[redacted]

Specimen collection date 03.07.2022
Date of Receipt 06.07.2022 14:16
Reporting Date 18.07.2022

GANZIMMUN GmbH - Hans-Böckler-Straße 109 - 55128 Mainz

[redacted]

DAkkS Deutsche Akkreditierungsstelle D-ML-13147-01-00

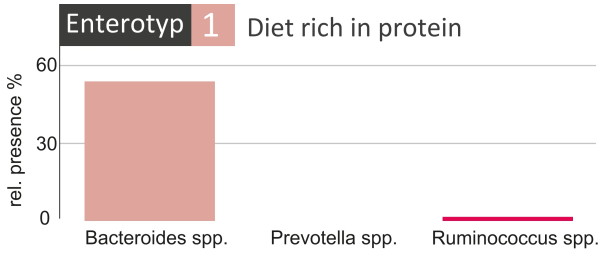
Laboratoryreport

Sample Material: faeces, microbiom special tube

Diversity

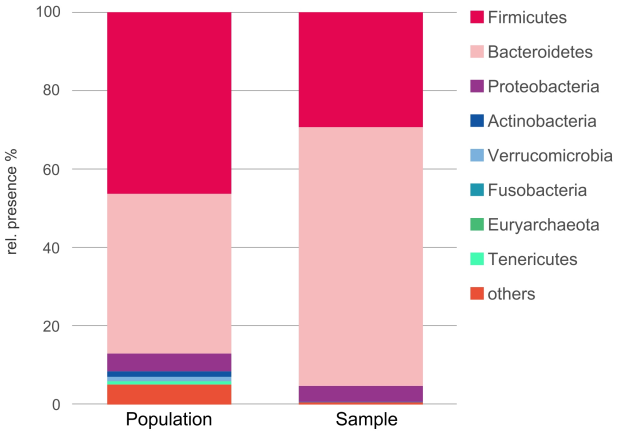
In the context of the microbiome, diversity refers to the diversity of the intestinal bacterial flora. It represents the stability and colonisation resistance.

Classification of the enterotype



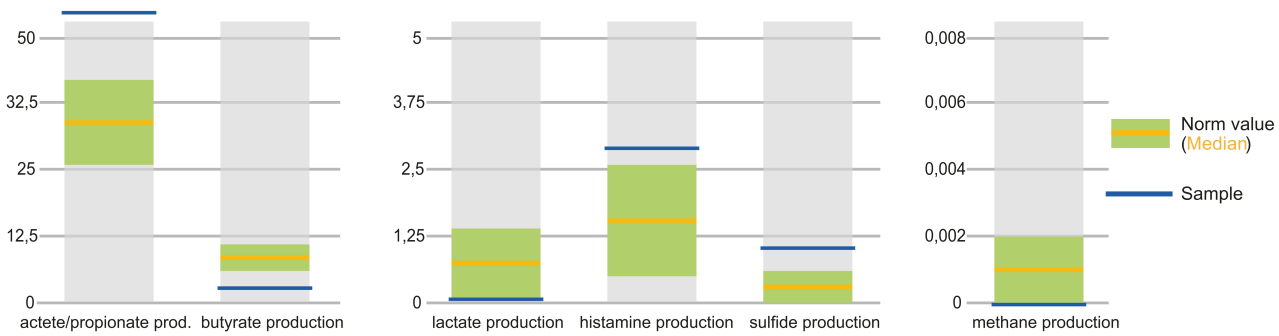
The intestinal microbiome can be divided into 3 enterotypes based on the predominant bacteria, which allow conclusions to be drawn about long-term eating habits.

Distribution diagram of bacterial strains



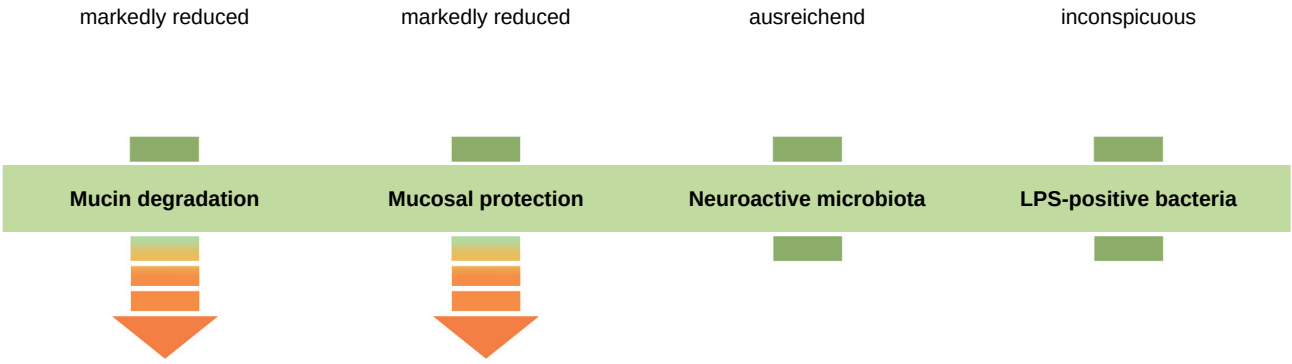
The frequency distribution reflects the proportions amongst the most common bacterial strains and compares your sample with the average distribution within the population.

Bacterial metabolism



The assignment to the groups was made on the basis of the known predominant metabolic performance of the bacterial species (modified according to Brown et al. 2011).

Functional bacterial groups



The arrow chart shows the measured deviation of the functional bacterial groups from the population values.

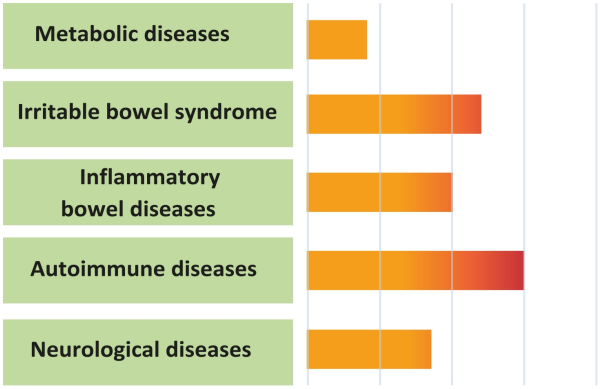
FODMAP-Index

The term FODMAP („Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols“) describes certain short-chain, easily fermentable carbohydrates and sugar alcohols, which are naturally present in numerous foods.

