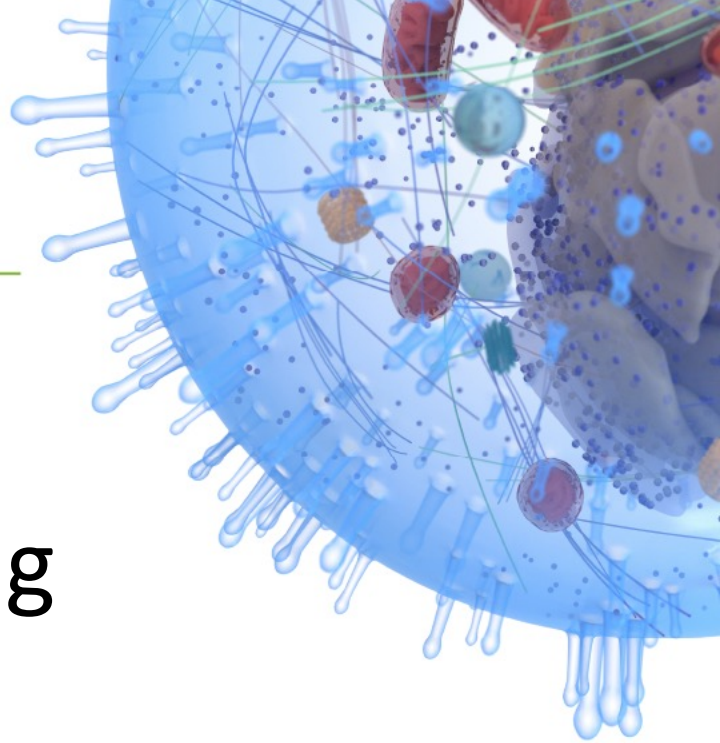
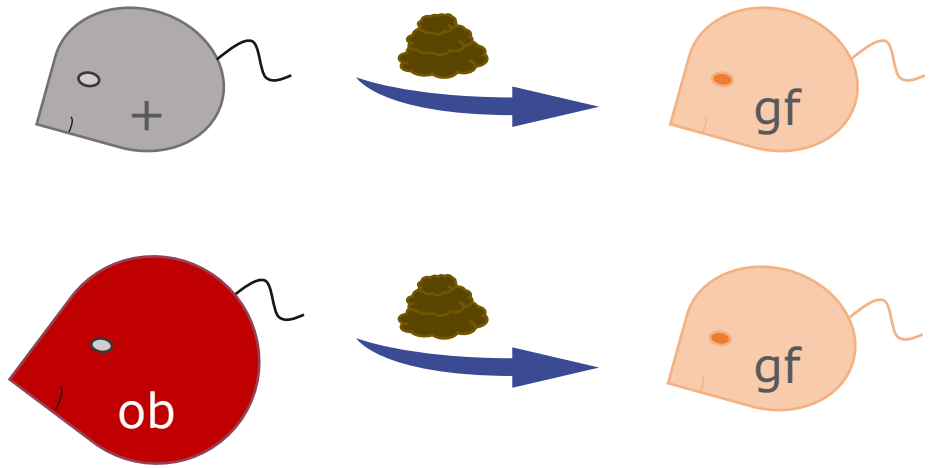


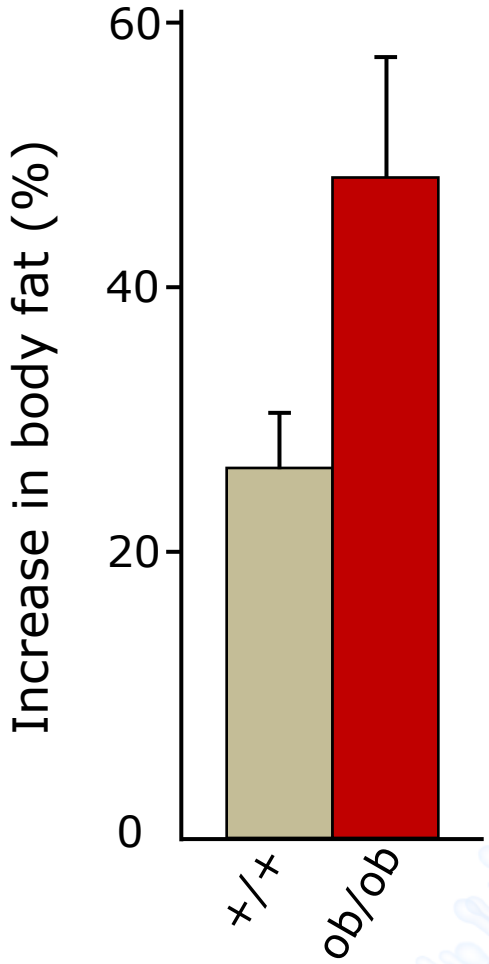
Analysis of the Gut Microbiome using Next-Generation Sequencing

