BIOCRATES
LIFE SCIENCES
The Deep Phenotyping Company

The Easiest Way to Assess Host-Microbiome Interaction

Biocrates® Bile Acids Kit

Quantitative Profiling of Human and Rodent Bile Acids

The Easiest Way to Obtain Information About Host-Microbiome Interactions

Bile Acids are much more than detergents needed for the digestion of dietary fats. They are important signaling molecules, and relevant for many biological processes.

Such as steroid hormones and oxysterols, bile acids are involved in **cholesterol metabolism**. Via enterohepatic circulation, bile acids pass through the intestines up to twelve times a day, where they interact with the microbiome. In the presence of specific bacteria, the bile acids are chemically modified.

Because the gall bladder and biliary tract are part of the digestive system, bile acids have been extensively studied in gastroenterology, hepatology, and gastroenterological oncology.

Growing evidence suggests that **dysbiosis** is a driving force in the development of disorders such as diabetes, cardiovascular disease and neurodegeneration. Thus, bile acids are of increasing interest for researchers of many specialities.

The Biocrates® Bile Acids Kit is the first ready to use solution to **quantify 20 bile acids** in a standardized and high throughput fashion.

Ready to Use ✓ 

High Throughput ✓ 

Reproducible ✓ 

Minimal Sample ✓ 

About Bile Acids

The **synthesis of primary bile acids**, and the conjugation of bile acids with tauring and glycine, **takes place in the liver.**
The microbiota induces modifications (dehydroxylation and/or deconjugation), leading to the formation of **secondary bile acids**. The majority of the bile acids are actively resorbed in the small intestine, while some passive re-uptake of bile acids occurs in the colon.

In bile acid metabolism, there are important differences between humans and rodents. While humans have no enzymes to reverse microbial dehydroxylation, mice do. Further, mice have a more diverse set of primary bile acids.

Bile acids exert several biological functions by interacting with several receptors. The most important signaling pathway is through the **Farnesoid X Receptor (FXR receptor)**, a class of nuclear receptors. Genes involved in triglyceride and glucose homeostasis are examples where expression levels are governed by FXR. Bile acids can also bind to the **PXR receptor**, and a range of **G protein-coupled receptors (GPCR)**. As the affinity of bile acid species to these receptors differs considerably, knowing about the composition of the bile acid pool is of great value.

Main Application Areas

Apart from the use in nutrition and gastroenterology, bile acids profiling is most relevant in complex diseases known to be associated with nutrition and lifestyle.

BA Class/ Indication	Primary Bile Acids	Secondary Bile Acids	Conjugated Primary Bile Acids	Conjugated Secondary Bile Acids
Oncology	Bile Acids have long been implicated in carcinogenesis of gastroenterological cancers. Deoxycholic acid (DCA) and Litocholic acid (LCA) seem to be important in the context, representing a shift towards secondary bile acids. The role of bile acids in other cancers is little investigated, with a role for bile acids in immunity, oxidative DNA damage and nitric oxide stress making an invovlement probable. Research suggests the FXR receptor may be expressed in cancer tissues, as a regulator of apoptosis.			
Cardiology	Chenodeoxycholic Acid is the most potent ligand to the FXR receptor, which is expressed in cardiovascular organs. CDCA may increase angiogenic signaling.	Litocholic Acid (LCA) may regulate cardiovascular function via the Vitamin D receptor. Ursodeoxycholic Acid (UDCA) reduces myocyte apoptosis.	Taurocholic Acid (TCA) and Glycocholic Acid (GCA) reduce contraction rate, amplitude, and action potential duration in cardiomyocytes through altered electrolyte dynamics.	Taurodeoxycholic Acid may reduce contractility of cardiomyocytes and upregulate NO production.
Diabetes	Chenodeoxycholic Acid (CDCA) is reduced in diabetes.	Deoxycholic Acid (DCA) is increased in diabetes. Litocholic Acid (LCA) potently regulates GLP-1 secretion, as well as energy metabolism via the TGR5 receptor.	Tauromuricholic acid has antagonistic action on FXR, potentially altering insulin-sensitivity, glucose utilization and gluconeogenesis.	Taurolitocholic Acid (TLCA) regulates GLP-1 secretion via the TGR5 receptor. Tauroursodeoxycholic acid (TUDCA) may play a (preventative) role in NAFL.
Neurology	Cholic Acid (CA), Chenodeoxycholic Acid (CDCA) and Litocholic Acid (LCA) are the most important bile acids in the central nervous system. A role of bile acids in cognitive decline upon liver failure is well established.			