FODMAP TYP3

A Low-FODMAP diet is recommended for the improvement of irritable bowel- like or gastrointestinal complaints.

Mikrobiome-associated risks



The stated risks **do not represent a diagnosis**, rather the statistical relationships between germs and specific clinical pictures determined in current scientific studies are presented here in relation to the determined microbiome.

GANZIMMUN DIAGNOSTICS

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Bioindicators

pH-value of faeces	6,5		5,5 - 6,5
Biodiversity (Shannon index)**	2,16		> 2,7
Firmicutes / bacteroidetes ratio**	0,4		0,9 - 1,5
Butyrate production**	3,1	%	6,0 - 11,0
Lactate production**	0,1	%	0,1 - 1,4
Acetate / propionate production**	57,3	%	26,0 - 42,0
Mucin degradation**	0.0	%	0,05 - 8,7
LPS-positive bacteria**	1,964	%	< 3,7

Bacterial strains (phyla)

Firmicutes**	29,314	%	42,0 - 52,0
Bacteroidetes**	65,940	%	34,0 - 45,0
Proteobacteria**	4,051	%	4,0 - 8,8
Actinobacteria**	0,138	%	0,3 - 1,6
Verrucomicrobia**	0,002	%	0,007 - 2,4
Fusobacteria**	0,000	%	< 0,004
Cyanobacteria**	0,003	%	0,02 - 0,6
Euryarchaeota**	0,000	%	< 0,002
Tenericutes**	0,006	%	0,005 - 0,200

Functional bacterial groups

Mucin-degrading microbiota

Akkermansia muciniphila**	0,002	%	0,003 - 2,1
Prevotella spp.**	0,006	%	0,006 - 5,1
Prevotella copri**	0,002	%	< 0,2

Mucosa protective mikrobiota

Akkermansia muciniphila**	0,002	%	0,003 - 2,1
Faecalibacterium prausnitzii**	0,002	%	1,5 - 5,2

Sulphate-reducing microbiota

Bilophila wadsworthia**	1,038	%	< 0,3
Desulfobacter spp.**	0,002	%	< 0,004

Desulfovibrio spp.**	0,024	%	< 0,2
Desulfuromonas spp.**	0,000	%	< 0,001

Neuroactive microbiota

Bifidobacterium adolescentis**	0.000	%	0,001 - 0,2
Bifidobacterium dentium**	0,009	%	> 0,001
Lactobacillus brevis**	0,000	%	> 0,001
Lactobacillus plantarum**	0,000	%	> 0,001
Lactobacillus paracasei**	0,000	%	> 0,001
Oscillibacter spp.**	0,073	%	< 0,3
Alistipes spp.**	2,776	%	2,2 - 6,7

Methane-producing bacteria

Methanobacteria**	0.000	%	< 0,002
Methanobrevibacter spp.**	0,000	%	< 0,001

LPS-positive microbiota

Citrobacter spp.**	0,000	%	< 0,001
Enterobacter spp.**	0,008	%	< 0,007
Escherichia spp.**	1,835	%	< 0,3
Klebsiella spp.**	0,000	%	< 0,002
Providencia spp.**	0,000	%	< 0,001
Pseudomonas spp.**	0,120	%	< 0,002
Serratia spp.**	0,002	%	< 0,001
Sutterella spp.**	0,000	%	< 2,9

Immunmodulation

Escherichia spp.**	1,835	%	< 0,3
Enterococcus spp.**	0,014	%	0,001 - 0,005

Fiber degrading microbiota

Bifidobacterium adolescentis**	0.000	%	0,001 - 0,2
Ruminococcus spp.**	1,666	%	2,2 - 4,8

Butyrate-producing microbiota

Butyrivibrio crossotus**	0,000	%	0,001 - 0,01
Eubacterium spp.**	0,134	%	0,2 - 1,6
Faecalibacterium prausnitzii**	0,002	%	1,5 - 5,2
Roseburia spp.**	1,320	%	0,3 - 1,5
Ruminococcus spp.**	1,666	%	2,2 - 4,8

Acetate-/ propionate-producing bacteria

Alistipes spp.**	2,776	%	2,2 - 6,7
Bacteroides spp.**	53,928	%	15,0 - 31,0
Bacteroides vulgatus**	26,707	%	1,0 - 8,9
Dorea spp.**	0,171	%	0,08 - 0,2

GANZIMMUN DIAGNOSTICS

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Lactate-producing / saccharolytic microbiota

Bifidobacterium spp.**	0,012	%	0,07 - 1,3
Bifidobacterium adolescentis**	0.000	%	0,001 - 0,2
Enterococcus spp.**	0,014	%	0,001 - 0,005
Lactobacillus spp.**	0,015	%	0,004 - 0,02

histamine-producing bacteria

Clostridium spp.**	1,094	%	0,9 - 2,2
Enterobacter spp.**	0,008	%	< 0,007
Hafnia alveii**	0,006	%	< 0,001
Klebsiella spp.**	0,000	%	< 0,002
Serratia spp.**	0,002	%	< 0,001
Escherichia spp.**	1,835	%	< 0,3

Clostridiaceae

Clostridium spp.**	1,094	%	0,9 - 2,2
Clostridium difficile**	0,008	%	< 0,001
Clostridium scindens**	0,000	%	> 0,001

Other microbiota

Fusobacterium nucleatum**	0,000	%	< 0,001
Oxalobacter formigenes**	0,000	%	> 0,001
Anaerotruncus colihominis**	0,155	%	0,04 - 0,1
Streptococcus spp.**	0,164	%	0,08 - 0,5

Fungi

Candida spp.**	0,000	%	< 0,005
Candida albicans**	0,000	%	< 0,005
Geotrichum candidum**	0,000	%	< 0,001
Saccharomyces cerevisiae**	0,199	%	< 0,2
Moulds**	negativ		negativ

Summary of molecular stool diagnostics, indication of:

- Detection of reduced biodiversity
- slightly reduced colonisation resistance

- disrupted mucosal protection
- minor microbiome-associated susceptibility to inflammation
- microbiome-associated tendency towards Leaky Gut
- possible bacterial miscolonisation of the small intestine (SIBOS)
- microbiome-associated health risks

Interpretation of findings intestinal microbiome

Diversity

Diversity refers to the diversity of species that occur in a microbiome. Physiologically, the microbiome has a high diversity, ie a high number of different species, and has a great ability to absorb changes and disturbances. Low diversity makes humans highly susceptible for various diseases, such as irritable bowel syndrome, food intolerances, chronic inflammatory bowel diseases and infections. The most important cause for low diversity is the use of antibiotics, the spectrum of which has a direct effect on reducing diversity.

