‘FUNCTIONAL MICROBIOMICS’ – Standardized Assessment of Nutrition-Microbiome-Host Interplay by Targeted Metabolomics

BIOCRATES LIFE SCIENCES
The Deep Phenotyping Company

Tuan Hai Pham, Ulf Sommer, Doreen Kirchberg, Xenia Iwanowa, Radu Talmazan, Martin Buratti, Barbara Wolf, Therese Koal

BIOCRATES Life Sciences AG, Innsbruck, Austria

Introduction

Microbiome research has reshaped our understanding of how microbes impact on diseases. Metabolomics allows the investigation of microbial metabolic activities, and is thus the ideal tool to assess **functional nutrition-microbiome-host interactions**.

Here, we discuss the application of a newly developed standardized, quantitative targeted metabolomics approach in kit format (**MxP® Quant 500**) for multiplexed analysis of host and gut bacteria-derived metabolites and lipids by mass spectrometry in blood and feces. Gut microbiota-associated pathways, including **choline metabolism, tryptophan/indole metabolism, bile acid metabolism, and fatty acid metabolism** are covered by the assay.

Material & Methods

Samples

A sample volume of 10 µl has been applied:

- Human EDTA plasma
- Human fecal homogenate (prepared with ethanol phosphate buffer optimal for high to medium polarity analytes)

Experimental Setup

The assay has been validated for human plasma and combines liquid chromatography-tandem mass spectrometry (LC-MS/MS) and flow injection analysis (FIA-MS/MS) carried out on triple quadrupole MS instruments using multiple reaction monitoring (MRM) technology.

Here, the samples were measured by using the novel BIOCRATES MxP® Quant 500 targeted metabolomics kit on a UHPLC-SCIEX 5500 system.

MetIDQ™ Software

+

MxP® Quant 500 Kit

UHPLC-SCIEX 5500 System
(also applicable to (U)HPLC-SCIEX 6500(+) and Waters TQ-S; others in preparation)

Metabolite Coverage

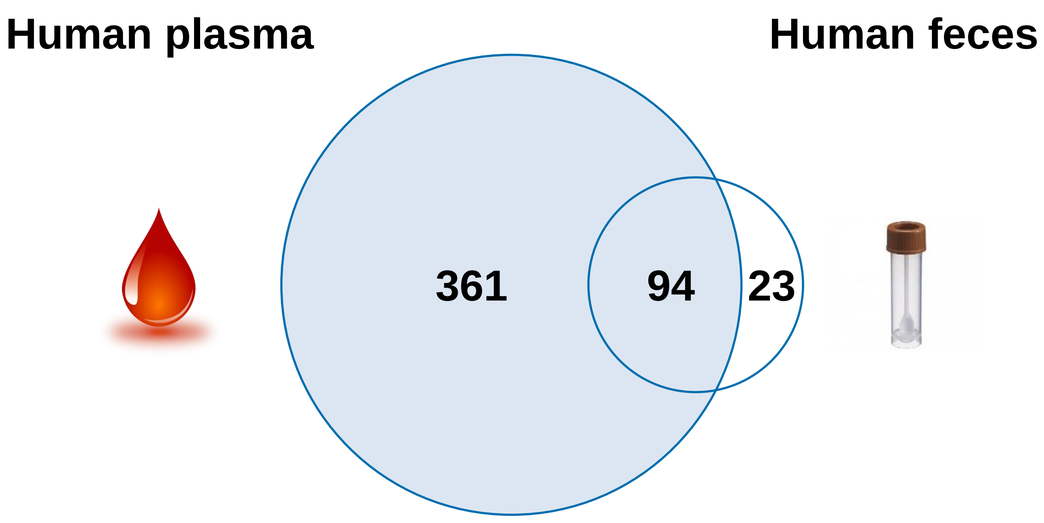
By using the kit up to 630 metabolites from 26 analyte classes can be analyzed, including numerous **microbiota-derived metabolites**:

- 106 small molecules from 13 analyte classes by LC-MS/MS
- Hexoses and 523 lipids from 12 analyte classes by FIA-MS/MS.

