

### Kit workflow and the MetIDQ™ software

#### Content

- Company overview
- Targeted metabolomics
- Standardized assay technology
- AbsoluteIDQ® p180 kit I
  - Key benefits
  - Analytical coverage and performance
  - How it works
- AbsoluteIDQ® p180 kit II
  - Setup
  - Workflow
  - Assay preparation
- MetIDQ™ software live demo



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## Company overview

Company profile Technology



## Company profile



Biotech company for targeted metabolomics kit technology in Innsbruck, Austria



> 15 years of experience, biocrates technology was applied for thousands of publications and by hundreds of clients in pharma, academia and health institutions



Fully integrated mass spectrometry-based solutions for metabolic & functional microbial phenotyping across all indication areas



Kit technology empowers quantitative analysis of thousands of endogenous metabolites. Statistical analysis and biological interpretation is available.



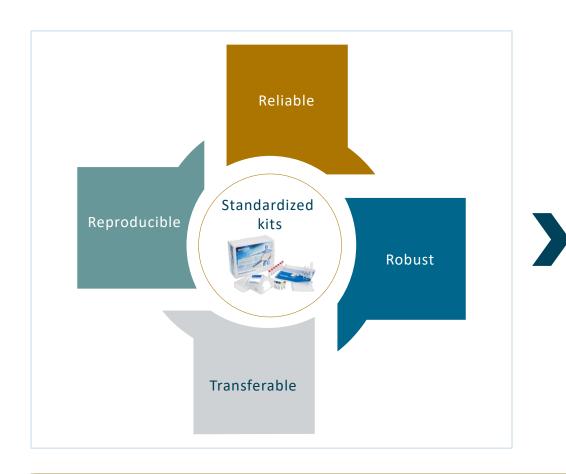
Our customer base includes global tier one pharma and academic customer as well as national health organizations.

A global community of more than 100 core facilities, CRO's, service customers & kit users to accelerate your science

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## Technology

biocrates develops, produces and distributes robust and high-performance kit tools for research purposes. This is THE standard for future diagnostic applications.





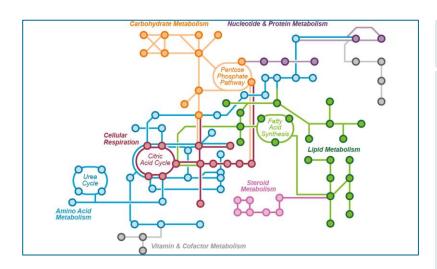
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## Targeted metabolomics

Metabolomics research
Why metabolomics matters
Metabolomic data interpretation

### Metabolomics research

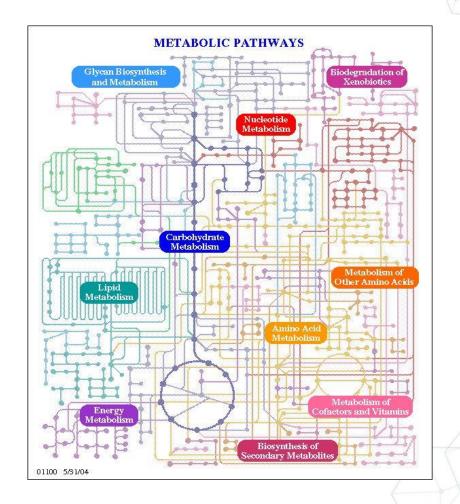
#### Metabolomics is the analysis of the transition of small molecules along defined pathways



- Metabolomics is the study/analysis of metabolites in biological systems
- Metabolites are low molecular weight organic molecules
- Metabolome is the total metabolites pool

#### Typical metabolites

- Sugars
- Nucleosides
- Organic acids
- Ketones
- Aldehydes
- Amines
- Amino acids
- Small peptides
- Lipids
- Sterols
- Terpenes
- Alkaloids



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### Why target metabolomics matters

#### Our approach

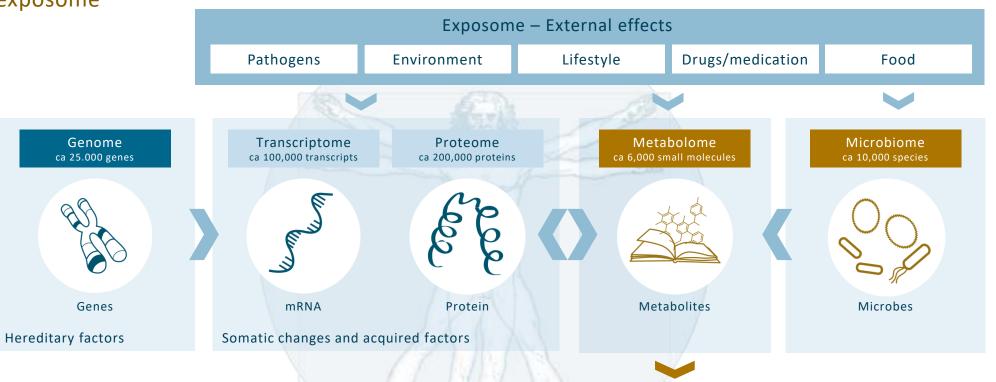
- Metabolomics is the most relevant tool to answer the multidimensional questions of system biology.
- From just a few microliters of sample material, the phenotype of an individual can be uncovered.
- Quantitative, standardized, and quality-controlled targeted metabolomics methods guarantee robust and reproducible results that are the cornerstones of all clinical research.
- biocrates provides mass spectrometry-based targeted metabolite analysis, with assays covering over
   1,000 metabolites from more than 30 metabolite classes.

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### Why metabolomics matters – The omics map

Metabolomics is functional readout of integrated genomic information under impact from

exposome



Metabolomics - The functional readout of nhenotype

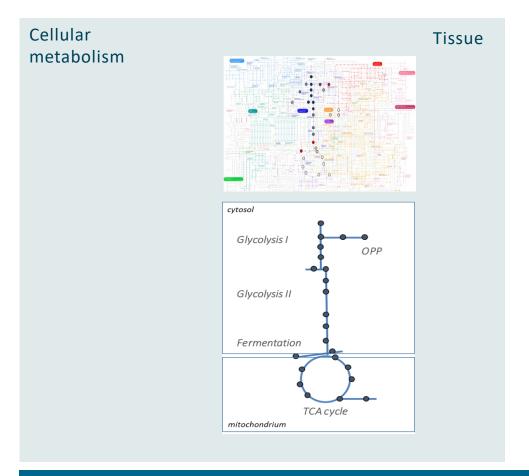
"What could happen? What could make it happen?"

