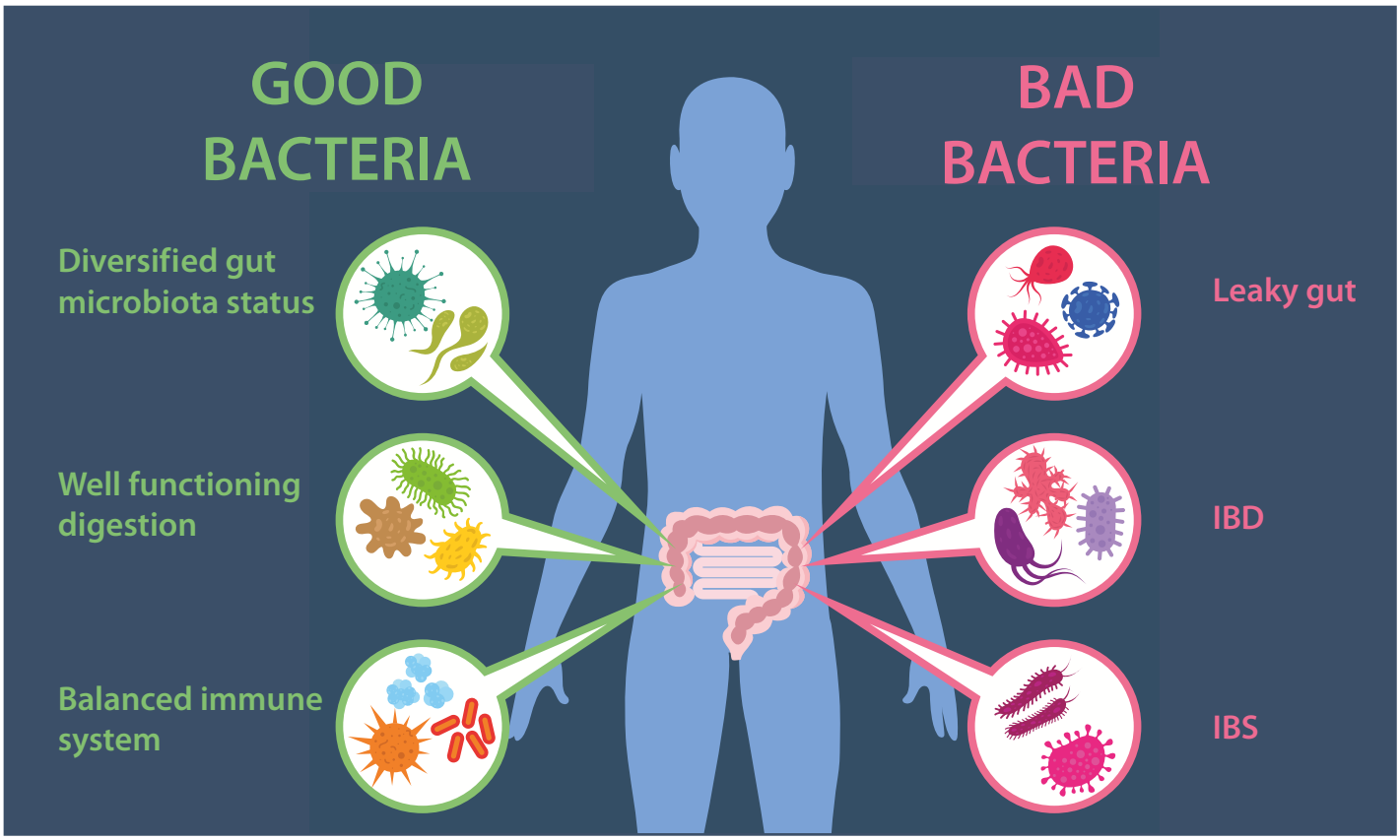
IDK® Microbiome

Microbiome and intestinal system



Specific Multiplex real time PCR and ELISA analytics

- Gut microbiome analysis / species ratio & quantification
- Obesity and diet monitoring
- Inflammation / pH regulation
- Parameters from α1-antitrypsin to zonulin
- Smart extraction system

For Research in

- Food & Health
- Nutrition & Immunology
- Internal Medicine & Gastroenterology

Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it

Diagnosis

An adult human being is settled by about 10^{14} bacteria (100 trillion!), not only, but predominantly in the gastrointestinal tract. The intestinal microbiota is a complex community of microorganisms in the gut and is unique like a fingerprint to each human. The intestinal microbiome means the entirety of the intestinal flora genes found in the intestinal flora. The genetic analysis of the microbiome and the determination of the proportionate composition of the intestinal flora are therefore an important diagnostic tool whose value is increasingly acknowledged.