Dr. rer. nat. Sibylle Blumenthal
Lab4more GmbH

Obesity in Mice

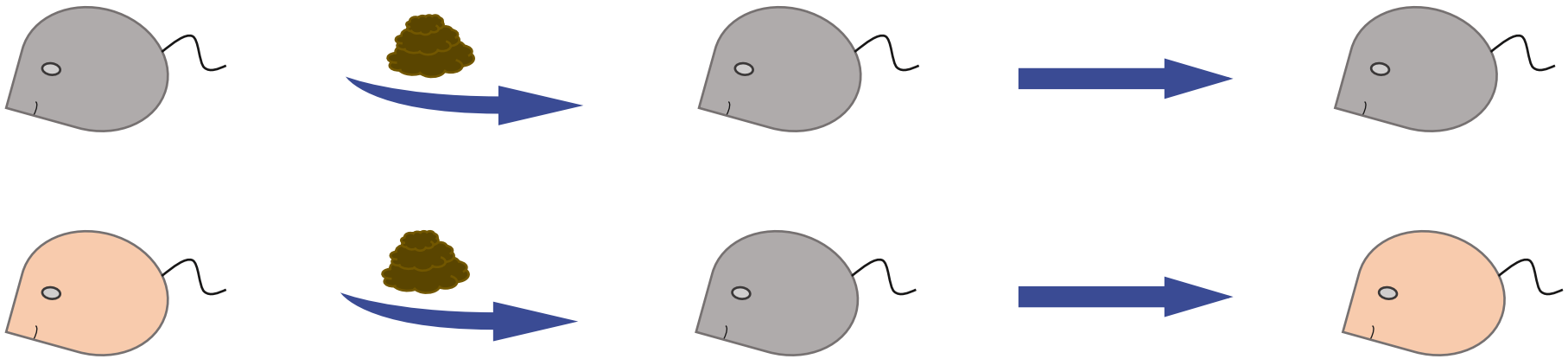


gf-mouse: germfree mouse
+ mouse: healthy mouse
ob-mouse: obese mouse



Turnbaugh et al., Nature. 2006, 444(7122):1027-31.

Anxiety/Depression in Mice



Mouse without anxiety / depression

Mouse with anxiety / depression

Nannan Li et al. (2019), Stress, 22:5, 592-602.

Why is the gut so important

Chronic inflammatory intestinal disorders
Ulcerative Colitis/Crohn's disease
IBS (inflammatory bowel syndrome)
Food intolerances
Leaky gut syndrome
Diarrhea / Constipation

Neuropsychiatric disorders
Depression
Dementia
Parkinson's disease
Autism
Anxiety

Metabolic syndrome
Obesity
Diabetes mellitus
Cardiovascular diseases
Non-alcoholic fatty liver