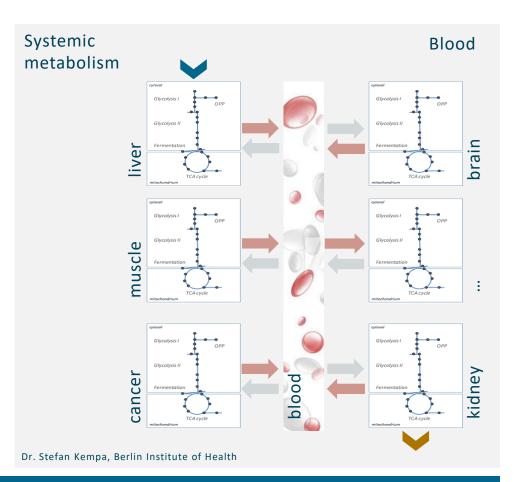
Causality - "What actually happens?"

Page 9 | biocrates For research use only | not for diagnostic procedures

### Why metabolomics matters – The source

Liquid biopsies provide access to pathophysiological status of individuals





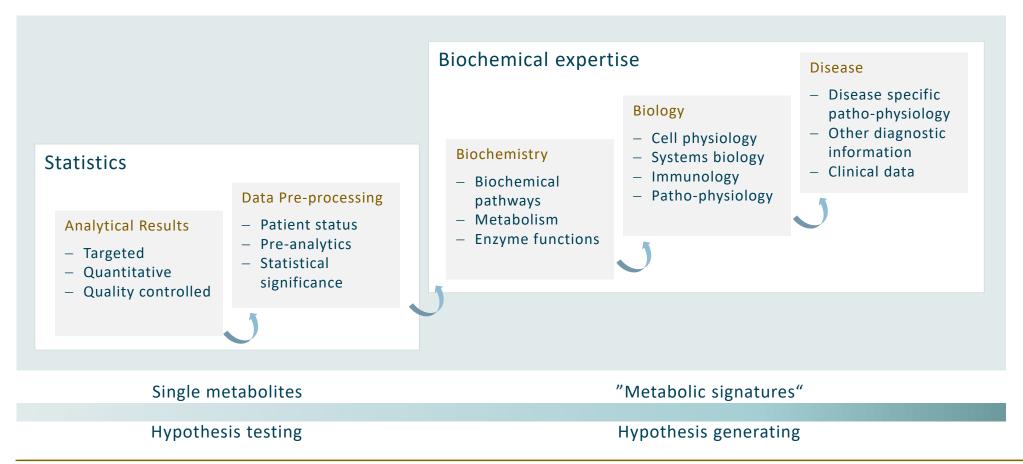
Systemic metabolism reflects energy homeostasis, immune response to disease and also functional impact of the

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### Metabolomics data interpretation

Disease know-how drives "metabolic signature"

Combined statistics and biochemical expertise required for state-of-the-art analysis



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## Standardized assay technology

Technological benefits
We standardized metabolomics
Scientific impact
Metabolomics benchmark



### Technological benefits

Connect the dots to bring research to a new level – Measure locally and connect science globally

biocrates ready to use kits



Standardized & reproduceable

Harmonized data format & data storage; simplified and biology-driven data analysis



Robust & quantitative

Comparability across instruments, labs and users Use in longitudinal, large-scale and validation cohorts



Decentralized multi-lab and multi-instrument approach

Local availability of platform

Pool data from multiple experiments, instruments & laboratory in one combined data base

Independent validation of third-party results

Total ownership of the results within a global network — Realizing the FAIR principles

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## Standardized targeted metabolomics

#### Standardized metabolomic data connect science globally



#### Kit components

- Reagents & consumables
- Methods & protocols
- Workflow manager
   MetIDQ

#### Laboratory environment

Inter-laboratory





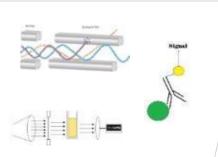
Inter-MS instrument







 All different technologies delivering absolute concentrations



#### Database MetIDQ

Harmonized & standardized data format in one data base

long time reliable for re-analysis



Combined analysis with other omics data (e.g. GWAS)

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## Scientific impact

### > 1,000 publications, > 27,000 citations and h-Index 79 support strong scientific impact of our



2018	European Heart Journal	Tomas et al.	Cardiology
2018	Cell Metabolism	Della Torre et al.	Hepatology
2018	Cell Metabolism	Raud et al.	Immunology
2018	Gut	Mayerle et al.	Oncology
2018	Alzheimer's & Dementia	Mahmoudian Dehkordi et al.	Neuroscience
2018	Alzheimer's & Dementia	Tynkkynen et al.	Neuroscience
2018	J Cachexia Sarcopenia Muscle	Pin et al.	Oncology
2018	Nature Communications	Mao et al.	Aging

Impact factor > 12.000



2018	PLoS Medicine	Varma et al.	Neuroscience
2018	JNCI	Lu et al.	Oncology
2018	PNAS	Willis et al.	Systems Biology
2018	PNAS	Skene et al.	Lifestyle
2017	Journal of Experimental Medicine	Bakiri et al.	Oncology
2017	European Respiratory Journal	Naz et al.	Pulmonology

Impact factor > 9.500



2018	EMBO Molecular Medicine	Jia et al.	Pulmonology
2018	PLoS Biology	Diessler et al.	Somnology
2018	Cancer Research	Mycielska et al.	Oncology
2018	BMC Medicine	DiBattista et al.	Epidemiology
2018	BMC Medicine	Lau et al.	Epidemiology
2018	Am J of Human Genetics	Romano et al.	Systems Biology
2018	EMBO Reports	Audano et al.	Systems Biology
2018	JASN	Li et al.	Nephrology
2018	Theranostics	Schaarschmidt et al.	Hepatology
2018	Int Journal of Epidemiology	Iqbal et al.	Epidemiology
2018	Cell Reports	Walters et al.	Aging
2018	International Journal of Cancer	Yuan et al.	Oncology

Impact factor > 7.000

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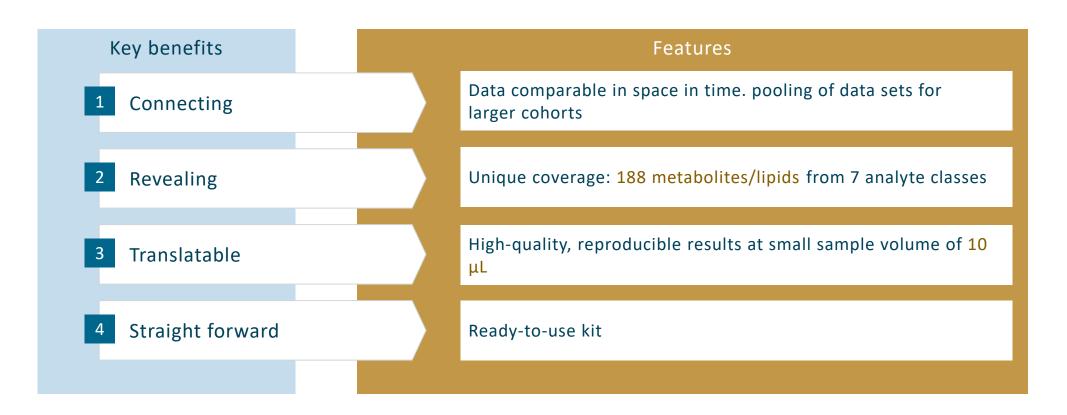
## AbsoluteIDQ® p180 kit

