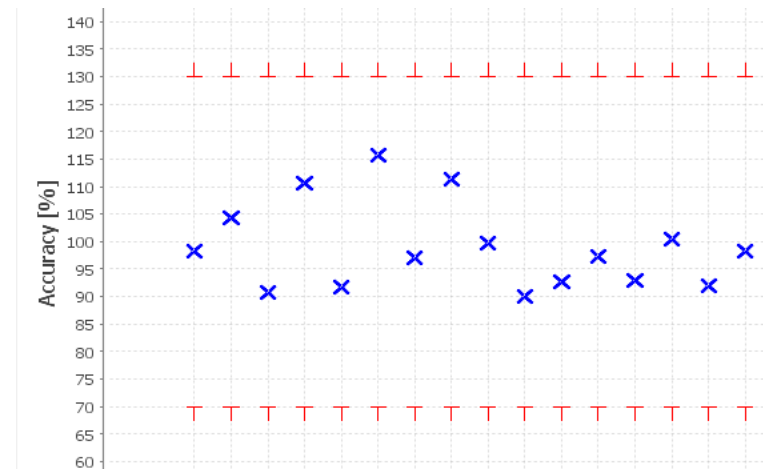
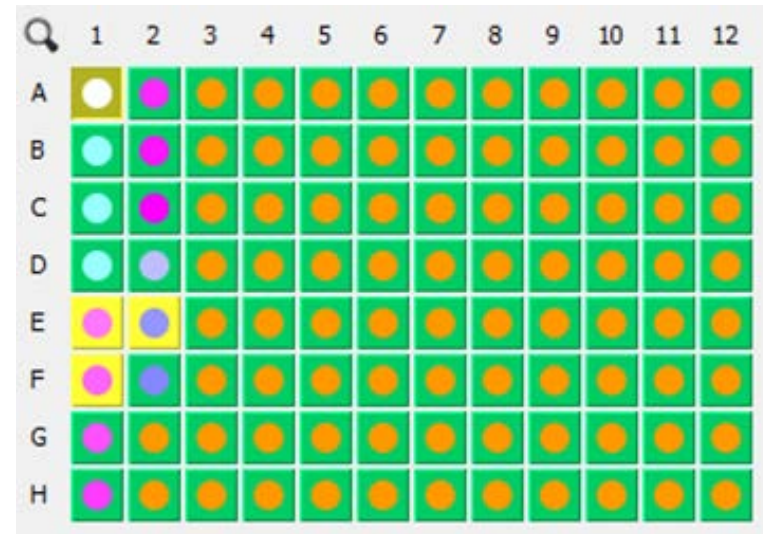
- Transparent display of generated data
- Analytical approval of the plate
- Inter-plate monitoring of QC results and precision

4. Data Evaluation and Export

- Data normalization (optional)
- Export of results in various file formats

5. Statistical Analysis (optional)

- Basic statistics & data visualization



Transparent, Automated Workflow

1. Information Management

- Sample registration, plate layout design
- Worklist generation

2. Quantification & Data Processing

- Automated signal identification & quantification
- Data merging

3. Automated Technical Validation

- Transparent display of generated data
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4. Data Evaluation and Export

- Data normalization (optional)
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5. Statistical Analysis (optional)

- Basic statistics & data visualization



Met/DQ™ Software

- ✓ Transparent, automated & guided process
- ✓ Provides quantitative results

- Reduced complexity & process safety
- Efficient & time saving
- High confidence in results

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Absolute/DQ® p400 HR Kit

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

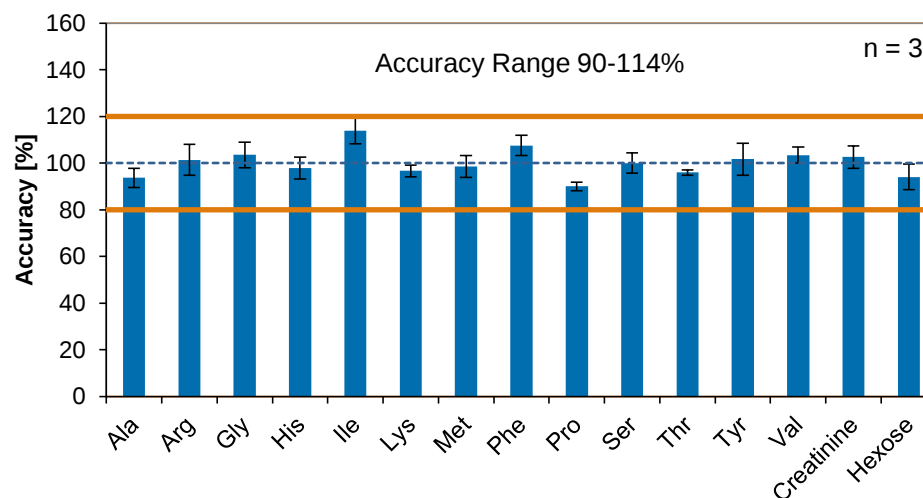
David Wishart (2015)