Other diseases
Allergies
Asthma
Rheumatic disorders

Microbiome - Microorganisms

- Yeast/fungi
- Viruses
- Parasites
- Archaea
- Bacteria

Illustration by Charis Tsevis

Microbiome - Bacteria

38
Trillion
Bacteria

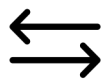
30
Trillion
Cells

7.9 Billion
World Population

Functions of the intestine

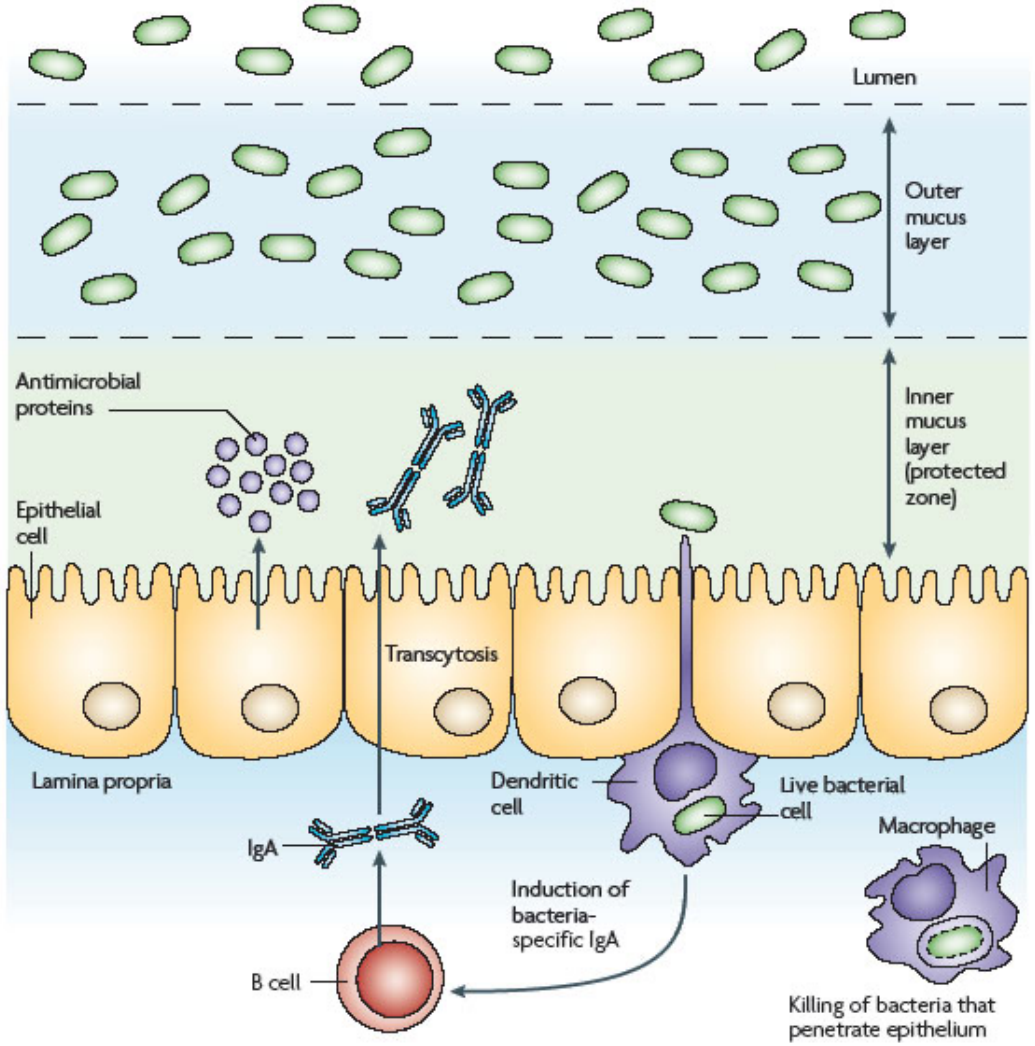
Not just digestion

Functions of the intestine



- Gut barrier
  - Selective permeability
  - Protective function

Gut barrier



Microbial barrier

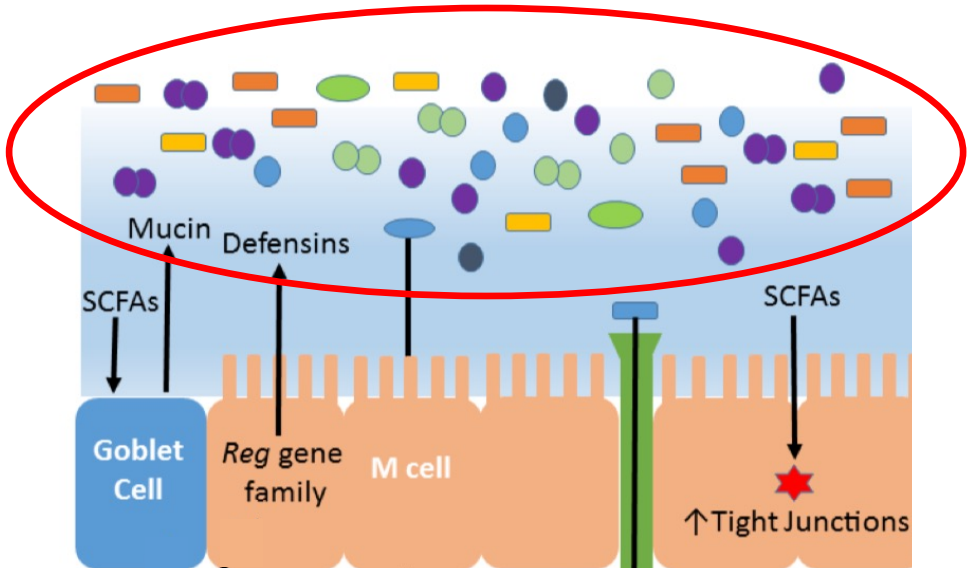
Mucus layer

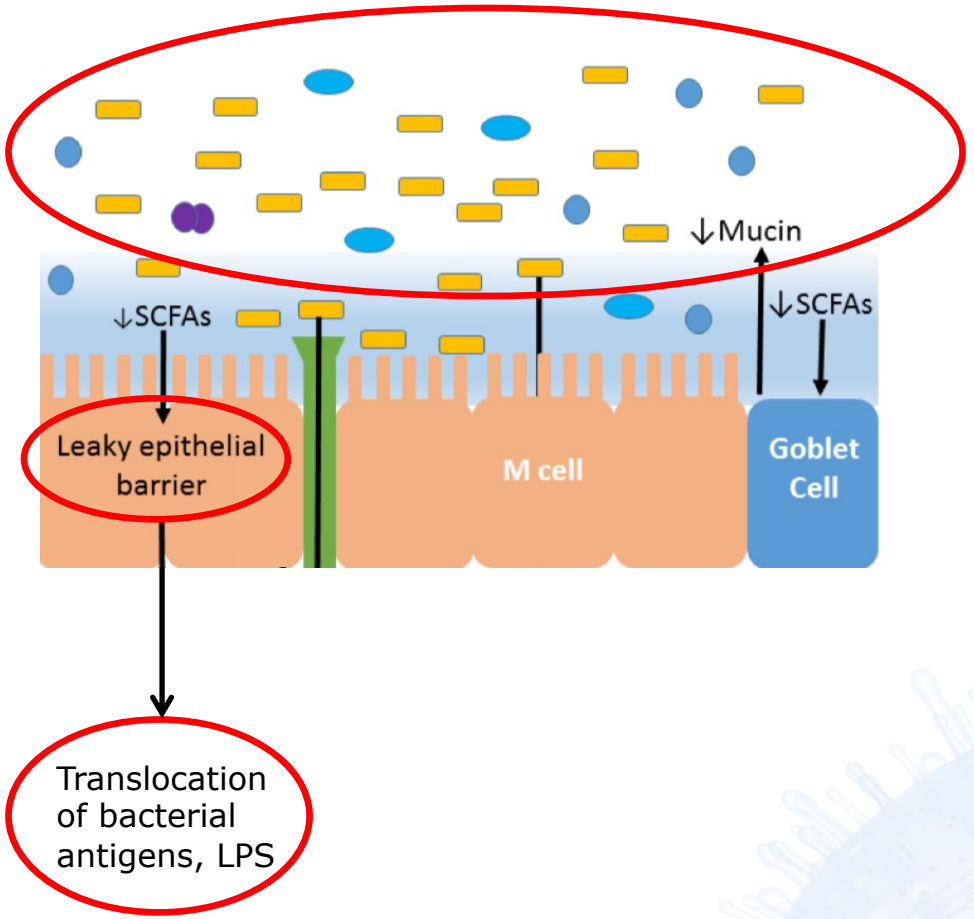
Epithelial layer

Gut-associated lymphoid tissue (GALT)

Hooper, L.V. et al 2009; Nature Reviews Microbiology volume 7.