Key benefits
Analytical coverage
How it works
Analytical performance
Matrices



## p180 kit – Key benefits

Standardized quantitative metabolomics – Up to 188 quantitative metabolic features



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## p180 kit – Key benefits



All in one box



Excellent longitudinal & inter-laboratory reproducibility



High throughput & small sample volume of 10  $\mu$ L



Standardized, simple & automated workflow



### Kit components:

- Reagents & Consumables
  - Kit plate
  - Standardized samples: internal standards, calibration standards, and QCs
- Instrument methods
  - Ready to use LC-MS methods
  - Method optimization not required
- Documentation and support
  - Detailed user manuals
  - Video tutorials
  - Application notes for multiple sample matrices
  - biocrates support by specialists
- Workflow manager MetIDQ™ software
  - Project, sample, and plate management
  - Automated quantification and quality assessment
  - Data normalization
  - Export of results for easy data analysis

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## p180 kit – Analytical coverage

188 metabolites | lipids from 7 analyte classes

### LC part

42 small molecules (2 classes)

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



analytical column

### FIA part

146 lipids & hexose (5 classes)

- Acylcarnitines (40)
- Phosphatidylcholines (76)
- Lysophosphatidylcholines (14)
- Sphingomyelins (15)
- Monosaccharide (1)



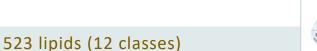
## Quant 500 kit – Analytical coverage

#### 630 metabolites/lipids from 26 analyte classes

#### Nutrition-microbiome-host interaction

#### 107 small molecules (14 classes)

- Alkaloids (1)
- Amine oxides (1)
- Amino acids (20)
- Amino acid related (30)
- Bile acids (14)
- Biogenic amines (9)
- Carbohydrates and related (1)
- Carboxylic acids (7)
- Cresols (1)
- Fatty acids (12)
- Hormones and related (4)
- Indoles and derivatives (4)
- Nucleobases and related (2)
- Vitamins and cofactors (1)



- Acylcarnitines (40)
- Phosphatidylcholines (76)
- Lysophosphatidylcholines (14)
- Sphingomyelins (15)
- Cholesteryl esters (22)
- Ceramides (28)
- Dihydroceramides (8)
- Hexosylceramides (19)
- Dihexosylceramides (9)
- Trihexosylceramides (6)
- Diglycerides (44)
- Triglycerides (242)

+ 234 Sums & Ratios

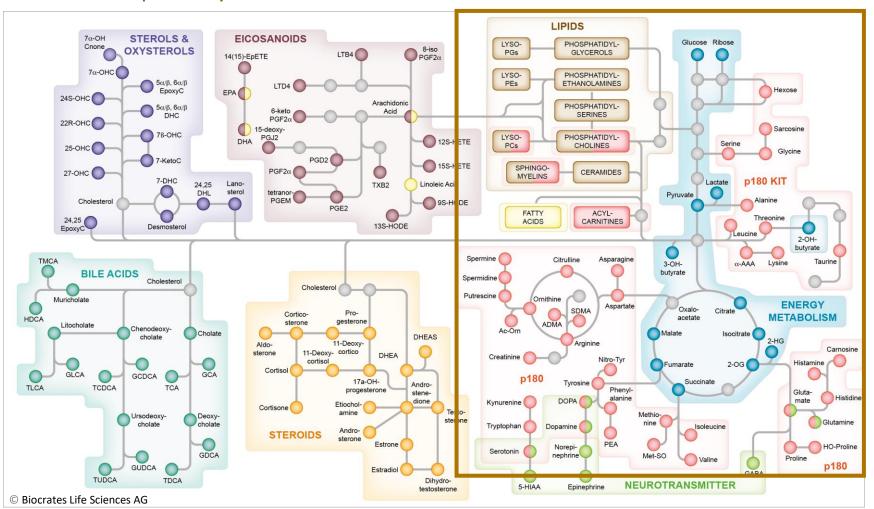
MetabolNDICATOR

produced or modified by gut microbiota



## p180 kit – Analytical coverage – Pathways

#### Broad coverage of metabolic pathways



p180 kit

### p180 kit – How it works, the workflow

#### 80 samples in 22 hours

#### Workflow initiation

- Create a new project workflow in MetIDQ™
- Register your samples

#### Data collection

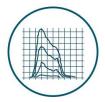
- Run plates with LC- and FIA-MS
- Instrument-specific methods are provided

#### Statistics & Interpretation

Perform advanced statistics:
 export from MetIDQ™ to statistics software of choice













#### Sample preparation

- Perform system suitability test
- Derivatize and extract your samples
- Pipette samples, standards, and
   QCs onto the plate

#### Quantitation & Validation

- Automated peak identification and quantification
- Automated sensitivity & accuracy check
- In-built data normalization suite

#### **Insight & Understanding**

- Make decisions based on standardized and reproducible metabolomic data
- Compare your results with published kit data from other laboratories

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## Quant 500 kit – How it works, the workflow

### 80 samples in 42 hours

#### Workflow initiation

- Create a new project workflow in MetIDQ™
- Register your samples

#### Data collection

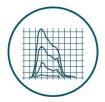
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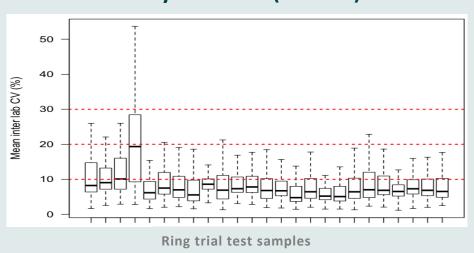
## p180 kit – Analytical performance

#### Ring trial across 6 international laboratories

# Inter-laboratory reproducibility of a targeted metabolomics platform for analysis of human serum and plasma

Alexandros P. Siskos, <sup>1§</sup> Pooja Jain, <sup>1§</sup> Werner Römisch-Margl, <sup>2</sup> Mark Bennett, <sup>3</sup> David Achaintre, <sup>4</sup> Yasmin Asad, <sup>5</sup> Luke Marney, <sup>6</sup> Larissa Richardson, <sup>6</sup> Albert Koulman, <sup>6</sup> Julian L. Griffin, <sup>6</sup> Florence Raynaud, <sup>5</sup> Augustin Scalbert, <sup>4</sup> Jerzy Adamski, <sup>7,8,9</sup> Cornelia Prehn, <sup>7</sup> Hector C. Keun<sup>1\*</sup>

### **Inter-Laboratory Precision (CV in %)**



#### Number of laboratories: 6

- Imperial College, UK
- Cambridge University, UK
- Institute of Cancer Research, UK
- Intern. Agency for Research on Cancer,
   France
- Helmholtz Zentrum München, Germany
- Biocrates, Innsbruck, Austria