<http://www.wishartlab.com/system/images/W1siZiIsIjwMTMvMDMvMDYvMTBfMzJfMDBfNjE2X3dpc2hhcnQuanBnll1d/wishart.jpg>



NIST SRM 1950



<https://www.bioc.cam.ac.uk/people/images/griffin.jpg/@images/2b4df4dc-9f1a-4e50-a5f4-c2ce9a93f0a8.jpeg>

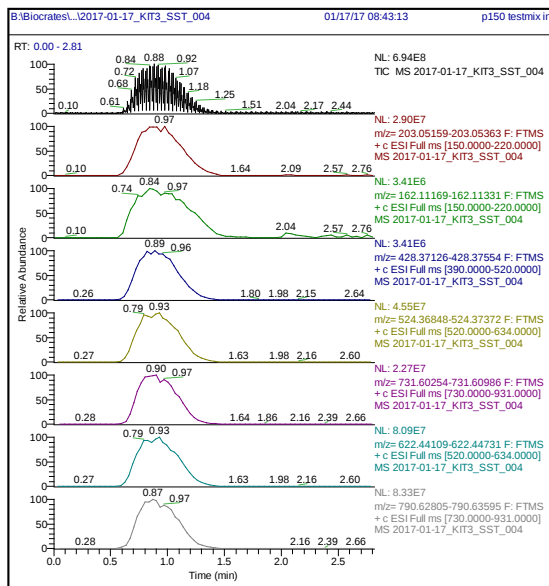
*"If we could get over this barrier [to not quantitate our data] then it will make **exchanging data between labs and studies** so much easier, and we could really move forward as a community."*

Jules Griffin (MetaboNEWS, Sept 2017)

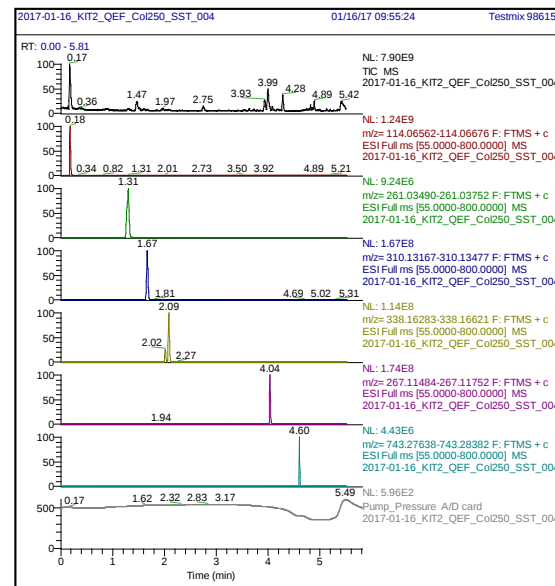
Absolute/DQ® p400 HR Kit

Analytical Performance: Inter-day Comparability

FIA-MS



LC-MS



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

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

✓ Inter-day comparability

Absolute/DQ® p400 HR Kit

Outstanding Inter-Lab Comparability

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LC-MS Part

- ✓ **3 labs, 5 instruments** (Q Exactive™ Focus & HF)
- ✓ Reference lab 1 (Q Exactive™ Focus)
- ✓ 6 individual human **plasma samples**