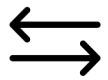
Intestinal Mucosa – Leaky Gut and Bacterial Translocation



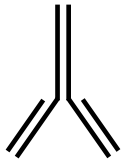


Barko et al., 2018

Functions of the intestine

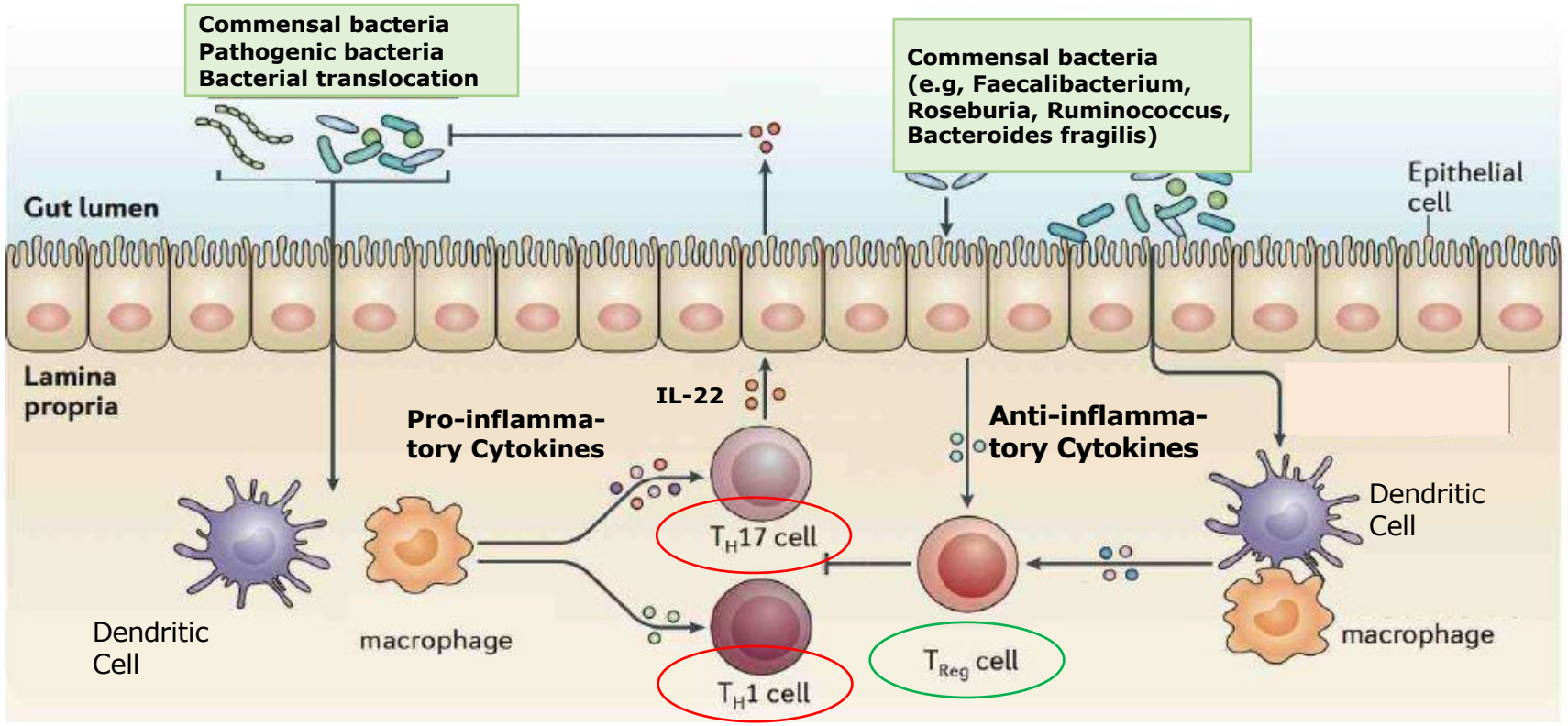


- Gut barrier
  - Selective permeability
  - Protective function



- Development and maintenance of the immune system
  - Activation of the immune system
  - Induction of antimicrobial peptides
  - Stimulation of cytokine and antibody production

Development and maintenance of the immune system



Th17 and Th1:

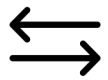
pro-inflammatory

Treg:

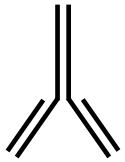
anti-inflammatory

Kamada N. et al., Nat. Rev. Immunology v.13, p.321–335(2013)

Functions of the intestine



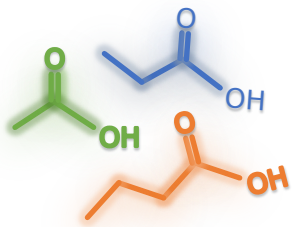
- Gut barrier
  - Selective permeability
  - Protective function



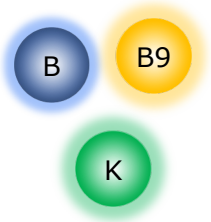
- Development and maintenance of the immune system
  - Activation of the immune system
  - Induction of antimicrobial peptides
  - Stimulation of cytokine and antibody production

- Digestion / Metabolic functions
  - Short-Chain Fatty Acids (SCFAs)
  - Vitamins and enzymes
  - Neurotransmitters

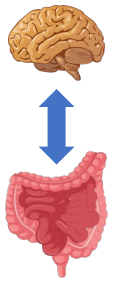
Digestion / Metabolic functions



- Short-chain fatty acids from dietary fiber (butyrate, acetate, propionate)
  - Strengthening of the gut barrier
  - Stabilization of tight junctions
  - Suppression of inflammatory mechanisms