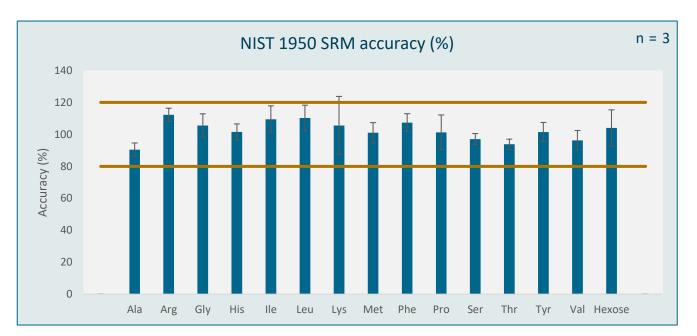
### **Inter-laboratory performance:**

**CV 7.6 %** 

Anal. Chem. 2017, 89, 656-665

## p180 kit – Analytical performance

#### Proven analytical accuracy





NIST SRM 1950

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

David Wishart (2015)

"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 2011)

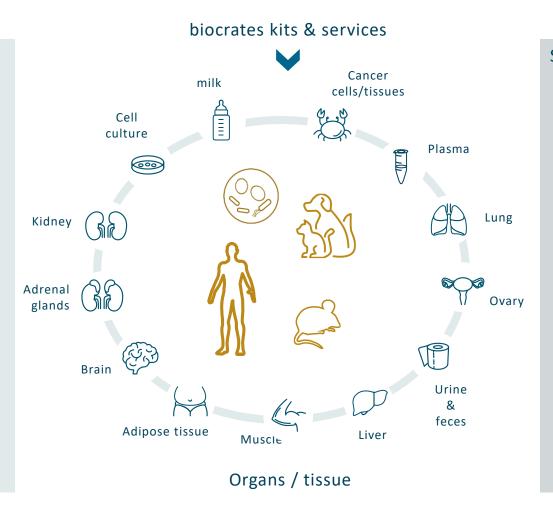
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## p180 kit – Matrices and species

#### Connecting biological systems

#### Matrices

- Plasma, serum (only 10 μL)
- Dried blood spots
- Cell culture medium
- Tissue
- Tumor tissue:
- Lung lavage (BALF)
- Skin samples
- Blister liquid / skins
- Feces
- Follicular fluid
- Milk
- Urine
- CSF
- Saliva
- Cells
- Cell culture supplements

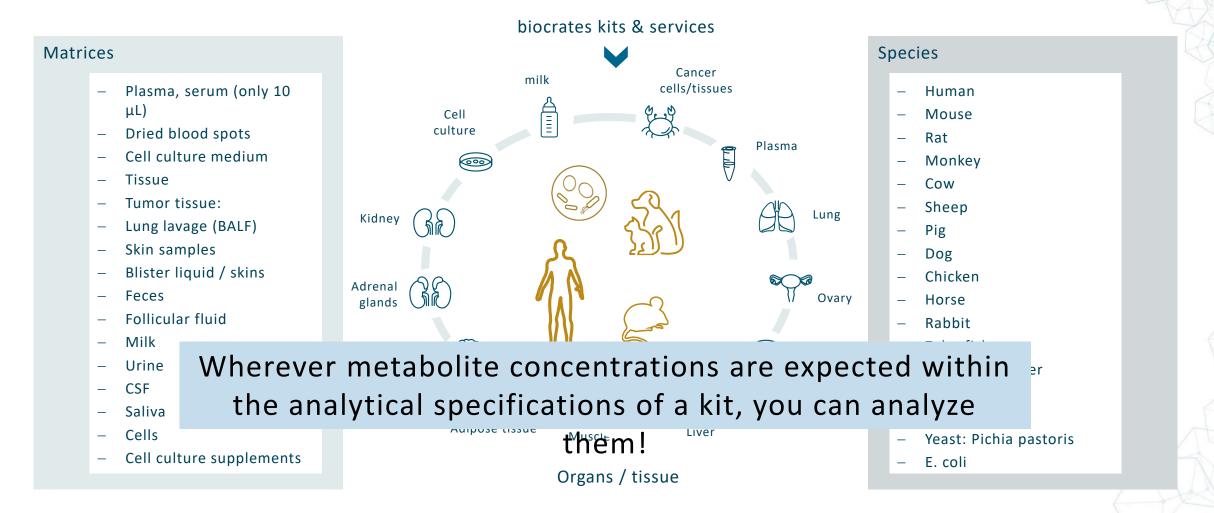


#### **Species**

- Human
- Mouse
- Rat
- Monkey
- Cow
- Sheep
- Pig
- Dog
- Chicken
- Horse
- Rabbit
- Zebrafish
- Chinese hamster
- C. elegans
- Soy
- Yeast: Pichia pastoris
- E. coli

## p180 kit – Matrices and species

#### Connecting biological systems



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## AbsoluteIDQ® p180 kit II

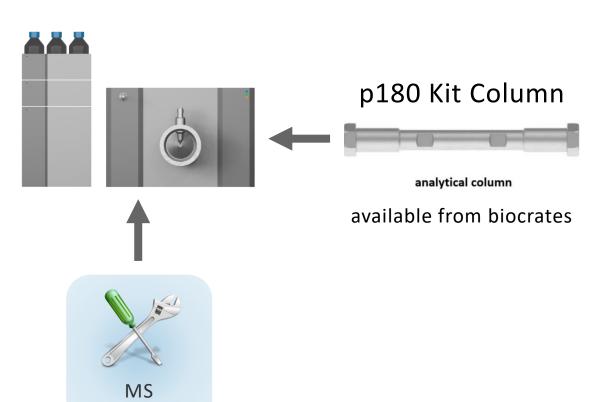
Setup
Workflow – preparation, measurements, data processing

Assay preparation – hints and tricks

### Setup – first time kit use only:

I. LC-MS instrument method setup

methods included



### Method setup:

- ✓ Instrument methods included in kit No tuning or optimization required
- ✓ Install methods according to user manual
- ✓ Assistance by customer support on request

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### Setup – first time kit use only:

### II. System suitability test (SST)



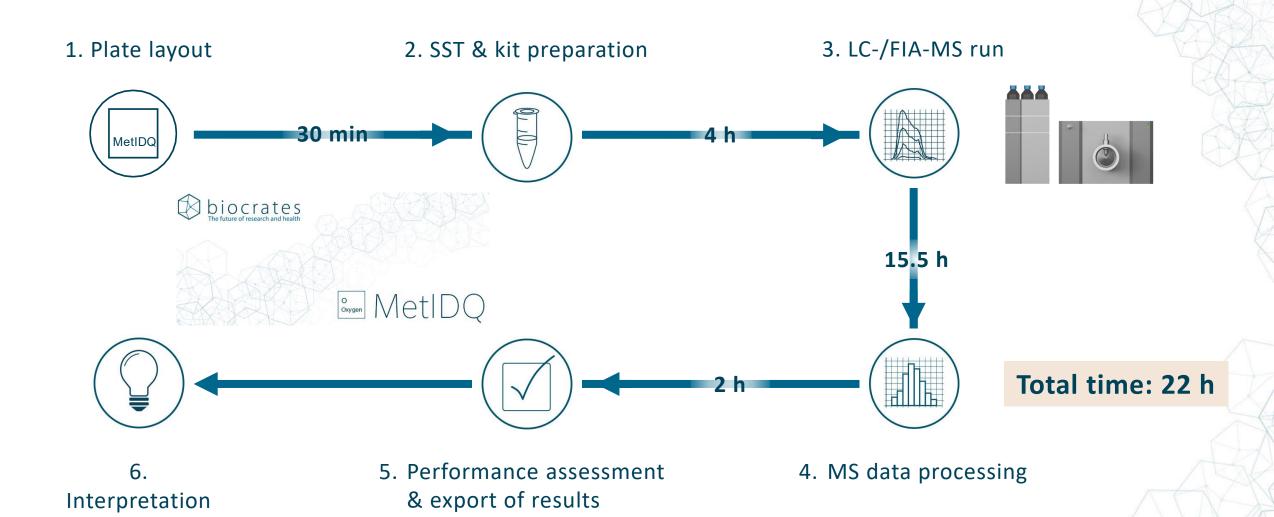




testmix FIA

### Method setup:

- ✓ Ready to use testmix
- ✓ Instrument cleaning instructions
- 1. Inject testmix
- 2. Adjust retention times (RTs)
- 3. Check instrument performance (SST)
- 4. Performance assessment together with customer support
- 5. Run the starter kit

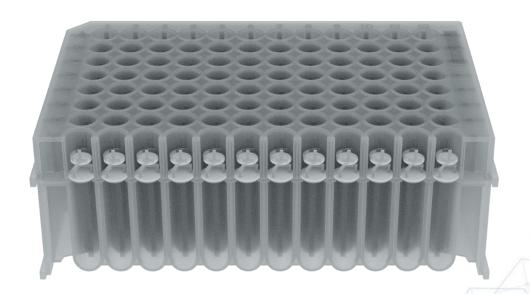


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### 1. Plate layout

### Kit plate design:

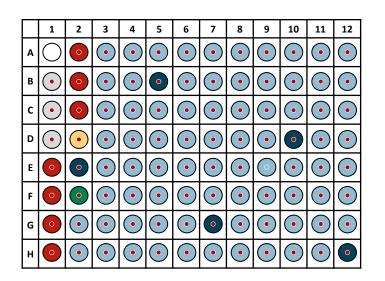
- All kits use 96 well plates
- Some wells are used for kit specific samples
  - blank
  - zero
  - calibration standards
  - quality controls (QCs)



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### 1. Plate layout

### Kit plate design:



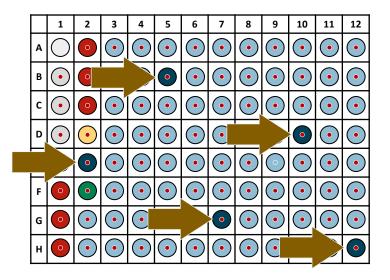
- blank
- zero samples
- calibration standards
- internal standards
- Up to 82 samples
  QCs in 3 levels:
- Olow
- medium
- high

78 – 82 samples can be analyzed per kit

### 1. Plate layout



Best normalization performance = best longitudinal comparability



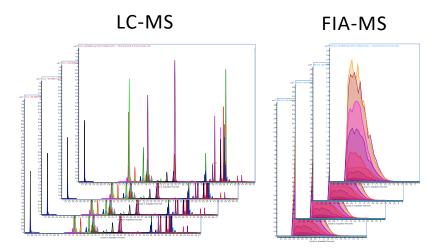
78 samples can be analyzed per kit

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### 1. Plate layout

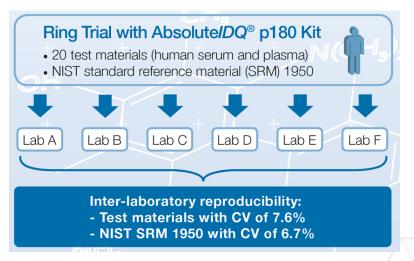
### 5x QCs replicates required for:

- Highly robust, quantitative results
- Excellent accuracy & precision





- Outstanding inter-instrument, interlaboratory, long-term reproducibility
- Applicable to multi-center, longitudinal epidemiological studies



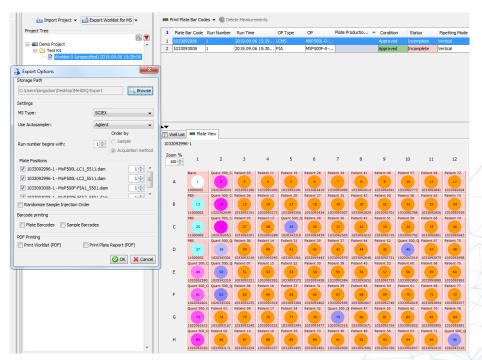
Siskos et al., Anal Chem 2017; 89(1):656-65

### 1. Plate layout



- 1. Register samples
- 2. Create plate layout
- 3. Export layout for kit pipetting and LC-MS runs

MetIDQ™ software



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#### 2. SST & kit preparation

System suitability test (SST)

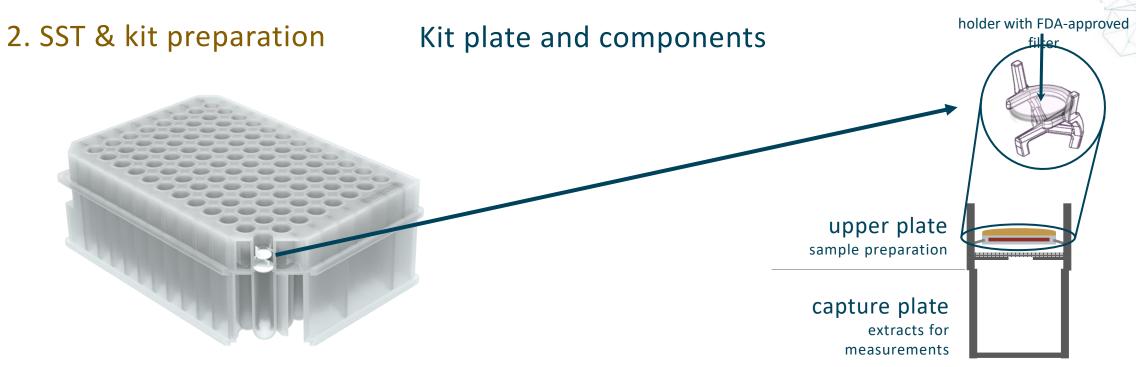






testmix **FIA** 

- ✓ If required, follow instrument cleaning instructions
- ✓ Use testmix
- 1. Inject testmix
- 2. Check retention times (RTs)
- 3. Check instrument performance (SST)
- 4. Run the kit