FODMAP-Index

The term FODMAP („Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols“) describes certain short-chain, easily fermentable carbohydrates and sugar alcohols, which are naturally present in numerous foods. Depending on the composition of their intestinal microbiome, patients with irritable bowel-like or gastrointestinal complaints may benefit from a low-FODMAP diet.

Literature:
Staudacher H. The impact of low fodmap dietary advice and probiotics on symptoms in irritable bowel syndrome: a randomised, placebo-controlled, 2 × 2 factorial trial. Gut 2015; 64:A51.
Halmos E. P. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. Gastroenterology. 2014; 146(1):67-75.

Enterotype determination

The enterotype of your stool sample corresponds to type 1.

The intestinal microbiome can be divided into three so-called **enterotypes**. They are independent of age, gender, body weight and nationality. Studies indicate that long-term dietary patterns, e.g. consumption of animal fats and proteins, could cause enterotypes to switch. First associations between enterotype III and artherosclerotic disease have also been described (Karlsson FH et al. (2012) Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat. Commun. 3:1245).

The **enterotype I** is characterised by an abundance of *Bacteroides spp.*. They are involved in generating energy from carbohydrates and proteins by fermentation, and also in the biosynthesis of biotin.

Bioindicators

Firmicutes/Bacteroidetes ratio

With **over 90%,** the Firmicutes and Bacteroidetes strains are the two dominating bacterial groups in the human intestine.
By **breaking down undigested food components**, the intestinal **Firmicutes** bacterial strains can provide the human body with short-chain carbohydrates and fatty acids as an **additional energy source**.
Numerous studies have shown that the ratio between Firmicutes and Bacteroidetes correlates with human body weight. An increased proportion of Firmicutes causes increased resorption of carbohydrates by the human intestinal mucosa.

Mucosaprotective flora

The mucosal protective flora of your sample is **decreased** . A high-fiber diet, prebiotics with inulin and, if dysbiosis is proven, appropriate probiotics can be used to increase the number of mucosal protective germs, *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* .

Akkermansia muciniphila is a gram-negative obligate anaerobic rod. This is a mucin-cleaving bacteria that plays a central role in maintaining ***Faecalibacterium prausnitzii*** by metabolic cleavage products, among others. Current studies have shown that this bacteria has beneficial effects on various health factors. Studies were also able to demonstrate that *Akkermansia muciniphila* has an **anti-inflammatory effect** and is beneficial for maintaining an **intact intestinal barrier**.

Faecalibacterium prausnitzii is a gram-negative obligate anaerobic rod of the Firmicutes strain. This bacteria is one of the three most frequent anaerobic bacteria in the intestinal flora. Changes in the specific bacterial species of the intestinal flora were found in patients with **inflammatory bowel disease, irritable bowel syndrome** and **coeliac disease.** One of these changes is a reduced count of *Faecalibacterium prausnitzii* bacteria. Various studies demonstrated that this bacteria has an important effect on cells of the immune system. It is further known that inflammatory processes in the intestines can be significantly reduced by the production of butyric acid. It is known that *Faecalibacterium prausnitzii* is one of the most abundant butyric-acid producing bacteria in the colon.
Overall, *Faecalibacterium prausnitzii* reduces intestinal inflammatory processes and is beneficial for inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis.

Several current studies have demonstrated a positive relationship between high counts of ***Akkermansia muciniphila*** bacteria and the following conditions:

- Low body weight
- Low body fat proportion
- Reduced metabolic endotoxaemia by bacterial lipopolysaccharides
- Reduced adipose tissue inflammation
- Reduced insulin resistance (type II diabetes)

Several studies determined the following **immunological effects** of ***F. prausnitzii***:

- Inhibition of transcription factor NF-KB → inhibition of the pro-inflammatory interleukin 8 (IL-8)
- Production of butyric acid, which further inhibits NF-KB
- Differentiation of regulatory T cells → increasing the anti-inflammatory interleukin 10 (IL-10), reducing the pro-inflammatory interleukin 12 (IL-12)

Neuroactive Microbiota

Neuroactive microbiota are microbiota that participate in the metabolism of neuroactive substances or form such substances.
Because **tryptophan is the precursor of serotonin** , the increased microbial count of Alistipes may interfere with the balance of the serotonergic system in the gut.
Oscillibacter produces **valeric acid** as the main metabolite. **Valeric acid** has a structural similarity to **gamma-aminobutyric acid (GABA)** and can like GABA bind to and inhibit the GABAa receptor. Bacteria that can form the neuroactive **gamma-aminobutyric acid (GABA)** include: *Bifidobacterium adolescentis, Bifidobacterium dentium, Lactobacillus brevis, Lactobacillus plantarum and Lactobacillus paracasei.*

Butyrate-producing bacteria

Butyrate-producing bacteria include mainly *Faecalibacterium prausnitzii, Eubacterium spp., Roseburia spp., Ruminococcus spp.* and *Butyrivibrio crossotus.*
These types of bacteria reduce intestinal inflammatory processes by promoting the formation of regulatory T cells and by inhibiting the production of pro-inflammatory cytokines by macrophages and dendritic cells. Butyrate also increases the oxygen consumption of colonocytes and exacerbates the phenomenon of mucosal "physiological hypoxia", which contributes to supporting the intestinal barrier

function. It inhibits proliferation of cancer cells and induces apoptosis.
A reduction in the number of butyrate-producers can promote inflammatory processes, increase intestinal mucosal permeability (Leaky Gut), and promote the manifestation of inflammatory diseases (Crohn's disease, ulcerative colitis), irritable bowel syndrome, food intolerances and coeliac disease.