- Vitamins and enzymes
  - B-Vitamins
  - Vitamin K
  - Folic acid

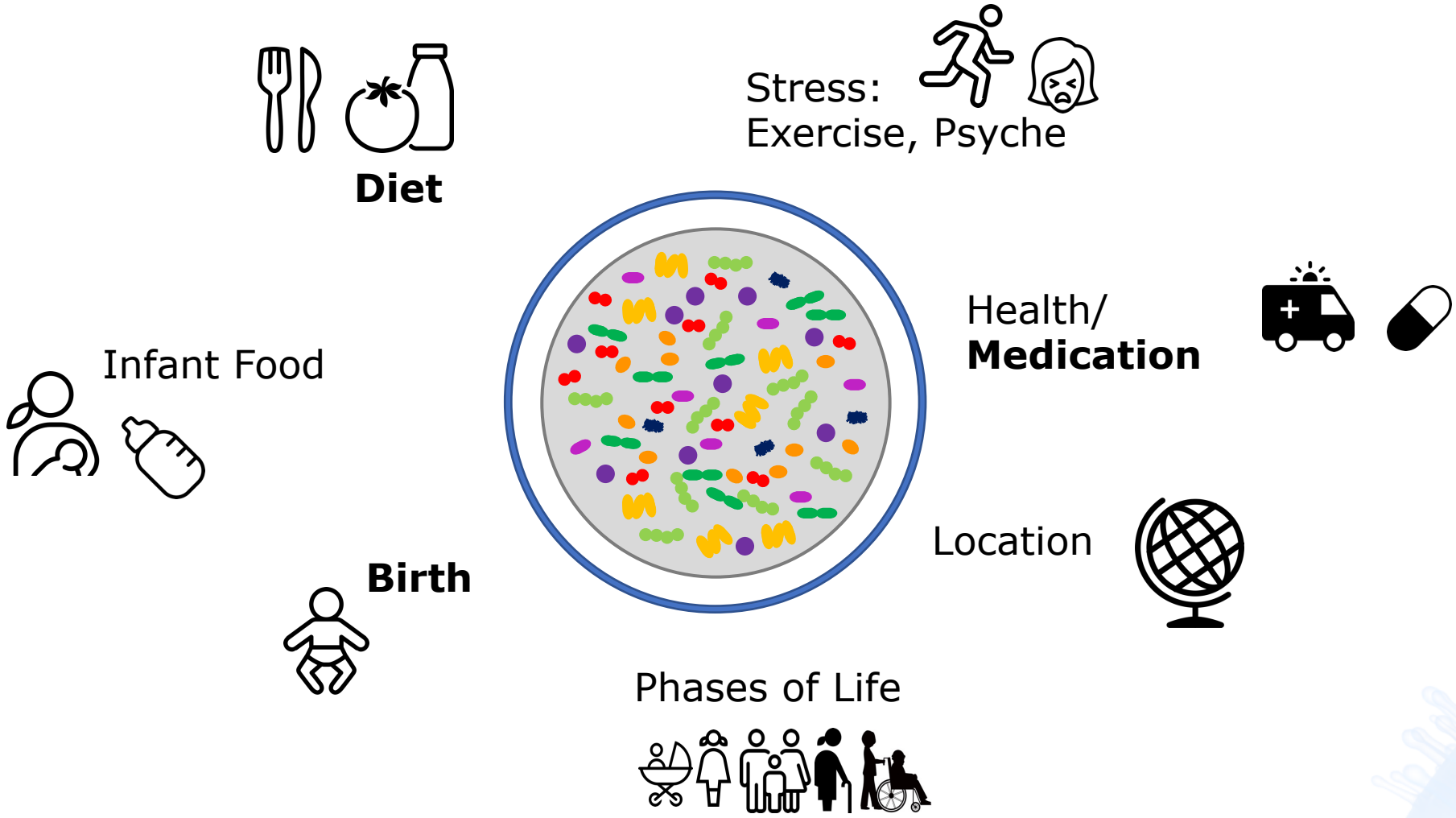


- Neurotransmitters (Gut-Brain Axis)
  - i.e. Dopamine, GABA, Serotonin
  - Secretion of cytokines
  - Promotion of inflammation in the CNS