#### Advantages:

- Internal standards on plate
- Easy and fast sample preparation
- Efficient derivatization, drying and extraction
- Precipitated proteins are filtered

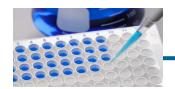


- Calibration standards (7 levels)
- Quality controls (3 levels)
- Testmix (2 vials)

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#### 2. SST & kit preparation

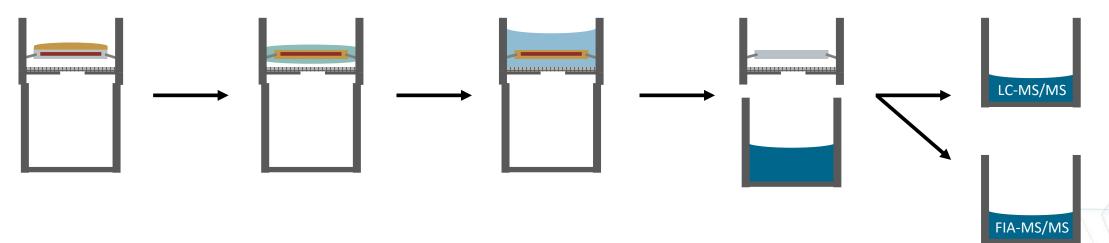
#### Kit preparation



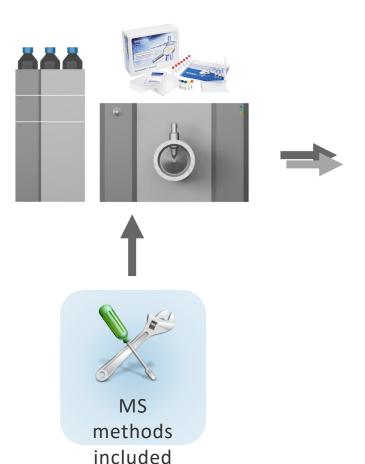
#### 4 h

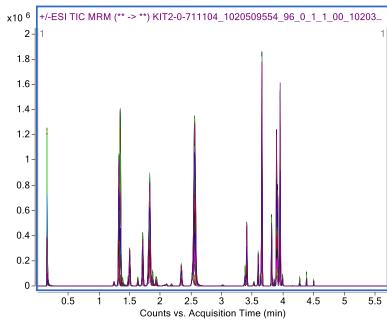
- 10 μL sample
- 10 μL ISTDs
- 30 min N<sub>2</sub> drying
- PITC derivatization
- 25 min incubation
- 60 min N<sub>2</sub> drying

- Extraction
- 30 min shaking
- Filtration
- Centrifugation or positive pressure
- Split extract
- Transfer to empty plates and dilute separately



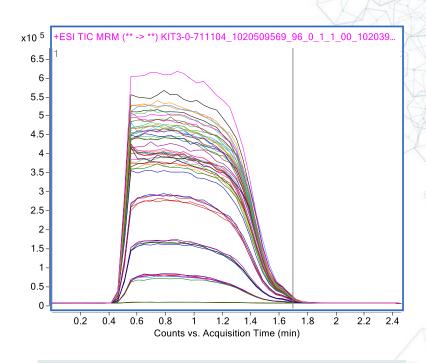
#### 3. LC-/FIA-MS run





#### LC-MS

- 6 min / sample
- 42 analytes
   amino acids, biogenic amines

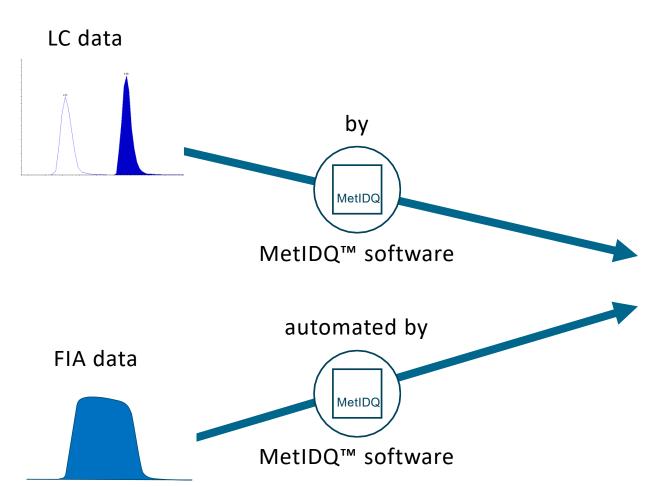


#### FIA-MS

- 3 min / sample
- 146 analytes
   acylcarnitines, hexose, glycol-&
   lysophospholipids, sphingomyelins

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#### 4. MS data processing



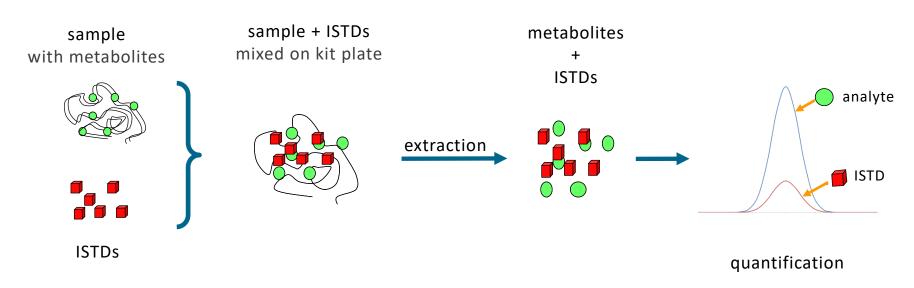


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#### 4. MS data processing

Quantification uses internal standards (ISTDs)

= "automated" intra-plate normalization

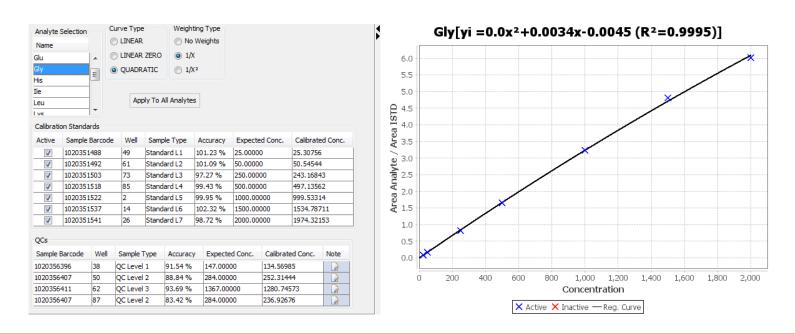


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#### 4. MS data processing

LC part:

- 7-point calibration + internal standards
- Internal standards → intra-plate normalization
- External standards (pure metabolites) → 7-point calibration
- Quality controls → performance assessment + inter-plate normalization



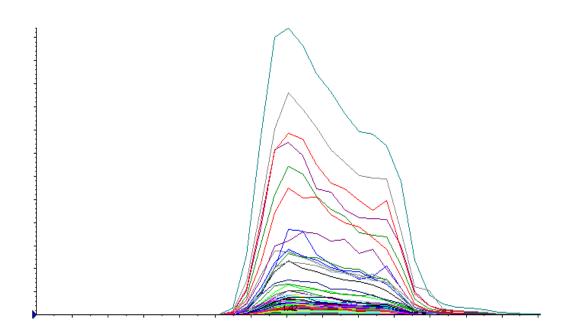
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#### 4. MS data processing

FIA part: — 1-point calibration + internal standards

- Internal standards → intra-plate normalization
- Quality controls → performance assessment + inter-plate normalization



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#### 4. MS data processing

Performance: LC part



quantitative
accurate & precise

Inter-plate data comparability:

- QC normalization recommended



quantitative
accurate & precise



relative-quantitative precise! accuracy?