Mucin-degrading bacteria

Mucin-degrading bacteria include mainly *Akkermansia muciniphila* and *Prevotella* species. These types of bacteria can degrade mucin and are essential for the regeneration of the physiological mucin layer. In this way, they support the maintenance of an intact intestinal barrier by butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*.

Sulphate-reducing bacteria

Sulphate-reducing bacteria, such as *Desulfovibrio spp., Desulfomonas spp.* and *Desulfobacter spp.*, are anaerobic bacteria that produce energy via sulphate reduction and form large amounts of sulphides. The metabolite of these bacteria is hydrogen sulphate, which has cytotoxic properties. Hydrogen sulphate can inhibit butyrate oxidation that is essential to supply energy to colonocytes. Proliferation of sulphate-reducing bacteria can result in chronic inflammation of the intestinal epithelium.

Methane-producing bacteria

Methane-producing bacteria, such as *Methanobrevibacter spp.* and *Methanobacterium spp.* are part of the Archaea domain. They are characterised by their ability to convert primary and secondary bacterial fermentation products, such as hydrogen and carbon dioxide, into methane. They therefore play a significant role in optimising the energy balance. In addition, methane has an inhibitory effect on intestinal motility, which can lead to worsening of chronic constipation. These bacteria can also activate dendritic cells in the gut mucosa and induce the production of TNF alpha and other pro-inflammatory cytokines.

Saccharolytic bacteria

Saccharolytic bacteria in the intestine are responsible for cleaving complex poly- and oligosaccharides, such as resistant starch. The lactic acid formed during cleavage is used by other bacteria such as *Ruminococcus bromii* or *Faecalibacterium prausnitzii* as the basis for producing butyric acid. *Bifidobacterium adolescentis* thereby plays a key role, which was investigated in a study with healthy subjects (Venkataraman et al. Microbiome 2016).

LPS-bacteria

LPS-positive bacteria are gram-negative bacteria that carry lipopolysaccharide (LPS) as a so-called endotoxin and, after penetrating into the intestinal mucosa, activate inflammatory processes, as is the case with Leaky Gut. The activation of the immune system can result in low-grade chronic inflammation ("silent Inflammation").

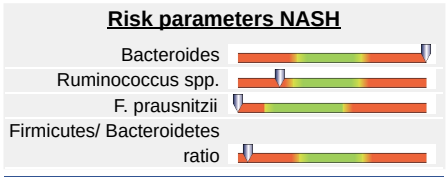
Microbiome-associated health risks

The specified risks represent **no diagnosis** , rather the statistical relationships between germs and specific clinical pictures taken from current scientific studies in relation to the determined microbiome.

Metabolic diseases	Irritable bowel syndrome	Inflammatory bowel diseases	Autoimmune diseases	Neurological diseases
Obesity	Irritable bowel	Chronic-inflammatory bowel diseases	Coeliac disease	Depression
Type 2 diabetes mellitus	Leaky Gut syndrome	Colorectal carcinoma	Rheumatoid arthritis	Chronic fatigue syndrome
Cardiovascular diseases	Histamine intolerance	Dysbiosis	Psoriasis	Autism spectrum disorder
Non-alcoholic steatohepatitis	Food intolerance	Colonisation resistance	Allergy / asthma	Parkinson's disease
Alcoholic steatohepatitis	SIBOS	Gastrointestinal susceptibility to infections	Type 1 diabetes mellitus	Alzheimer's disease

Metabolic diseases

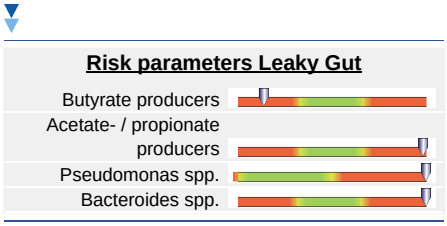
Non-alcoholic steatohepatitis - NASH



Several studies already observed a correlation between intestinal bacteria and the development of non-alcoholic steatohepatitis. A shift in the metabolic function of intestinal bacteria is predominantly caused by dysbiosis. In the intestine, it leads to an increase in the permeability of intestinal mucosa for lipopolysaccharides (LPS) and ultimately causes chronic inflammation. The extent of LPS permeability can be determined by measuring the soluble LPS receptor protein sCD14 in the serum. It was further determined that the concentration of bacterial metabolites in the blood, such as trimethylamine which is metabolised in the liver to trimethylamine-N-oxide (TMAO) correlates with the severity of steatohepatitis.
According to studies, the relative frequency of the bacteria *Bacteroides spp.* and *Ruminococcus spp.* correlated with NASH. A similar effect was observed when *Prevotella spp.* and *Faecalibacterium prausnitzii* were reduced.

Irritable bowel syndrome

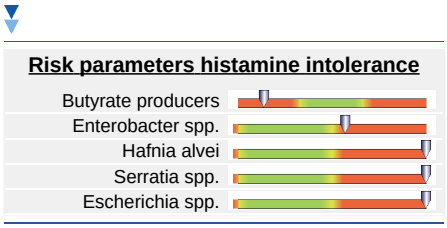
Leaky Gut syndrome



The scientific findings on the causes and consequences of increased intestinal mucosa permeability are playing an important role in the diagnostics and therapy of gastrointestinal complaints. The transfer of bacterial antigens is believed to be involved in metabolic processes or autoimmune diseases. The new findings demonstrate that a balanced ratio between butyric acid-producing and mucin-degrading bacteria (mucosa protection ratio) plays an important role. When the balance is disrupted and the diversity reduced, bacterial lipopolysaccharides (LPS) can enter the human circulatory system and lead to pathological conditions. The regulatory protein zonulin is a suitable marker to better assess the permeability of the intestinal mucosa.