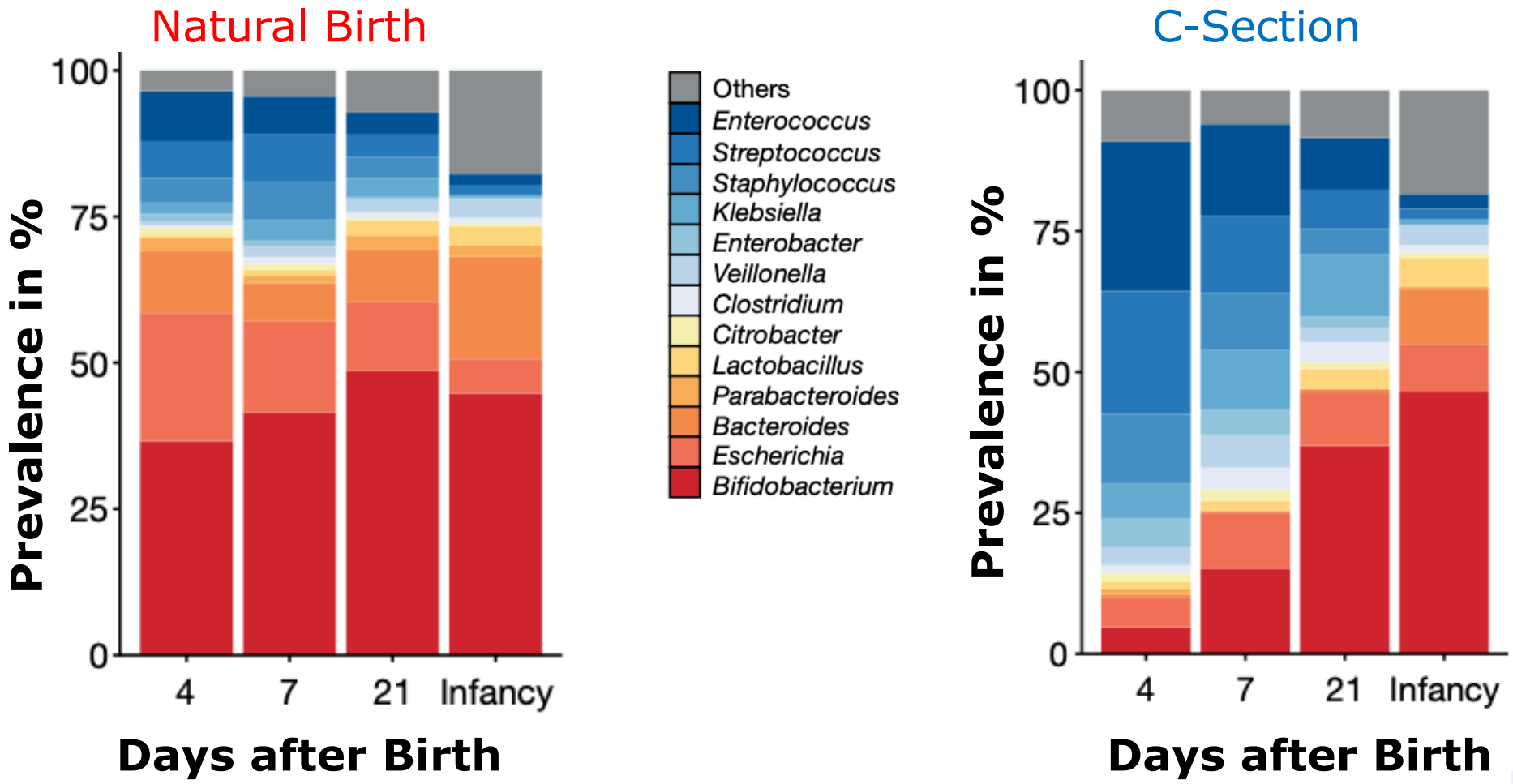
Effects on the Gut Microbiome

How do we get a healthy gut?

Effects on the Gut Microbiome



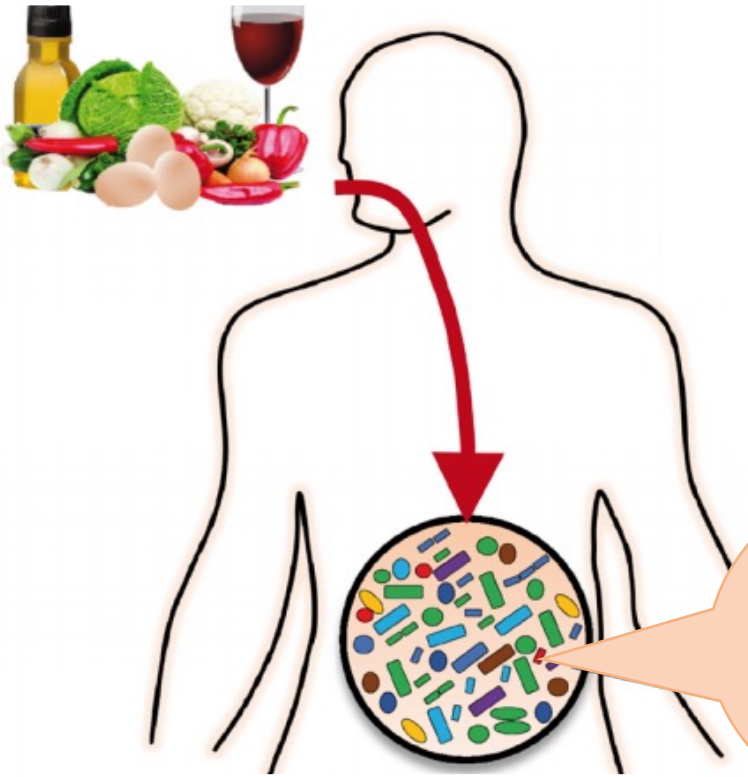
Birth (natural vs. Caesarian section)



Shao, Y. et al., Nature 2019, 574(7776):1-5.

Diet and Nutrition

Mediterranean Diet



Gut microbiome

↑ Diversity

↑ *Bacteroidetes, Lactobacilli, Bifidobacteria, Faecalibacterium, Oscillospira, Roseburia, Ruminococci, Clostridium cluster XIVa;*

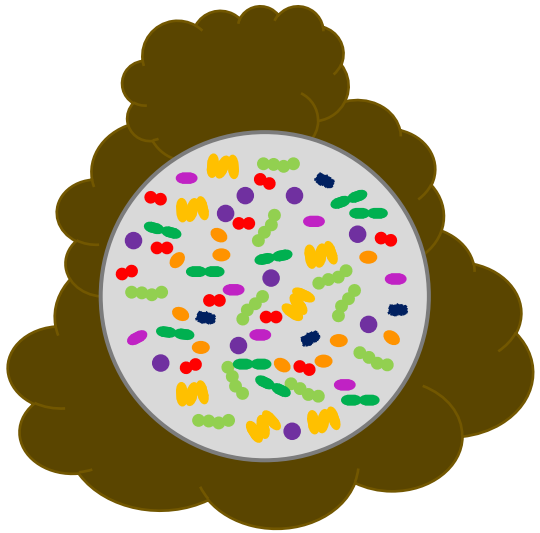
↓ *Firmicutes, Proteobacteria*

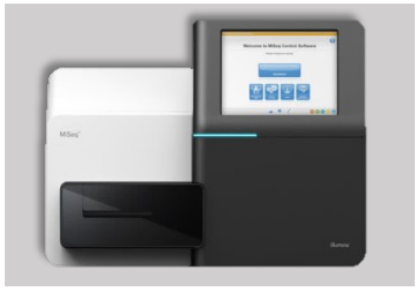
Nagpal R, Shively CA, Register TC et al. F1000Research 2019, 8:699.