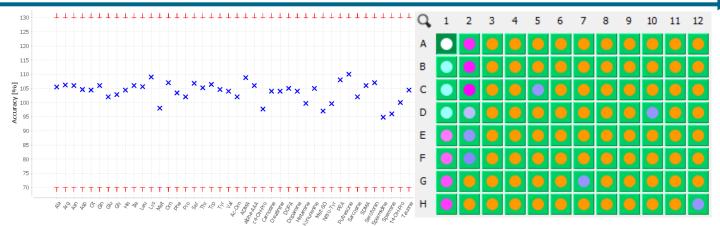
Inter-plate data comparability:

FIA part

QC normalization required

#### 5. Performance assessment & export of results





Thr	Trp	Tyr	Val	1-Met-His	3-Met-His	5-AVA	AABA	A
Aminoacids								
1.69	1.62	1.36	5.07	0.015	0.004	0.043	0.696	١
148	48.6	69.7	197	4.86	5.51	2.51	17.3	
306	230	239	411	9.87	11.5	4.79	32.9	,
150	50.4	66.8	153	7.04	2.54	0.671	4.83	١
111	39.6	103	121	3.90	1.88	0.259	10.1	
160	72.2	69.3	162	9.10	3.49	0.072	6.27	١
123	51.9	63.1	167	6.89	2.34	0.026	5.70	
91.5	46.6	40.3	107	7.78	2.92	0.082	8.43	
182	72.3	57.0	150	4.84	1.80	0.043	4.80	
122	42.5	42.3	123	4.10	1.44	1.18	4.91	
115	56.5	41.7	113	6.42	2.22	0.025	8.21	
161	59.0	87.4	177	6.01	2.67	0.043	8.16	
117	41.2	75.6	153	7.28	2.85	0.020	3.54	

#### Automated technical validation:

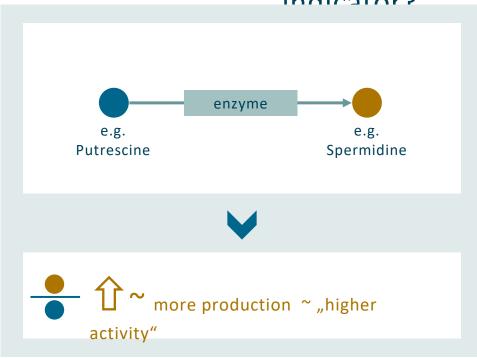
- blank checking cleanliness of system
- ✓ sensitivity checking internal standard intensities
- accuracy checking quality control results
- monitoring inter-plate monitoring of QC results and CVs

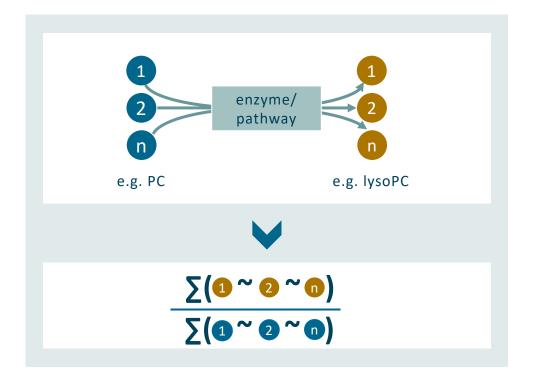
Analytical approval of the plate
Data normalization (recommended)
Export of results table in various file

#### 6. Interpretation

#### What is a metabolism

indicator?





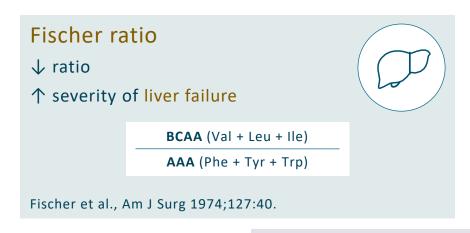
A metabolism indicator can act as a proxy for enzyme activity

Metabolism indicators allow to measure enzymatic activity with metabolomics

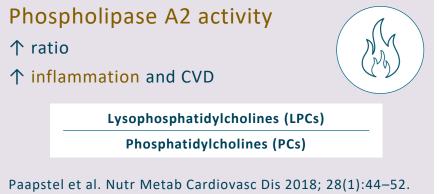
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#### 6. Interpretation

#### Make data coherent – Why quantitation matters







AAA: aromatic amino acids, BCAA: branched-chain amino acids, IDO: indoleamine 2,3-dioxygenase, CVD: cardiovascular disease

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Assay preparation – hints and tricks



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#### Assay preparation – hints and tricks

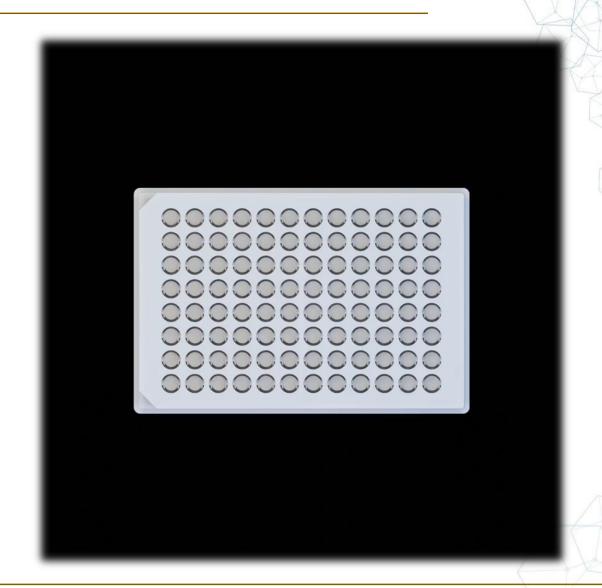
Kit plate - pipetting of samples:

- biocrates kit plate used
- sample volume: 10 μL



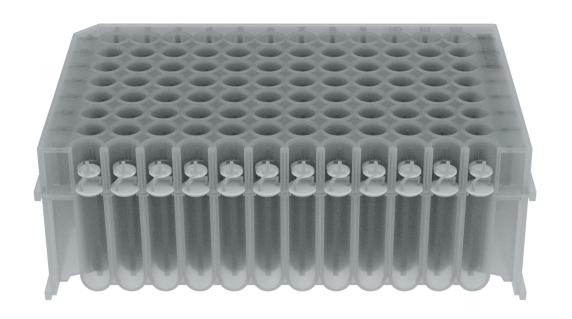


Assay preparation – hints and tricks

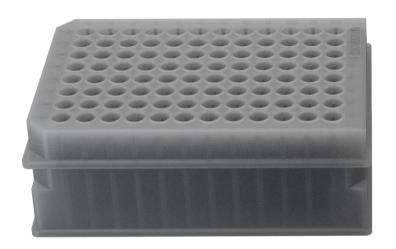


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Assay preparation – hints and tricks



### Assay preparation – hints and tricks



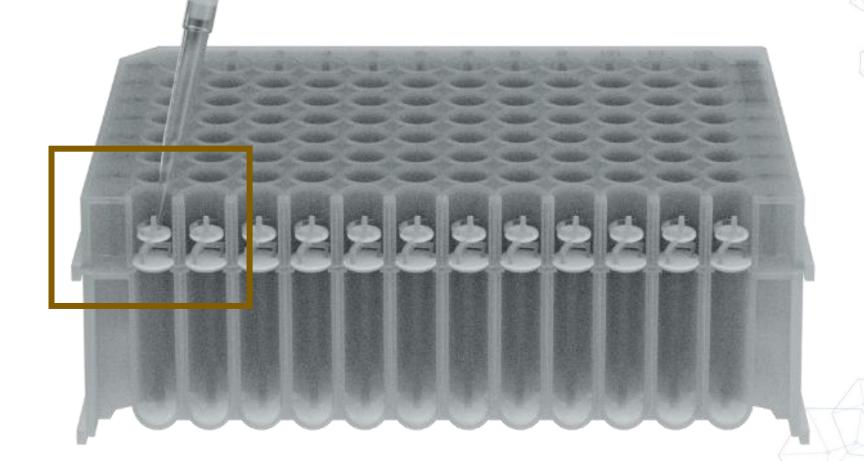


Assay preparation – hints and tricks



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Assay preparation – hints and tricks



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#### Assay preparation – hints and tricks

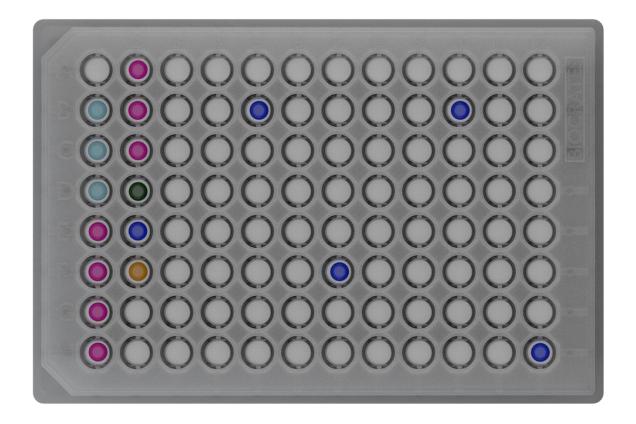
#### Standard and maximum sample volumes:

- Standard sample volume: 10 μL
  - for plasma and serum
  - unless otherwise noted in SOP
- Further matrices: what is the optimal sample volume?
  - perform pilot test using a small number of samples
  - try different sample volumes, e.g. 10, 20, 30 μL
  - for assistance, customer should contact customer support



Assay preparation – hints and tricks

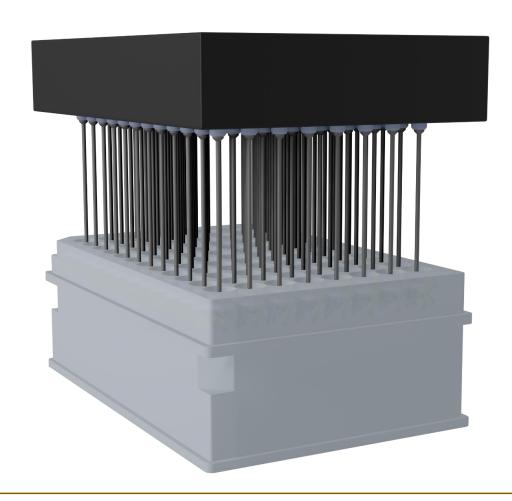
After sample preparation...



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### Assay preparation – hints and tricks

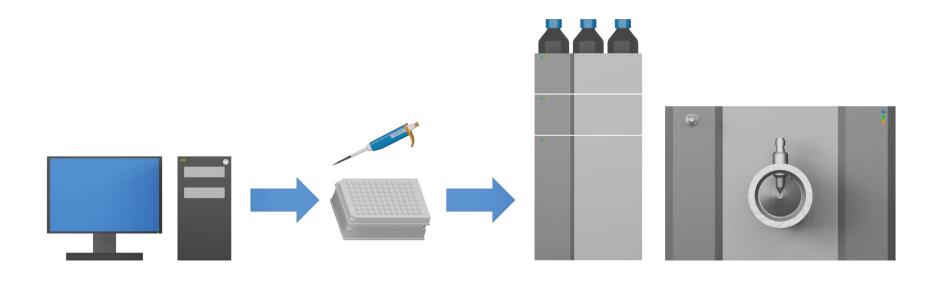
...drying of kit plate



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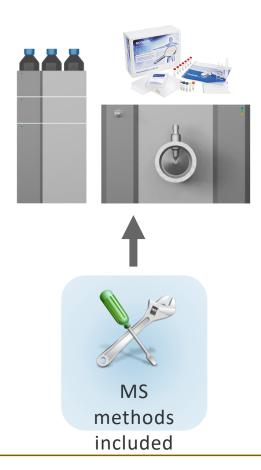
# p180 kit – summary

#### Lab workflow



## p180 kit – summary

#### Measurements



#### Instrument methods included in kit:

- Ready to use
- No tuning or optimization required

#### Method setup:

- Install methods according to user manual
- Adjust retention times (RTs) using testmix
- Instrument test: perform SST
- Assistance by customer support on request

## p180 kit – summary



All in one box



Excellent longitudinal & inter-laboratory reproducibility



High throughput & small sample volume of 10  $\mu$ L



Standardized, simple & automated workflow



#### Key features:

- 10 μL sample volume
- multiple matrices per plate
- up to 80 samples per kit
- 4 h sample preparation time, 2 hours handson
- one day sample analysis run time
- 2 h data automatized analysis time



high throughput

p180: up to 500 samples per week

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## MetIDQ™ software live demo



#### Workflow manager for targeted metabolomics

- Before sample measurement:
  - register samples and projects,
  - generate plate layout and worklist for mass spectrometer
- After sample measurement:
  - quantification and data processing
  - export of results



### MetIDQ™ software live demo



## 3 minutes break...



3 2 1

Stay tuned!



### Contact

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