The healthy composition of "good bacteria" can become dysbalanced by various factors, including stress, poor diet, lack of sleep and ageing. There are simple lifestyle changes that can dramatically improve digestion and help to avoid digestive problems, such as heartburn, bloating, diarrhoea, constipation, IBS, and nausea.

Molecular genetic diagnosis with real time PCR allows the analysis of the diversity of the microbiome. The quantification of clinically relevant types of bacteria allows for statements about dysbiosis. Only with this examination, an adapted therapy can start, for instance a specific diet with pre- and probiotics.

IDK® Microbiome

Immundiagnostik's approach for routine testing of intestinal parameter panels and microbiota is an all-in-one solution with a PCR and ELISA panel, bioinformatical reports with results interpretation and explanation as well as recommendations for health maintenance and therapy!

Microbiota-Intestinal System ROUTINE TESTING

	No.	Cat. No.	Item
		Enterotype	
Panel	1	KG1912-96	MutaPLEX® Bacteroides, PCR
		KG1913-96	MutaPLEX® Prevotella, PCR
			MutaPLEX® Ruminococcus, PCR
			MutaPLEX® Lachnospiraceae, PCR
		Mucin- / Butyrat Forming	
Panel	2	KG1911-96	MutaPLEX® Akkermansia muciniphila, PCR
			MutaPLEX® Faecalibacterium prausnitzii, PCR
		Mucin- / Butyrat- / H_2S-Forming	
Panel	3	KG1911-96	MutaPLEX® Akkermansia muciniphila, PCR
			MutaPLEX® Faecalibacterium prausnitzii, PCR
		KE19005	MutaPLATE® SRB (Sulfate Reducing Bacteria), PCR
		Firmicutes/Bacteroidetes Ratio (Obesity, Irritable Bowel Syndrome)	
Panel	4	KG1912-96	MutaPLEX® Bacteroides, PCR
			MutaPLEX® Bifidobacterium, PCR
			MutaPLEX® Eubacterium rectale, PCR
		Indigestion (Maldigestion)	
Panel	5		Pancreatic Elastase
	6	K 7878W	Bile acids
		Indigestion (Malabsorption)	
Panel	7		Calprotectin
	8		α1-Antitrypsin (Alpha-1-Proteinase-Inhibitor)
		Mucosal Immunity	
Test	9	K 8870	Immunglobulin A, secretory [sIgA]
		Mucous Membran Permeability (Leaky Gut)	
Test	10	K 5600	Zonulin
		Allergy / Pseudoallergy / Histamin Intolerance	
Panel	11	K 8213	Histamin
	12	K 6811	EDN
		Inflammatory Markers	
Panel	13		Calprotectin
	14		α1-Antitrypsin (Alpha-1-Proteinase-Inhibitor)
	15	K 6870	Lactoferrin
	16	K 6900	Lysozyme

Documentation

Examples for Medical Findings

Medical Profile for

Name	Sample	Date of Birth	02.05.1966	Order ID	11566990
First Name	John	Sex	Male	Order Date	31.08.2018
Sampling Date	09.08.2018 00:00	Validation Date	System	Findings Status	Final Report
Sample Material	Fe	Validation on	31.08.2018	Findings Date	31.08.2018

Test	Result	Unit	Standard Range	Previous Result
Profile				
Stool Properties				
Colour	brown			NA) VISU
Consistency	mushy			NA)
Mucin-/Butyrat-/H_2S Forming				
Akkermansia muciniphila	< 1,0 x 10^7	CFU/g faeces	> 5,0 x 10^9	NA) QPCR
Faecalib. prausnitzii PCR	6,3 x 10^9	CFU/g faeces	> 2,0 x 10^10	NA) QPCR
Firmicutes-Bacteroides-Ratio				
Eubacteria	1,2 x 10^9			NA) QPCR
Determination of Maldigestion				
Pancreatic elastase	174,00	µg/g	> 200	NA) ELISA
Mucosal Immunity				
Secretory IgA	<167	µg/ml	510 - 2040	NA) ELISA
Leaky gut				
Zonulin	13,50	ng/ml	< 55	NA) ELISA
Inflammation of the Intestinal Mucosa				
Calprotectin	<17,9	mg/l	< 50	NA) ELISA
Alpha 1-Antitrypsine	<1,8	mg/dl	< 27,5	