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

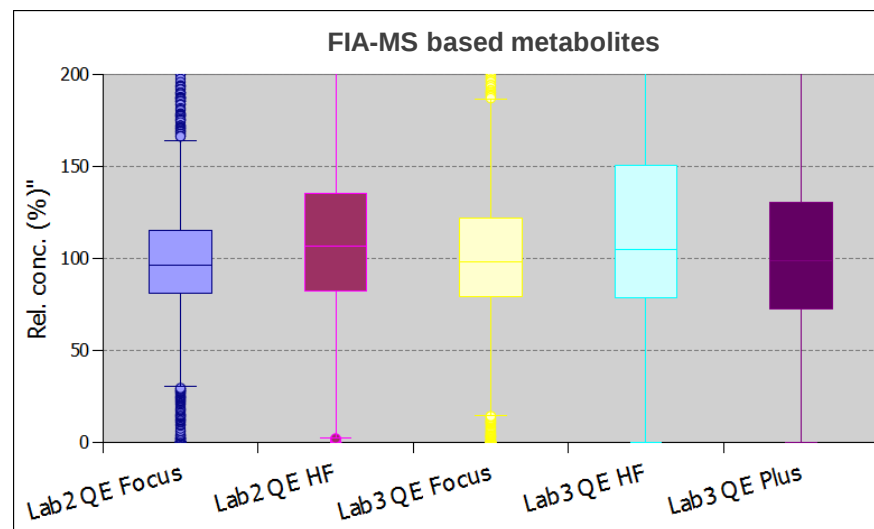
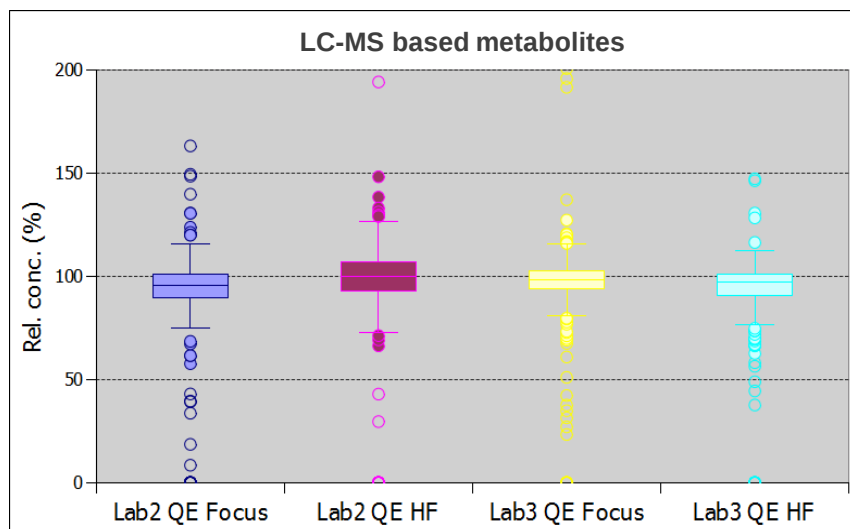
Resolution for LC-MS:
70k (Q Exactive™ Focus)
60k (Q Exactive™ HF)

FIA-MS Part

- ✓ **3 labs, 6 instruments** (Q Exactive™ Focus, HF & Plus)
- ✓ Reference lab 1 (Q Exactive™ Focus)
- ✓ 6 individual human **plasma samples**

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

Resolution for FIA-MS:
70k (Q Exactive™ Focus)
120k (Q Exactive™ HF)
70k (Q Exactive™ Plus)



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



CrossMark

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 [...].”



Worldwide Unique Ring Trials

Absolute/DQ® p180 Kit

- ✓ 6 laboratories, 3 instruments
- ✓ 7.6% CV
- ✓ Siskos et al. (2017), Anal Chem, 89: 1

Biocrates® Bile Acids Kit

- ✓ 12 laboratories, 4 instruments
- ✓ 8.3% CV
- ✓ Pham et al. (2016), J Appl Lab Med, DOI: jalm.2016.020537