Table: Indications and relevant metabolites (literature available upon request)

Key Benefits

- **Provides comprehensive information - 20 analytes covered:**
  - Primary bile acids (5): produced by the liver
  - Primary conjugated bile acids (5): primary bile acids transformed by liver enzymes
  - Secondary unconjugated bile acids (4): primary bile acids transformed by bacteria
  - Secondary conjugated bile acids (6): produced by bacterial and liver transformation
- **High throughput:** up to 1,000 samples per week
- **Suitable for translational studies:** human and rodent bile acids covered
- **Low sample amount:** 10 to 20 µL serum/plasma
- **Ready to use**, standardized Kit
- **Proven reproducibility** (Pham TH et al., Journal of. Applied Lababoratory Medicine 2016)

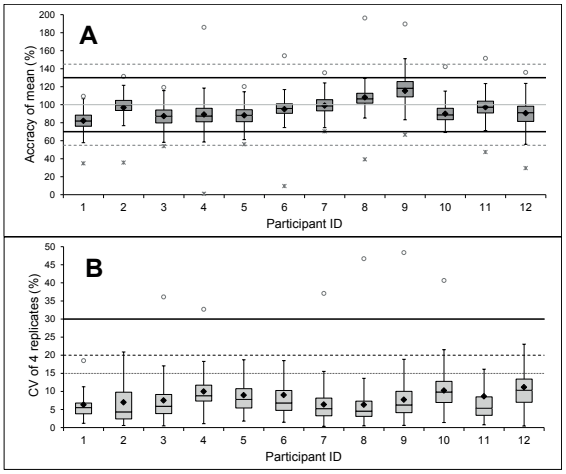


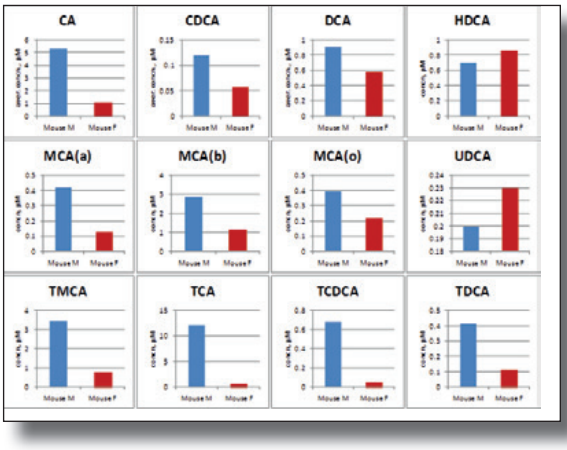
Figure: Accuracy (A) and Reproducibility (B) of Biocrates® Bile Acids Kit across 12 laboratories

Relevance of Bile Acids Profiling

Shortcomings of clinical routine

The measurement of the total bile acids pool is clinical routine. However, the total amount of bile acids in blood does not take into consideration that the origin and biological function of individual bile acids is quite different. For example, the affinity of bile acids to the FXR receptor differs more than 250-fold.
As the total bile acid concentration may stay unaltered even if composition of bile acids is considerably changed, the knowledge of the composition of the bile acids pool can provide important information about production and metabolism of bile acids by both the microbiome and the liver.

Mouse plasma: male vs. female



Bile acids and immunity

The gut is the largest immune organ of higher organisms, to which the microbiome contributes. Bile acids have immune modulatory properties, and as the figure on the side shows, individual bile acids levels can be quite different between the sexes. This may contribute to the gender difference in the incidence of auto-immune disorders.

Bile acids and toxicity

As hepatotoxicity is among the main reasons for drug attrition, bile acids profiling has become a standard procedure in pharmaceutical research. Liver injury leads to increased bile acid levels and altered bile acid pools. In this context it is important to keep the differences between humans and rodents in mind.

Technology

The Biocrates Bile Acids Kit allows for the analysis of up to 20 bile acids in a single run. To do so, it uses liquid chromatography-mass spectrometry, a technology widely available at clinical, academic and industry laboratories. The Kits have been validated on multiple LC-MS platforms from leading vendors.

We will be pleased to advise you on the detailed requirements to measure the Kits in your own laboratory. Should you not have suitable instrumentation, you can run the Kit via a partner laboratory, or in Biocrates' Metabolic Phenotyping Services laboratory in Innsbruck, Austria.
Contact us for further information!