Methods

How do we analyze the bacteria in a stool sample?

Methods for Stool Analysis





Bacterial culture

Method: Stool is inoculated onto culture media

Detection: Growth of bacteria

PCR

Method: Bacterial DNA is extracted

Detection: Targeted amplification of individual genes

NGS

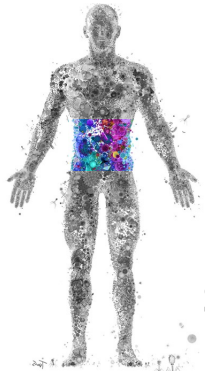
Method: Bacterial DNA is extracted

Detection: Sequencing of all genes

Next Generation Sequencing

Analysis of all bacteria in the stool sample

Procedure in the Lab

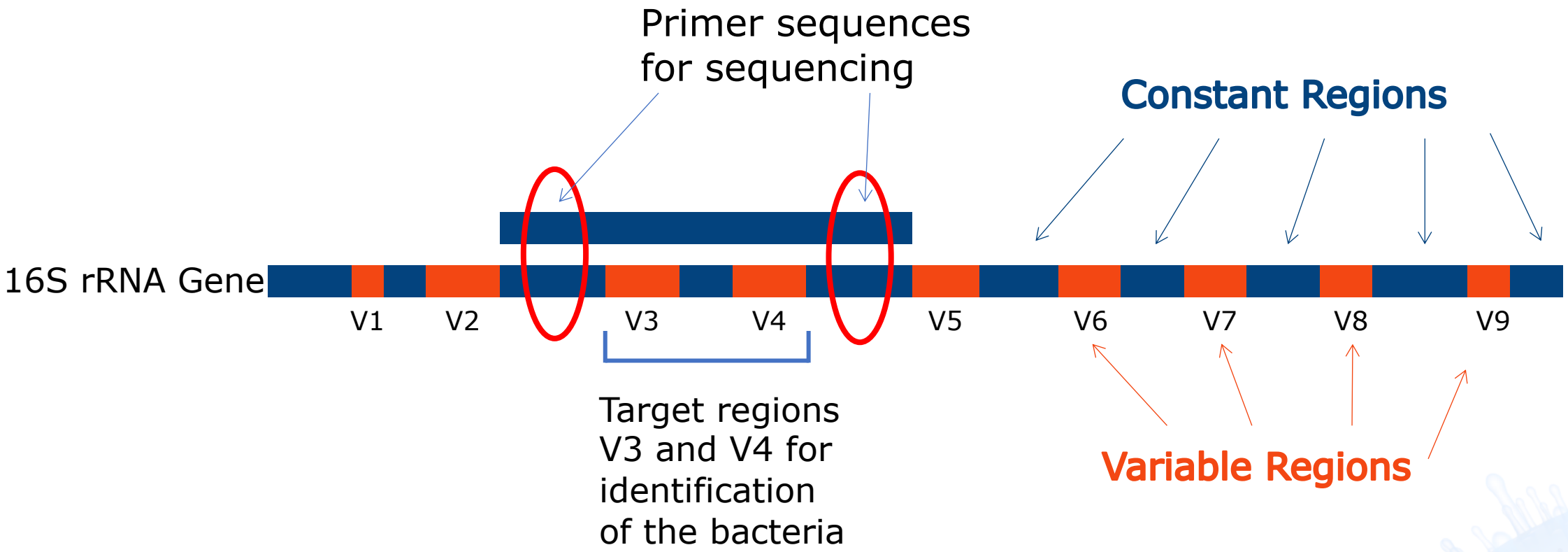


Stool sample

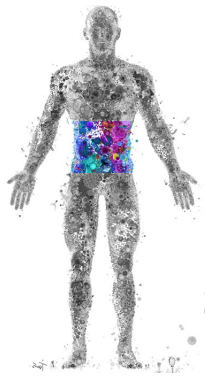
DNA-extraction

16S-Sequencing of bacterial DNA

16S ribosomal RNA Gene (16S rRNA)