- ✓ **First ring trial scheduled for Absolute/DQ® p400 HR Kit**

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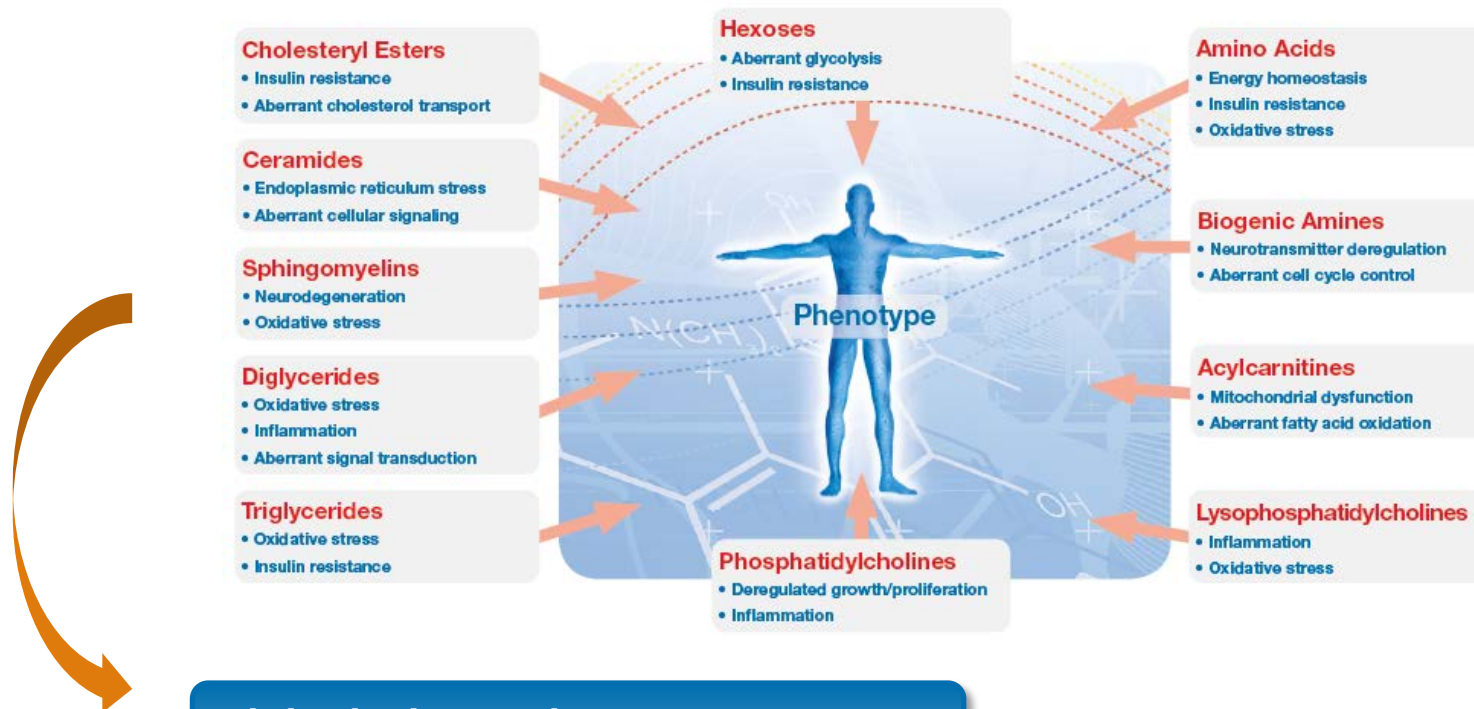
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Absolute/DQ® p400 HR Kit



Biological Functions

- ✓ **Membrane composition**
- ✓ **Cellular signaling**
- ✓ **Mitochondrial functions**
 - Mitochondrial beta oxidation of fatty acids
 - Bioenergetic processes



Oncology
Cardiology
Neurology
Aging
Diabetes
...

Absolute/IDQ® p400 HR Kit

Multiple Rate-Limiting Enzymes Covered

Rate-Limiting Step

Slowest step of an enzymatic reaction that determines the rate (speed) at which the overall reaction proceeds.