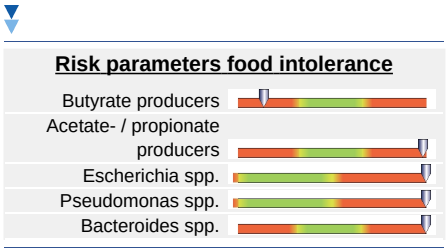
Histamine intolerance

Histamine plays a central role in allergic reactions and is a mediator for inflammatory processes. Elevated faecal histamine concentrations can be caused by an increase in histamine intake with the food or by enhanced intestinal putrefaction activity and histamine synthesis by the intestinal bacteria. This bacterial metabolic activity is caused predominantly by a high number of Proteobacteria. When diversity is reduced at the same time, symptoms like those seen with histamine intolerance can appear. An adequate number of butyric acid-producing bacteria, such as *Faecalibacterium prausnitzii* and highly diverse intestinal bacteria can causally counteract these symptoms.



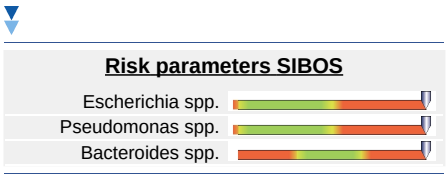
Food intolerance

Current research results on the causes and consequences of a reduced intestinal barrier show that under physiological conditions most food antigens are resorbed by the intestinal epithelium and are intracellularly degraded into small peptides by its digestive enzymes without triggering pathological immune reactions. If the physiological conditions are disrupted, as in cases with reduced diversity and a strong increase in bacteria of the *Escherichia, Klebsiella* and *Pseudomonas* genus, incompletely digested food components can transfer into the circulatory system where they can trigger potentially pathogenic immune reactions. An example is non-coeliac gluten sensitivity (NCGS), whose clinical manifestation is very similar to that of coeliac disease. In contrast, important protective mechanisms of mucosal integrity are supported by the muco-protective flora, such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*.



Small Intestinal Bacterial Overgrowth Syndrome (SIBOS)

The term SIBOS summarises an intolerance to certain carbohydrates or proteins. In cases with lactose or fructose intolerance, an analysis using the hydrogen breath test can support a diagnosis. According to studies, the causes can be non-physiological conditions of the bacteria colonising the intestine. Thus, a significantly elevated relative frequency of *Eschericha spp., Klebsiella spp.* and *Pseudomonas spp.* in the intestine may cause SIBOS. The diagnosis is supported when in addition obligate anaerobic bacteria, such as *Bacteroides spp.* and various species of the genus *Clostridium*, are strongly increased and diversity decreased.



Further diagnostics for the risk area irritable bowel syndrome

Due to the identified risk of irritable bowel syndrome, the following **additional laboratory diagnostic tests** are recommended:

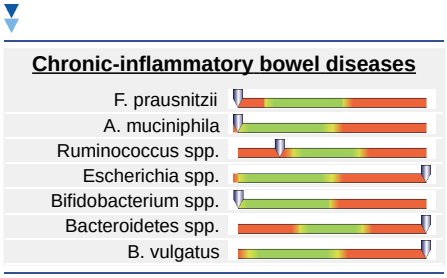
- Parasites (immunologic) in the stool
- Histamine metabolite in urine
- PreScreen allergy in serum
- Breath test (fructose and lactose)

Inflammatory bowel diseases and susceptibility to infection

Chronic-inflammatory bowel diseases

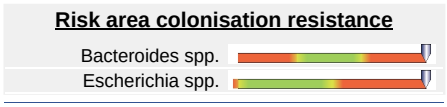
The bacteria of the intestinal tract use different mechanisms to elicit, promote or inhibit inflammatory processes in the intestine. These mechanisms are based mainly on the production of metabolites and toxins that can have a direct pro-inflammatory effect or an indirect effect by influencing the production of various cytokines.

Bacteria of the intestinal flora, that have a pro-inflammatory effect in the intestine and were detected at an increased frequency include, amongst others, *Fusobacterium nucleatum, Escherichia spp., Desulfovibrio piger, Methanobrevibacter smithii, Bacteroides thetaiotaomicron* or *Bacteroides vulgatus*. This also includes the **enterotype 1,** which is characterised by *Bacteroides spp.* as the predominant bacteria. A reduced bacterial diversity can also promote



inflammatory processes in the intestine.
Bacteria with an anti-inflammatory effect, such as *Akkermansia muciniphila, Faecalibacterium prausnitzii, Roseburia spp., Ruminococcus spp., Odoribacter spp., Bifidobacterium spp.* or *Streptococcus thermophilus* have a relatively low frequency of detection. Reduced diversity is an additional factor that promotes inflammatory processes in the intestine.

Colonisation resistance

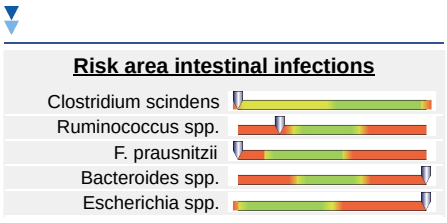


The intestinal microbiome contributes to colonisation resistance against pathogenic bacteria and viruses through four interconnected functions:

(1) Direct inhibition of neighbouring bacteria by production of toxic compounds.
(2) Maintenance of the mucosal layer and the underlying intestinal epithelium.
(3) Regulation of the immune response.
(4) Efficient utilisation of nutrients, such as mucopolysaccharides, by commensal bacteria that can help limit the expansion of less well-adapted invasive bacteria.

The investigation of your microbiome reveals **reduced colonisation resistance**. The chance of infections by pathogenic bacteria and viruses is therefore higher.

Gastrointestinal susceptibility to infections



Campylobacter infections

The different susceptibility for an infection with *Campylobacter* depends on the species composition of the intestinal microbiome. People with a higher variety (diversity) of their microbiome and with a high frequency of bacteria from the genuses of *Dorea* and *Coprococcus* are significantly more resistant against a *Campylobacter* infection than people with a low diversity and low frequency of these bacteria. On the other hand, bacteria such as *Bacteroides, Escherichia coli* and *Streptococcus* increase sensitivity towards such infections.
The analysis of your sample reveals **reduced resistance** of your microbiome **against infections by enteropathogenic *Campylobacter*** species.