Laboratory-Id N°. 11566990
Received 31.08.2018
Report 03.09.2018
Last Name **Sample**
First Name **John**
Date of Birth 02.05.1966

Stool Diagnostics

Akkermansia muciniphila

The molecular-genetic stool analysis of Mr. John Sample indicates reduced **bacterial counts** of **Akkermansia muciniphila**.
Akkermansia muciniphila is a strictly anaerobic growing, gram-negative bacterium, v
(mucosa mucus) but at the same time encourages the mucosa to produce more n
active molecules. High Akkermansia counts indicate a thick mucin layer. Low
counts often come along with reduced **mucus production** enabling pathogens,
penetrate the mucosa and thus may cause inflammations.
By decomposing mucus Akkermansia muciniphila provides important nutrie
prausnitzii – the most important butyrate producer. **Butyric acid** plays a key
intestinal epithelia cells and protects the mucosa. Recent studies showed that
the Akkermansia counts

- achieving **stabilization of the mucosa barrier** (Leaky Gut) and
- **an anti-diabetic effect** by supporting hormone production (GLP-2).

Faecalibacterium prausnitzii

We determined **reduced Faecalibacterium prausnitzii** counts in the stool of
Inflammatory irritations of the intestinal mucosa are often characterized
proteins in stool (alpha-1-antitrypsine, lysozyme or calprotectin). A very se
mucosa irritations may also be the absence of an intestinal bacterium - **Fa**
cal bacteria prausnitzii are **firmicutes**, clostridia of the old clostridium-le
most frequent intestinal bacteria of humans. The bacterial counts are r
gram of stool. Faecalibacterium prausnitzii only seems to occur in humans
are no age-dependent differences in regard to the population.

Faecalibacterium prausnitzii develops **butyrate** and secretes substance
influence on intestinal cells by blocking **NF-κB-activation** and **IL-8 prod**
Butyric acid can protect from CED and intestinal cancer [Sokal et al.
prausnitzii counts are not only found in case of **Crohn's disease** or **i**
whole variety of other diseases, which come along with inflammatory m

- **Diseases of the autistic spectrum**
- **Adiposity**
- **Colon irritabile**

As **inflammation degree** and **Faecalibacterium prausnitzii count**
determination of the bacterium in stool offers a diagnostically con
about the mucosa's butyrate supply and the presence of inflammat
prausnitzii cannot be cultivated; therefore its determination is carrie
procedures.

Determination of Maldigestion

Digestive function of the pancreas
Pancreatic elastase 1 correlates to the digestive function of the exocrine pancre

Laboratory-Id N°. 11566990
Received 31.08.2018
Report 03.09.2018
Last Name **Sample**
First Name **John**
Date of Birth 02.05.1966

Therapy Recommendations

The measurable bacterial counts of Akkermansia muciniphila and F. prausnitzii were reduced. To achieve an increase prebiotics in form of galactose (GOS) or fructose-oligosaccharides (FOS) should be given. The daily dose should not be less than 5 g. Especially in case of fructose malabsorption GOS should be preferred. By giving starch preparations an increase of Akkermansia muciniphila cannot be realized.

Due to the low sIgA values in stool one should try to increase sIgA production and secretions. Very suitable is in this case the microbiological therapy applying preparations with viable (Symbioflor I, II, Mutaflor) or inactivated bacteria (ProSymbioflor). Preparations with viable bacteria principally have a stronger simulating effect than those with inactivated bacteria.

Dietetic treatment
Because of the weak pancreatic function we recommend the application of digestive enzymes and a change of diet to fat reduced and low-fibre balanced nutrition. Digestive enzymes should always be taken with meals. 40,000-60,000 units lipase per meal seem sensible.
Fat-reduced and low-fibre diets are based on frequent small meals with maximum fat consumption of 70 g/day.

With kind regards

Dr. Franz Paul Armbruster

Attention: The recommendations given are only advice based on the compiled findings and possible clinical information. They are exclusively addressed to the therapist / physician and are not intended for direct transfer to the patient. The provided suggestions cannot replace diagnosis and therapy of the treating therapist or physician.