PLA2-Activity

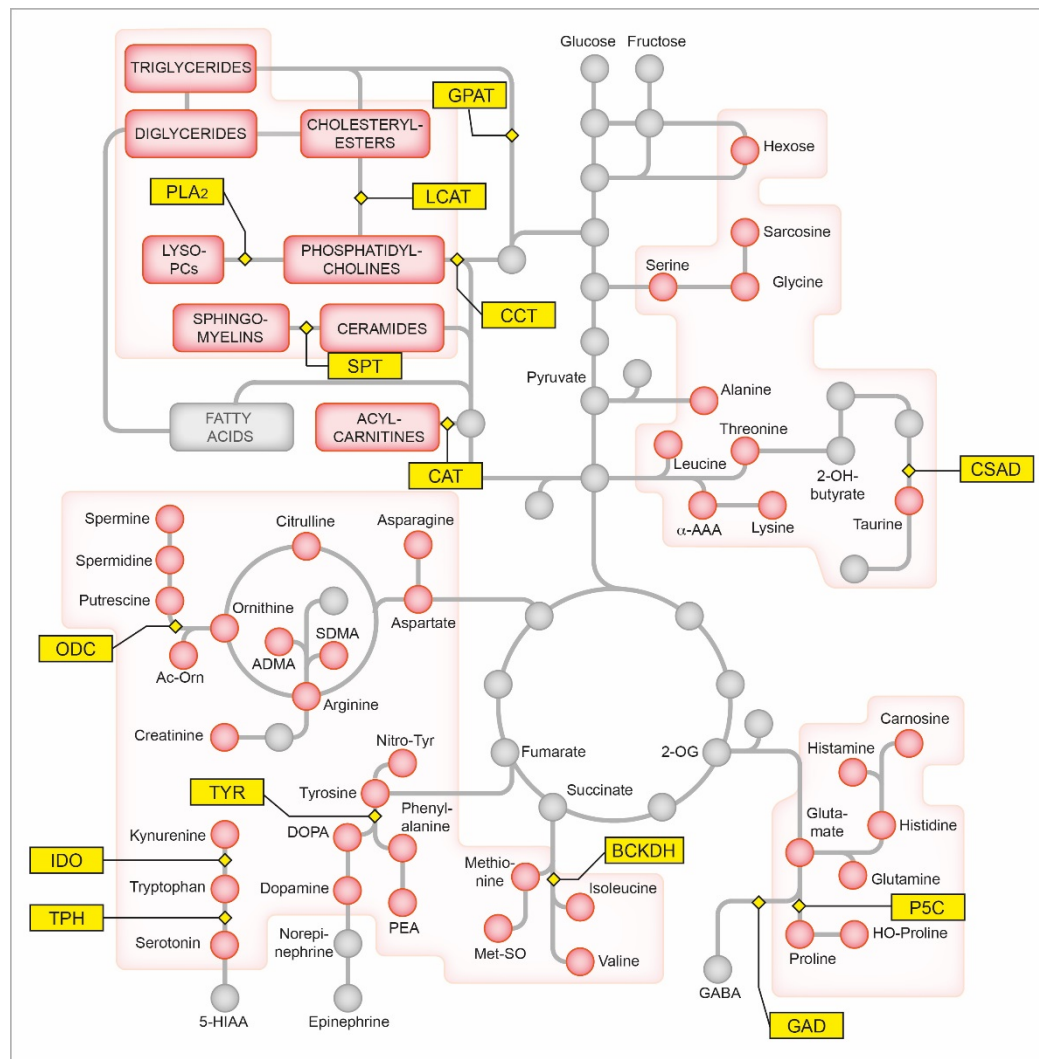
Ratio lysoPC/PC

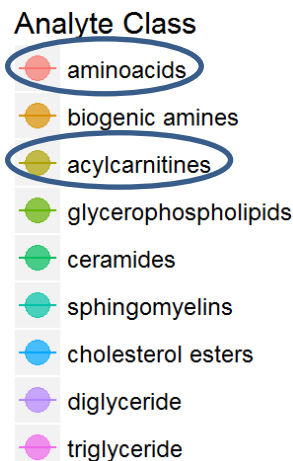
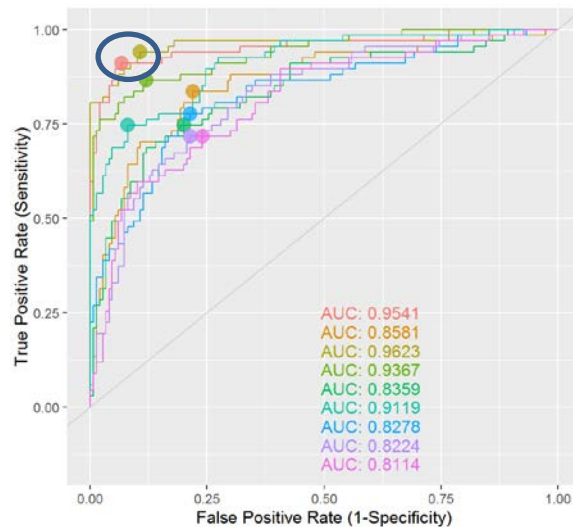
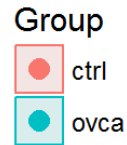
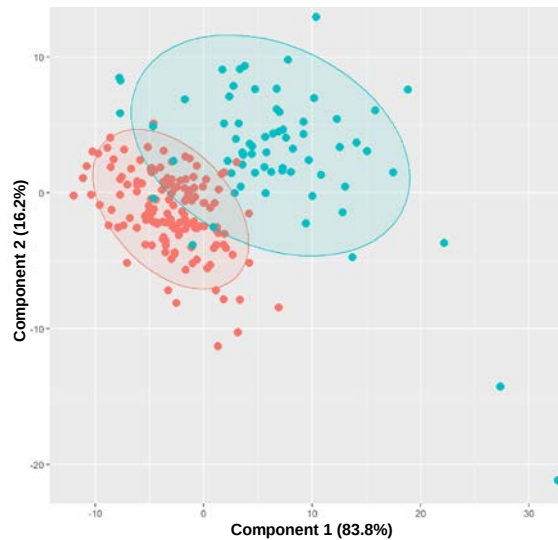
- lysoPC synthesis
- Inflammation