Clostridium difficile infections

Saccharolytic microbiome bacteria, such as *Bacteroides thetaiotaomicron*, release sialic acid and therefore promote the growth of *Clostridium difficile*. Antibiotic treatment further increases the concentration of free sialic acid and in addition triggers the production of succinate, which is associated with an additional growth advantage for *C. difficile*.
Due to the production of secondary bile acids, such as desoxycholate and lithocholate, which strongly inhibit the growth of vegetative *C. difficile* cells, the presence of *Clostridium scindens* in the intestine is conversely associated with resistance against *C. difficile* infections.
The analysis of your sample reveals **reduced resistance** of your microbiome **against infections by *Clostridium difficile*** .

Infections with rota virus and noro virus

In studies, microbiota analysis showed a significant negative correlation between the sensitivity against infections with noro viruses and rota viruses and the frequency of *Ruminococcus spp.* and *Faecalibacterium prausnitzii*. On the other hand, a positive correlation between these infections and the frequency of

Akkermansia muciniphila was determined.
The analysis of your sample reveals a **reduced resistance** of your microbiome **against infections with noro viruses and rota viruses**.

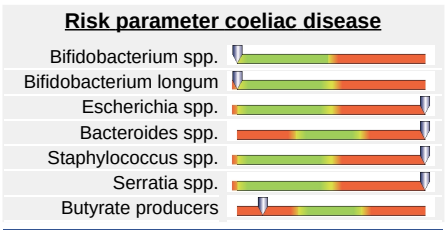
Further diagnostics for the risk area inflammatory bowel diseases

Due to the identified risk of inflammatory bowel diseases, the following **additional laboratory diagnostic tests** are recommended:
• Alpha-1 anti-trypsin
• Calprotectin
• Bile acids
• Pancreas elastase
• Secretory IgA
• Zonulin
• Haemoglobin-haptoglobin complex
• M2PK
• Blood in the stool

Autoimmune diseases

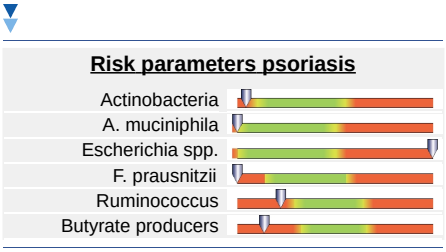
Coeliac disease

Coeliac disease is one of the most frequent autoimmune diseases in children and adults. The research group around Cheng et al. (BMC Gastroenterology 2013, 13:113) determined a significant accumulation of *Prevotella spp.* and *Serratia spp.* in affected people and a strongly reduced diversity in the faecal samples. In contrast, the samples from the healthy population were high in Clostridium spp. and Ruminococcus spp. If coeliac disease seems unlikely because of the absence of a genetic predisposition, non-coeliac gluten sensitivity (NCGS) could be present, which is accompanied by very similar symptoms.



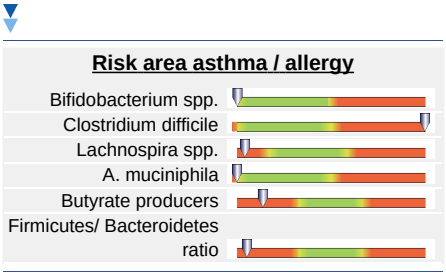
Psoriasis

Psoriasis is an inflammatory systemic autoimmune disease primarily visible through skin changes that also affects joints, ligaments, vessels and other organs. In analogy to other autoimmune diseases, a genetic predisposition is often present. The risk of developing one of the psoriasis forms can be increased by a reduced mucosa-protective and butyric acid-producing bacterial intestinal flora and by lower diversity. In a study that included patients and healthy subjects, a significant relationship between the frequency of the bacteria *Coprococcus spp.*, *Akkermansia muciniphila* and *Ruminococcus spp.* was observed in stool samples (Arthritis Rheumatol. 2015 January; 67(1): 128–139).



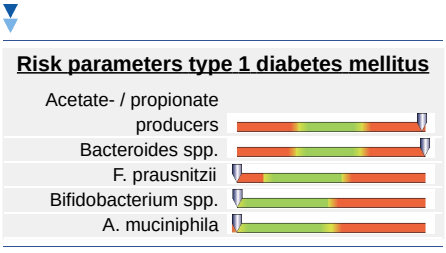
Allergy / Asthma

Allergic reactions can start as early as in childhood, remain in later years, disappear or reappear with increased intensity. Several studies emphasised the protective importance of a previous colonisation of the intestinal flora with *Lactobacillus spp.*, *Lachnospira spp.*, *Veillonella spp.* and *Bifidobacterium spp.* In contrast, a reduced diversity and the predominance of bacteria from the Proteobacteria strain and the gram-negative anaerobic *Bacteroides spp.* promote the development of inflammatory and allergic reactions.



Type 1 diabetes mellitus

Type I diabetes mellitus is considered to be an autoimmune disease. In studies with affected patients, a clear correlation between the disease and the relative frequency of bacteria from the genuses of *Prevotella, Clostridium, Veilonella, Bifidobacterium, Lactobacillus* and *Bacteroides* was determined (Murri et al. BMC Medicine 2013, 11:46). In a different study, researchers determined other correlations relating to the diversity and the ratio between short-chain fatty acid-producing bacteria (*Faecalibactrium, Ruminococcus, Bacteroides*) and mucin-degrading bacteria (*Prevotella* and *Akkermansia*) (PLOSONE October 2011, Volume 6, Issue 10,



e25792).

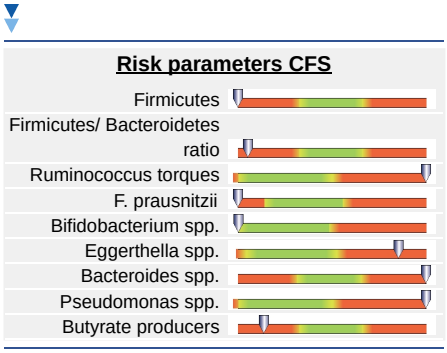
Further diagnostics for the risk area autoimmune diseases

Due to the identified risk of autoimmune diseases, the following **additional laboratory diagnostic tests** are recommended:

- Gluten sensitivity in serum
- DQ2/DQ8
- HLA-B27
- Large rheumatoid profile
- Autoimmune screen
- Asthma/rhinitis seasonal or year-round