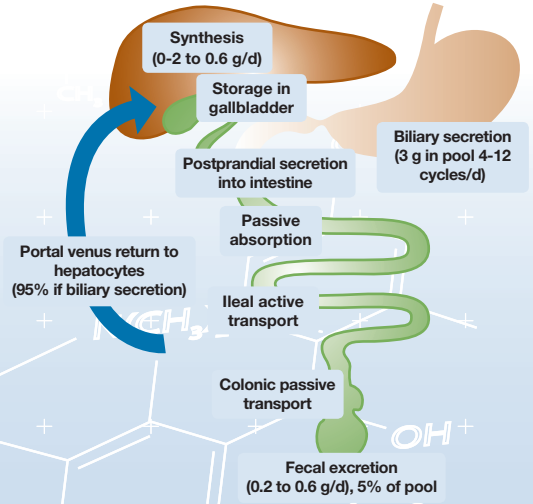


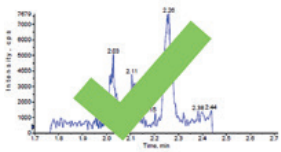
Figure 1: Based on Li and Chiang, Pharmacol Rev. 66:948-983, October 2014

Workflow (for measurement in your own laboratory)

Sample Preparation (4 hours)

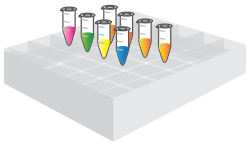
First Steps

Samples are registered in the Met*IDQ*® software (provided with the Kit). The testmix is used to ensure suitable system conditions.



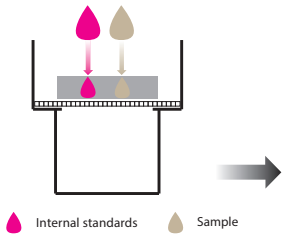
Preparation

Calibration standards and quality controls (provided with the Kit) are reconstituted. Samples are prepared.



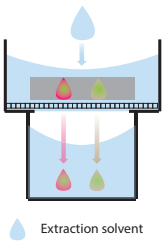
Loading

Samples and all reagents are pipetted onto the filter plate.



Extraction

Methanol is added to extract the bile acids.



Sample Processing (12-22 hours)*

Run Samples

Samples are measured with the provided (U)HPLC-MS/MS method.

Quantify

Data are processed with the provided quantitation method.

* Depending on analytical platform

Data Analysis (3 hours)**

Quality Control

Results for calibration standards, quality controls and samples are evaluated.

Reporting

Statistical analyses can be performed in Biocrates' software tool Met*IDQ*®, or in other statistical softwares.

** Minimum time for quality control and export of data

Covered Analytes

Type of BA[1)]	Analyte	Name	Internal Standard	Human plasma/ serum	Mouse plasma
P	CA	Cholic acid	d5-CA	✓	✓
P	CDCA	Chenodeoxycholic acid	d5-CDCA	✓	✓
P	MCA(a)	Alpha-Muricholic acid	d5-CA		✓
P	MCA(b)	Beta-Muricholic acid	d5-CA		✓
P	MCA(o)	Omega-Murichoclic acid	d5-CA		✓
S	DCA	Deoxycholic acid	d5-CDCA	✓	✓
S	LCA	Lithocholic acid	d4-LCA	✓	✓
S	HDCA	Hyodeoxycholic acid	d4-HDCA(b)		✓
S	UDCA	Ursodeoxycholic acid	d4-HDCA(b)	✓	✓
CP	GCA	Glycocholic acid	d5-GCA	✓	✓
CP	GCDCA	Glycochenodeoxycholic acid	d4-GLCA	✓	
CP	TCA	Taurocholic acid	d5-TCA	✓	✓
CP	TCDCA	Taurochenodeoxycholic acid	d5-TCDCA	✓	✓
CP	TMCA (a+b)	Tauromuricholic acid (sum of alpha and beta)	d5-TUDCA	✓	✓ **
CS	GDCA	Glycodeoxycholic acid	d4-GLCA	✓	✓
CS	GLCA	Glycolithocholic acid	d4-GLCA	✓	✓
CS	TDCA	Taurodeoxycholic acid	d5-TCDCA	✓	✓
CS	TLCA	Taurolithocholic acid	d4-GLCA	✓	✓
CS	GUDCA	Glycoursodeoxycholic acid	d4-GUDCA	✓	✓
CS	TUDCA	Tauroursodeoxycholic acid	d5-TUDCA	✓	✓ *

[1)] P = primary bile acid, S = secondary bile acid, CP = conjugated primary bile acid, CS = conjugated secondary bile acid

* Partial coelution with THDCA under UHPLC conditions, quantifiable only under HPLC conditions
** semi-quantitative for Waters Xevo™ TQ MS
Generally present at very low concentrations (close to or < LLOQ) in healthy samples
Generally present concentrations well above LLOQ in healthy samples

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Products

Product	Product number
• Biocrates® Bile Acids Kit (96)	9120052120813
• Biocrates® Bile Acids Kit (56)	9120052120905
• Biocrates® Bile Acids Kit (U)HPLC Column	9120052120868
• Biocrates® Bile Acids Kit (U)HPLC Precolumn	9120052120875
• Biocrates® Bile Acids Kit (U)HPLC Setup Box	9120052120882

The Biocraets Bile Acids Kit is designed for research use only.
Not for in-vitro diagnostic use

If you want to learn more, contact us!

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