IDO-Activity

Ratio Kyn/Trp

- Tryptophan breakdown
- Immune modulation





Collaboration: Prof. Burwinkel, Heidelberg University, Germany

- ✓ Identification of **key metabolites** of **ovarian cancer** in plasma
- ✓ **AA and AC** are the top markers
- ✓ **Importance of affected pathways** in tumor pathophysiology and other diseases

Sample Description	Sample Number
Healthy Controls	150
Ovarian Cancer Patients	69



- ✓ **Unique metabolite portfolio of Absolute/IDQ® p400 HR Kit provides added value**

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Metabolomics enables precision medicine: “A White Paper, Community Perspective”

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„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 (PKU)**
→ 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 or C8/C12 > 1.6

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



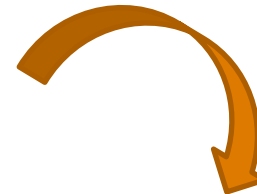
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$$\frac{\text{Val} + \text{Leu} + \text{Ile}}{\text{Phe} + \text{Tyr} + \text{Trp}} \rightarrow \frac{\text{BCAA}}{\text{AAA}}$$

Fischer ratio

↑ BCAA/AAA ratio with
↓ severity of liver failure



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

- The **Absolute/DQ® p400 HR Kit** is a **ready-to-use** solution designed for targeted metabolomics on Q Exactive Orbitrap HRAM MS.
→ *Ready to go 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 controlled, and targeted** 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 assures **longitudinal and inter-lab comparability**.

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

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Glenn Damkroeger
Dirk Brouwer
Neli Jochim
Heather Sumner
Klaus Lindpaintner

Thank you!

Contact us for more information

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We provide the
Phenotype to the Genotype

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Universal Selling Points of p400 HR

How to transport the messages?

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Feature	USP / Benefit
Standard Instrumentation	No additional investments required
Broad Installed base	Easy accessibility
408 endogenous metabolites from 11 metabolite classes - 365 lipids & 43 small molecules	Unique broad Metabolic and Lipid Coverage gaining insights in to Cell Signaling & Structural Information + Bioenergetics (Acyl-carnitine, AS & Biogenic Amines)
Complete Standardized & Quality controlled All-in-one Solution	Ready to use & user friendly (No instrument tuning and method development)
transparent streamlined Workflow Manager Met/DQ guided process	High confidence into analytical results
Automated Signal identification, -quantification and technical validation	High confidence into analytical results
96-well plate format, solid phase mediated sample preparation	High-throughput, multiplexing, small sample volume
Quantitative Results with a High Accuracy, Intraday comparability & multi center + instrument reproducibility	Robust Platform necessary for Longitudinal & Inter-lab/study comparability