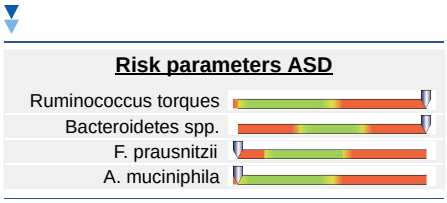
Neurological disorders

Chronic Fatigue Syndrome – CFS



Chronic fatigue syndrome, also called myalgic encephalomyelitis, is a disease pattern that is often not sharply defined and is characterised by mental and physical fatigue and in some cases muscle ache after only minor exertion. The causes for this mental illness are mostly complex in nature and are attributed to immunology, post infection or to the bacterial composition of the intestinal flora and its metabolites, such as short-chain fatty acids. A study including affected and healthy subjects frequently observed the relationship between CFS and irritable bowel syndrome (IBS). The intestinal flora of affected patients was characterised by a low frequency of bacterial species, such as *Faecalibacterium prausnitzii, Eubacterium spp.* and *Odoribacter spp.*, while *Clostridium spp.* and *Ruminococcus torques* were markedly increased. A correlation between the severity of the symptoms was even established for some bacteria. A low number of the *Alistipes* genus was connected to higher vitality and motivation, while the symptoms were significantly more severe when the frequency of *Faecalibacterium prausnitzii* was low (Nagy-Szakal et al. Microbiome (2017) 5:44).

Autism Spectrum Disorder - ASD



The autism spectrum disorder comprises early childhood autism, Asperger's syndrome, and several rare atypical forms. An early diagnosis can help the patient develop compensation mechanisms that can overcome shortcomings in social communication. In a study including patients and healthy control subjects, a significant correlation was observed between the permeability of the intestinal mucosa, measured by means of zonulin in the serum, and the degree of the Autism Rating Score (Esnafoglu et al. J Pediatr. 2017 May 11). The integrity of the intestinal mucosa is significantly influenced by the intestinal bacteria. Thus, a strong reduction in butyric acid-producing bacteria such as *Faecalibacterium prausnitzii* can cause an insufficient regeneration of colonocytes and promote the permeability of the intestinal mucosa. Predominance of Proteobacteria, and in particular a higher density of the *Sutterella* genus, is often observed in autistic patients. A 10-fold amount of *Ruminococcus torques* from the Firmicutes strain was recorded in samples of affected patients compared to samples from healthy subjects (Wang et al. Molecular Autism 2013, 4:42). Another study observed a significantly lower number of mucin-degrading *Akkermasia muciniphila* and the short-chain fatty

acid-producing *Bifidobacterium spp.* in children with autism.

Further diagnostics for the risk area neurological disorders

Due to the identified risk of neurological disorders, the following **additional laboratory diagnostic tests** are recommended:

- Adrenal stress index in saliva
- Large hormone profile (female/male)
- Thyroid profile
- Total T3/reverse T3 ratio
- Q10
- Oxidative stress
- Vitamins B1, B2, B3, B5
- Methylmalonic acid in urine

Other risks

Calcium oxalate urinary stones

According to a study by the group from the Slone Epidemiology Centre of Boston University, the Harvard Medical School and the Neurological Clinic of Duke University, the intestinal tract bacteria *Oxalobacter formigenes* can reduce the risk for developing kidney stones by up to 70%. The researchers report that the protective effect is most likely based on the metabolisation of oxalate in the digestive tract. In contrast, the absence of this bacteria can increase the risk for forming these kidney stones.

Medically validated by Dr. med. Irina Neumann

All parameters marked with an * are tested at our accredited laboratory partners.
** study not accredited

Basic principles of microbiome therapy

The development, diversity and stability of the intestinal microbiome are sensitive to peoples' lifestyle and dietary habits. Therefore, the intestinal microbiome must always be viewed as a product of lifestyle. The opposite conclusion can be derived from the fact that long-term stabilisation of the intestinal microbiome is only possible when improper nutrition and other unfavourable living conditions are eliminated.

Microbiome therapy is therefore not only based on **long-term dietary changes** but also on the administration of **prebiotic preparations**. This therapy biologically stabilises intestinal environmental conditions. At the same time, it results in the desired adaptation of the microbiome. This clearly shows that the focus should not be on the administration of viable microbes in form of **probiotics**, but that a suitable presentation of substrates for the desired modulation of the microbiota should be prioritised instead.

The prerequisite for a highly diverse physiological intestinal microbiome is therefore a long-term, varied, low-fat, fibre-rich diet containing secondary plant substances that corresponds, for example, to a vegetarian whole food diet!

According to the *German Society of Nutrition [Deutsche Gesellschaft für Ernährung]* (DGE) vegetarian food with lots of fruit, vegetables and whole-grains – if possible organically farmed - is recommended in any case. At the same time, "microbiome-healthy nutrition" is characterised by avoiding artificial food additives, such as preservatives, food stabilisers, artificial flavours, dyes etc. as much as possible.

Substrates promoting a **physiological microbiome**

Fibres (prebiotics) such as:

* Psyllium husks
* Flaxseed
* Acacia fibres
* Wheat bran
* Resistant starches (e.g. resistant dextrin)
* Fructo-/galacto oligosaccharides
* Amylopectin / citruspectin
* Whole-grain millet
* Buckwheat
* Buckwheat
* Baobab fruit (African monkey bread tree)

Secondary plant ingredients from the polyphenol group such as:

* (Epi)catechin (green tea)
* Procyanidines (red grapes)
* Flavanoles (cocoa)
* Tannins (tea)

Substrates that promote a **non-physiological microbiome**:

- Too much protein (irrespective of the source; inflammatory proteins are also available as a substrate for the putrefying flora)
- Too much fat
- Refined carbohydrates/starch

Factors that disrupt the development of a "healthy" microbiome

This is in contrast to the more unfavourable nutritional habits in our populations, which often start as early as in infancy by use of formula. In adolescence and adulthood stress, this is followed by a disrupted sleep-wake rhythm, excess consumption of industrially-produced food, excess consumption of carbohydrate-rich food and the regular intake of additives, such as artificial flavours, dyes, sugar substitutes and food stabilizers. Alcohol and various toxic residues in food also prevent the development of a healthy microbiome. Moreover, **unnecessary antibiotic therapies** are often an important cause for the development of dysbioses. Preventative, probiotic or symbiotic therapy should therefore be given during and after antibiotics administration.