Results

Microbiota Metabolome Analysis

Human fecal samples were homogenized using an ethanol phosphate buffer-based extraction protocol optimized for small molecule analysis. In comparison to human plasma samples, ~80 % of the metabolites and lipids overlap:



Matrix Comparison

Metabolites and lipids >LOD were quantified with high precision (LC-MS/MS: CV< 20%; FIA-MS/MS: CV<30%):

	Analyte Class	Total	Human Plasma	Human Feces*
Small molecules	Alkaloids	1	0	1
	Amine Oxides	1	1	0
	Amino Acids	20	20	18
	Amino Acid Related	30	19	14
	Bile Acids	14	13	13
	Biogenic Amines	9	3	7
	Carbohydrates and Related	1	1	0
	Carboxylic Acids	7	3	1
	Cresols	1	1	1
	Fatty Acids	12	5	8
	Hormones and Related	4	2	0
	Indoles and Derivatives	4	3	2
	Nucleobases and Related	2	1	2
	Vitamins and Cofactors	1	1	1
	Sub-total	**107**	**73**	**68**
Lipids	Acylcarnitines	40	17	2
	Glycerophospholipids (Lysophosphatidylcholines & Phosphatidylcholines)	90	76	9
	Sphingomyelins	15	14	1
	Cholesteryl Esters	22	18	4
	Ceramides	28	15	12
	Dihydroceramides	8	1	1
	Glycosylceramides (Mono-, Di-, & Trihexosylceramides)	34	19	6
	Diglycerides	44	8	7
	Triglycerides	242	214	7
	Sub-total	**523**	**382**	**49**
	TOTAL	**630**	**455**	**117**

*Fecal extracts have been prepared with ethanol phosphate buffer optimal for high to medium polarity analytes. Use of different homogenization protocols may yield higher numbers of detected metabolites and lipids.

Functional Microbiomics

Nutrition and microbiota are two important confounding factors that impact human metabolism. To study the role of diet or host-microbiota interactions on a functional level, analysis of the metabolome, particularly with respect to **gut-liver axis** or **gut-brain axis**, are crucial.

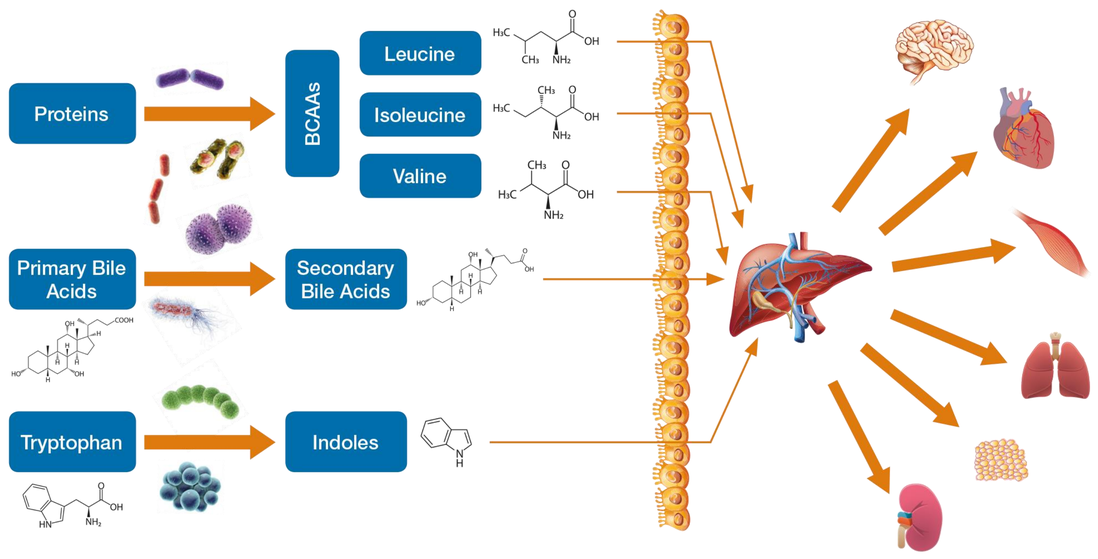


Figure: Adapted from Biral et al., Cell Mol Life Sci. 2018

Conclusions

- The **MxP® Quant 500 kit** validated for human plasma can also be applied to human feces, which allows for correlation studies.
- Metabolites and lipids quantified in human plasma **overlap to a large extent** with those in human fecal homogenate**.**
- **Optimization of extraction protocol** for fecal samples may yield higher numbers of detected lipids.
- The assay enables the analysis of **functional nutrition-microbiota-host interplay** as a basis for investigating the causal link between the microbiota and diseases.

BIOCRATES Life Sciences AG
Eduard-Bodem-Gasse 8
A-6020 Innsbruck, Austria

Tel: +43-512-57 98 23
Email: office@biocrates.com
Website: www.biocrates.com