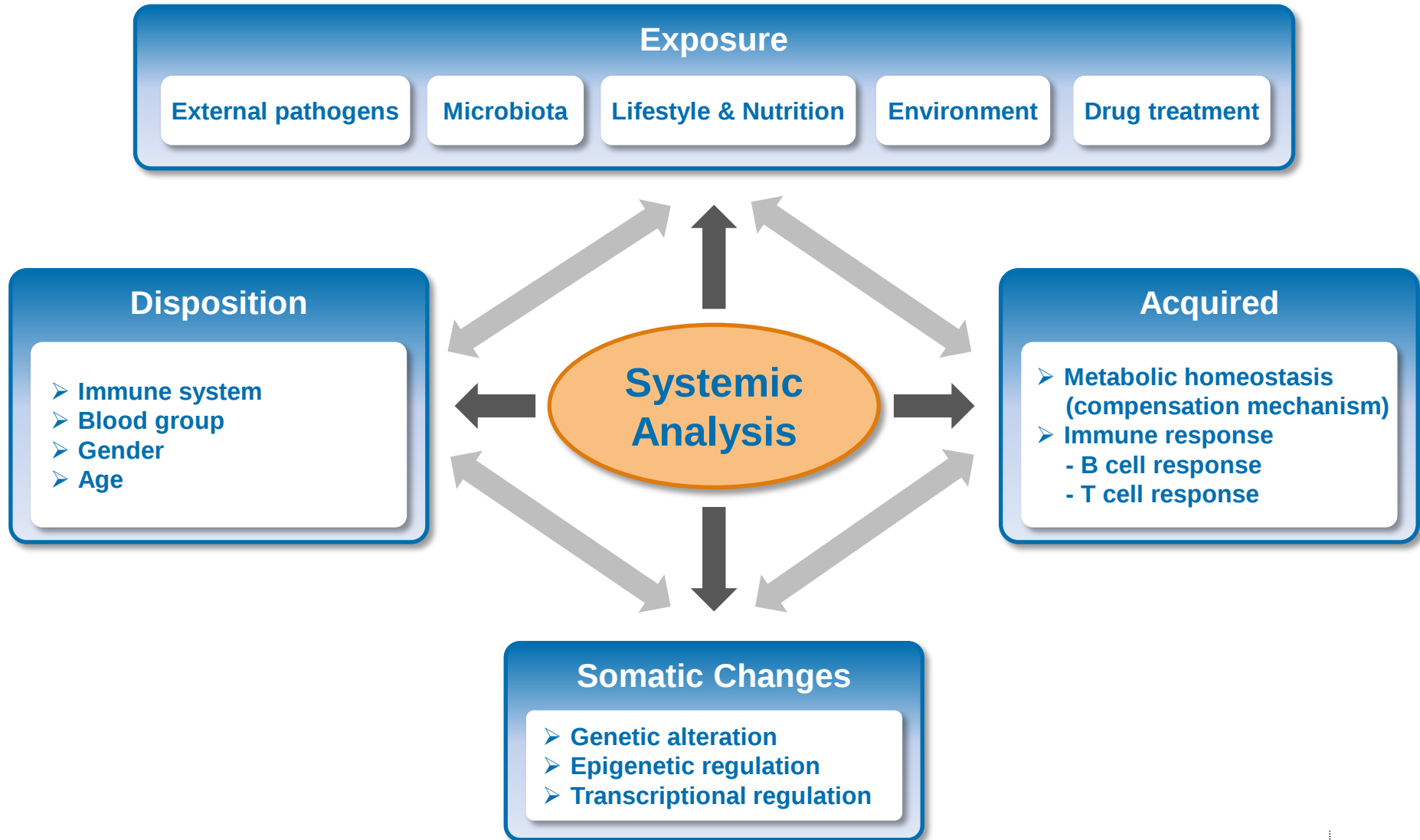
Metabolic Phenotyping

Surrogate Marker for Individual Phenotype

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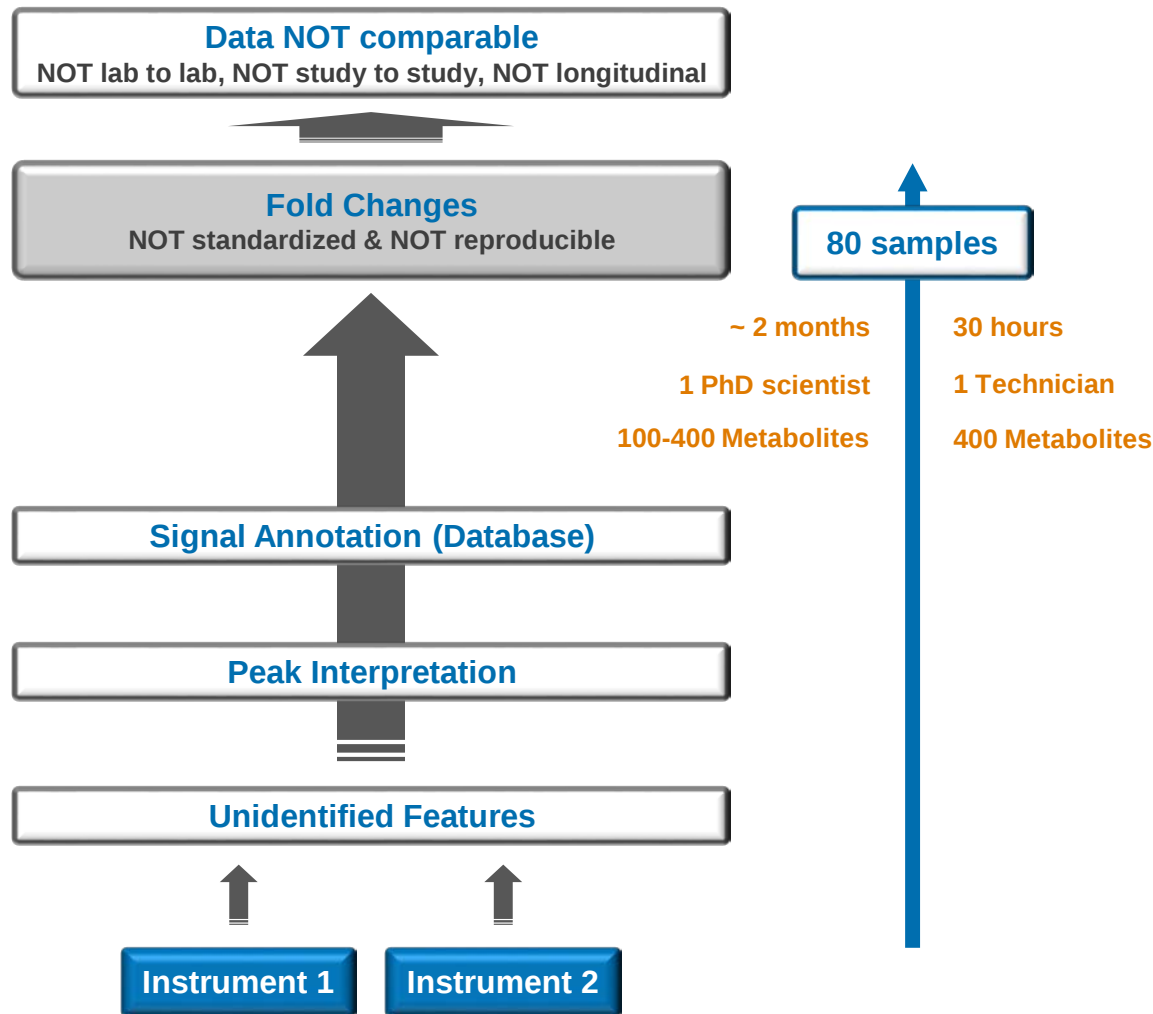
Targeted Metabolomics