Download

You can find additional information on therapy in the specialist brochure **Intestinal microbiome** in our download centre at www.ganzimmun.de

Fibres

Fibres are indigestible carbohydrates of plant-derived food that benefit only the

microbiome and are not a substrate for humans. This simple fact permits the direct deduction that an insufficient intake of fibres will inevitably result in "supply disruptions" for the microbiome, which cause major and exclusively harmful changes to the entire gastrointestinal microbiota, ultimately affecting the host at a correspondingly level.

Prebiotics

Prebiotics are components of food that are part of the soluble fibre group. They are composed of indigestible and natural fructooligosaccharides (FOS) or galactooligosaccharides (GOS), are stable in gastric acid and – corresponding to the above-mentioned principles about fibres – are available to the microbiome and non-human organisms as growth substrates. Thus, prebiotics selectively affect the growth and the metabolic performance of the intestinal microbiome in the colon. They therefore have a significant health-maintaining effect. Mixtures of different prebiotics as present in finished formulations in various combinations have proven effective.

Prebiotic oligosaccharides – the most important group of the prebiotics – are also contained in breast milk. They are the prerequisite for the development of a healthy microbiome in the child. This clearly demonstrates that the use of prebiotics can be appropriate even in childhood.

Secondary plant ingredients

Secondary plant ingredients are part of a substance group that is formed by plants among other things as defence substrates against pesticides and diseases, as growth regulators or as dyes. From the evolutionary perspective, it can be assumed that bioactive substances from plants play an essential role in maintaining and promoting human health and physical performance. This also appears to be true for the intestinal microbiome, which is modulated in particular by polyphenols. Substances such as **procyanidins**, and dyes such as **flavonoids** and **anthocyanins** are part of the group of polyphenols. A varied diet rich in fresh vegetables and fruit contains sufficiently high concentrations of secondary plant nutrients.

A current study* also proves the importance of secondary plant ingredients for the species ***Akkermansia muciniphila***. Polyphenols confer important substrates to *Akkermansia*, resulting in a survival advantage and thus contributing to its stabilisation and proliferation.

Literature:

* Anonye, B. O. 2017. Commentary: Dietary Polyphenols Promote Growth of the Gut Bacterium Akkermansia muciniphila and Attenuate High-Fat Diet-Induced Metabolic Syndrome. Front Immunol. 8:850.

Probiotics

Probiotics are viable, metabolically active microorganisms that survive the passage through the stomach due to their acid resistance and unfold specific and nonspecific effects in the intestine. They strengthen a patient's own physiological flora through their metabolic activity so that undesired bacterial species can be displaced. They inhibit putrefying bacteria, such as histamine producers, by competing for substrate and stabilising a physiological microbial intestinal environment.

The administration of probiotics during microbiome therapy serves to supplement the above-mentioned prebiotic measures in order to optimise the environmental conditions. With the help of the various bacterial compositions available today, the measures can be varied depending on the findings and the clinical symptoms.

Daily doses of **highly concentrated probiotics** (at least 1x 10^9) and the highest possible variety of bacterial species, like in the so-called **multi-species probiotics**, are required to achieve an efficient probiotic effect.

Therapy recommendations

[redacted]

Following recommendations are directed exclusivley to the treating doctor or threapist and are not intended for distribution to the patient.

Please note, that the recommendations include **alternative products from different manufacturers**, that are similar in terms of active ingredients, administration and indication. As a guide, please pay attention to the information in the corresponding columns, which are lagrely identical for alternative pharmaceuticals.

Product	Ingredients and administration	Indication	Note
ColonBalance® **Company / manufacturer:** Biogena Naturprodukte GmbH & Co. KG **Dosage:**1 x daily. 1 ML **Supplier:** www.biogena.com	**Ingredients:** resistant dextrin, pregelatinised waxy maize starch (amylopectin), acacia fiber (Fibregum™), citrus pectin **Administration:** Stir 1 measuring spoon (10 g) in about 100 ml of liquid and drink immediately, or stir into cereals, yoghurt, etc.	• to increase the fiber intake. • Serves as a substrate for the health-promoting bacterial strains and thus contributes to colonization resistance to yeasts and other pathogens. • Binds cholesterol and bile acids in the intestine and thus promotes their excretion.	
Darm Formula Plus **Company:** Biogena Naturprodukte GmbH & Co. KG **Dosage:** 3 capsules per day **Supplier:** www.biogena.com	**Ingredients:** black cumin seed extract, Curcuma longa extract, black pepper extract, inulin (fructooligosaccharide), niacin and vitamin B2 **Administration:** take with plenty of liquid	• to support a healthy intestinal microbiome and to maintain a normal intestinal mucosa function • inulin has a positive effect on microbiome diversity and supports the activity of butyrate formers	
OPC Polymax® 250/30 **Company / manufacturer:** Biogena Naturprodukte GmbH & Co. KG **Dosage:** 2 capsules per day **Supplier:** www.biogena.com	**Ingredients:** grape seed extract 145 mg, grape extract 117 mg, green-tee extract 140 mg, pomegranate-extrakt 140 mg, olive leaf-extract 120 mg, oligomere Proanthocyanidine (OPC) 60 mg, polyphenole (total) 500 mg **Administration:** take with plenty of liquid	• As a prebiotic to improve the composition and activity of the intestinal microbiome. • Inhibits potentially pathogenic bacteria such as Clostridium difficile • To improve the antioxidant status of oxidative stress, cardiovascular diseases, arteriosclerotic changes and increased blood fat levels (triglycerides, cholesterol and LDL).	
praelasan® Pulver **Company / manufacturer:** Nutrimmun GmbH **Dosage:** 3 measuring spoons per day **Supplier:** pharmacy Drug code (PZN): 9922267 (420 g powder = 30 portions)	**Ingredients:** prebiotic corn dextrin, psyllium husk, calcium, baobab fruit powder **Administration:** Stir 14 g of powder (3 measuring spoons) in 200 ml of water and drink before a meal.	• Nourishes the physiological microbiota, especially with a low-fiber diet and thus contributes Colonization resistance to fungi and other pathogens. • With a tendency to constipation.	Pay attention to increased fluid intake so that the contained fiber can swell in the intestine.