General Workflow for qPCR

- **Sample Collection** (Stool) with MutaSTAB®
- **Nucleotide Extraction** (total DNA) with MutaCLEAN® Complete Mag
- **Real time PCR:** Set up with our MutaPLEX® microbiome PCR-kits (exact individual Ct-values of each bacteria species)
- **Calculation of CFU:** Determination of the species ratio (quantified by standard curve, expressed in copy numbers = CFU)
- **Optional: Special Diagnostic Services** (SDS) in cooperation with IDK & additional experts (documentation and interpretation)

Indications and explanations

➲ Mucin / Butyrat / H_2S formation

Akkermansia muciniphila is a strictly anaerobic growing, gram-negative bacterium, which decomposes mucin (mucosa mucus), but at the same time encourages the mucosa to produce more mucus and anti-microbially active molecules. High Akkermansia counts indicate a thick mucin layer. Low Akkermansia muciniphila counts often come along with reduced **mucus production** enabling pathogens, pollutants and allergens to penetrate the mucosa and thus may cause inflammations.

By decomposing mucus Akkermansia muciniphila provides important nutrients for **Faecalibacterium prausnitzii** – the most important butyrate producer. **Butyric acid** plays a key role as energy supplier for intestinal epithelial cells and protects the mucosa.

➲ Firmicutes/Bacteroidetes Ratio

The **Firmicutes/Bacteroidetes** ratio undergoes an increase from birth to adulthood and is further altered with advanced age. A high ratio is associated with increased permeability of the intestinal mucosa, resulting in amplified paracellular efflux of food antigens and so leading to inflammatory disease symptoms beyond the gut. Highest ratio is associated with obesity as firmicutes are able to metabolise complex, "indigestible" carbohydrates and so providing the host additional energy. Firmicutes are represented by Eubacterium rectale and Bacteriodetes are represented by Bacteroides spec..

➲ Enterotype

There are mainly 3 enterotypes of microbiome in human beings: Enterotype 1 (ET-1) is characterised by high **Bacteroides** counts, Enterotype 2 (ET-2) by strong **Prevotella** population, Enterotype 3 (ET-3) shows strong **Ruminococcus** flora in combination with **Lachnospiraceae**. This leads to different patterns in the utilisation and metabolisation of fatty acids, proteins and carbohydrates as well as different abilities to synthesize vitamins and absorb micronutrients. Persons with different microbiome enterotypes need different vitamin or micronutrient supply. The predominant **ET-1** with > 50% in the population is found in persons using a "mixed-diet" using generally the entire food supply. These people feed on both fruits and vegetables, but also eat meat. Accordingly, they utilise animal fats / fatty acids, proteins / amino acids very good, but also use the carbohydrates contained in the diet. The **ET-2** is often found in vegetarians with rather low protein supply and is characterised by a high specialisation in carbohydrate utilisation. The rare **ET-3** (about 5%) is found in persons using pure "raw food" with high-fiber diet and these functional bacteria can use sugar molecules, which are indigestible for humans, for physiologically important butyric acid production.

➲ Determination of Maldigestion

Pancreatic elastase is mainly bound to bile salts during intestinal passage and is not degraded. The stool concentration reflects the secretory capacity of the pancreas. It is useful for diagnosis of exocrine pancreas insufficiency in case of unexplained diarrhoea, constipation, steatorrhea, flatulence, weight loss, upper abdominal pain, and food intolerances and for monitoring of exocrine pancreas function in cystic fibrosis, diabetes mellitus, or chronic pancreatitis.

➲ Mucosal Immunity

Secretory IgA (sIgA) is determined to proof an imbalanced immunological barrier on the intestinal mucosa or an autoimmune disease. High levels indicate an ongoing immune response, low levels indicate low production or high consumption.

➲ Leaky Gut

Zonulin binds to a specific receptor on the surface of intestinal epithelia and triggers a cascade of biochemical events which induce tight junction disassembly and a subsequent permeability increase of the intestinal epithelia, allowing some substances to pass through and activate immune reactions. It is suggested that increased levels of zonulin are a contributing factor to the development of celiac disease and other autoimmune disorders such as insulin dependent diabetes, multiple sclerosis and rheumatoid arthritis.

➲ Inflammation of the Intestinal Mucosa

α1-antitrypsin is generally elevated with allergy related diseases. Often higher concentrations in stool are a hint for enteral loss of serum α1-antitrypsin and for a higher permeability of the mucus of the gut.

Calprotectin is a calcium-binding protein secreted predominantly by neutrophils and monocytes. Faecal calprotectin is a marker for neoplasic and inflammatory gastrointestinal diseases – for diagnosis and therapy monitoring.

FOMB 7 2019-10-17