Overcoming Challenges of Untargeted Metabolomics

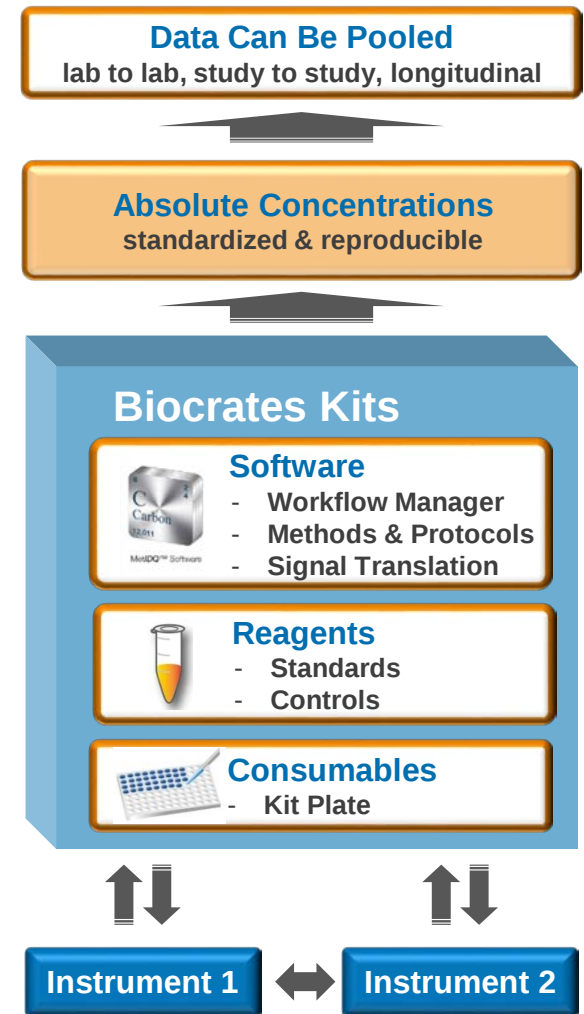
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Untargeted Metabolomics



Targeted Metabolomics

Cardiology

➤ SysVasc:

Identification of molecular targets for vascular disease treatment



➤ BiomarCaRE:

Biomarkers for cardiovascular risk assessment



Lifestyle & Genomics

➤ HELIX:

Impact of early environmental influences on health (incl. 6 European birth cohorts)



Neurology

➤ BrainAge:

Impact of prenatal stress on brain ageing



➤ ADNI (1, GO, 2):

Understanding of Alzheimer's disease and early diagnosis



Epidemiological Cohorts

- KORA (F3, S4, F4)
- UK Twins
- EPIC
- CHRIS
- Leiden Longevity Study
- PopGen
- ERF
- Fenland
- Others

Diabetes

➤ DeTecT2D:

Development of a new diagnostic tool for early detection of type 2 diabetes

Nephrology

➤ SysKid:

Understanding chronic kidney disease (diabetes and hypertension context)



Nutrition & Lifestyle

➤ Nutritech:

Effect of diet on "phenotyping flexibility"