Processing of the Data



Stool sample

DNA-extraction

16S-Sequencing of bacterial DNA

Report: Overview of the bacteria found in the stool sample with interpretation

```
TGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGTACAGAGGGTTGCCAACCCGCG
AGGgggAGCCAATCCCAGAAAAGCCGATCGTAGTCCGGATTGTTCTCCGCAACTCGAGAGCA
>BE326250111_37 JLK5VL137 orig_bc=ATCACG new_bc=ATCACG bc_diffs=0
GTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATACAATGGT
CGGTACAGAGGGTTGCCAACCCGCGAGGgggAGCCAATCCCAGAAAGCCGATCGTAGTCCG
>BE326250111_54 JLK5VL154 orig_bc=ATCACG new_bc=ATCACG bc_diffs=0
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GCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCATGCTGCGGTGAATACGTTCCCGGG
>BE326250111_91 JLK5VL191 orig_bc=ATCACG new_bc=ATCACG bc_diffs=0
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ACGTTCCCGGGCCTTGTACacacCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAGTG
>BE326250111_90 JLK5VL190 orig_bc=ATCACG new_bc=ATCACG bc_diffs=0
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>BE326250111_127 JLK5VL1127 orig_bc=ATCACG new_bc=ATCACG bc_diffs=0
```
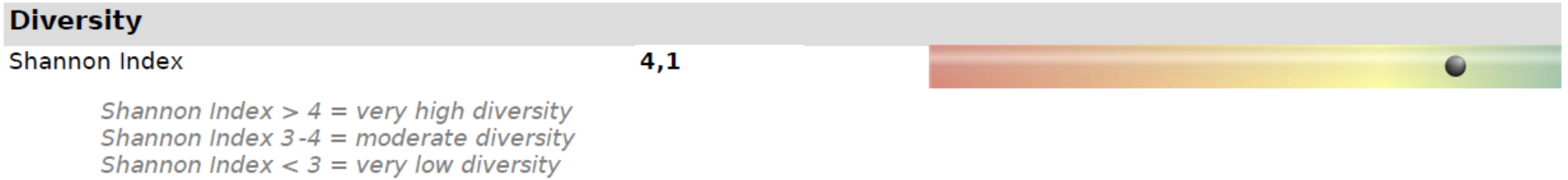

Identification of bacteria

Report

What is included in the report?

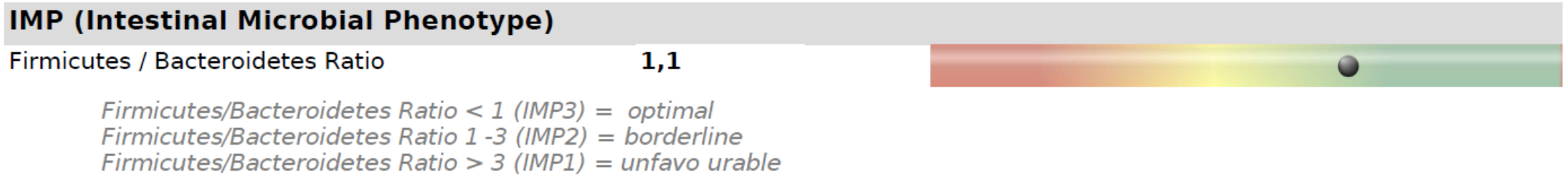
Diversity



Shannon Index – Abundance and evenness of the species

High diversity indicates a stronger and more stable microbiome

Intestinal Microbial Phenotype



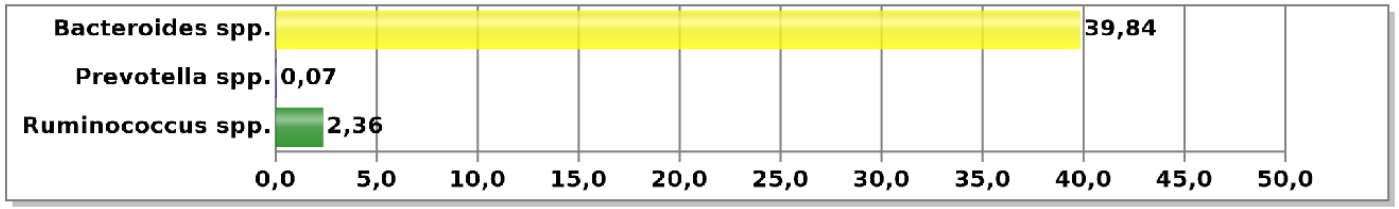
Intestinal Microbial Phenotype - Firmicutes/Bacteroidetes Ratio

A high IMP can lead to inflammatory reactions

Enterotype

Enterotype

1



Enterotype

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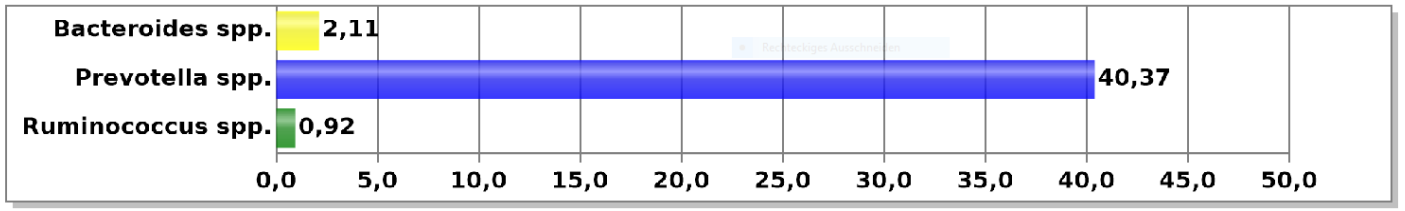
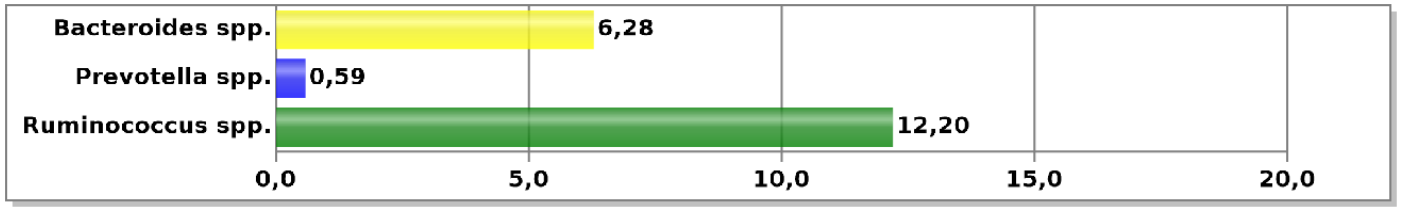


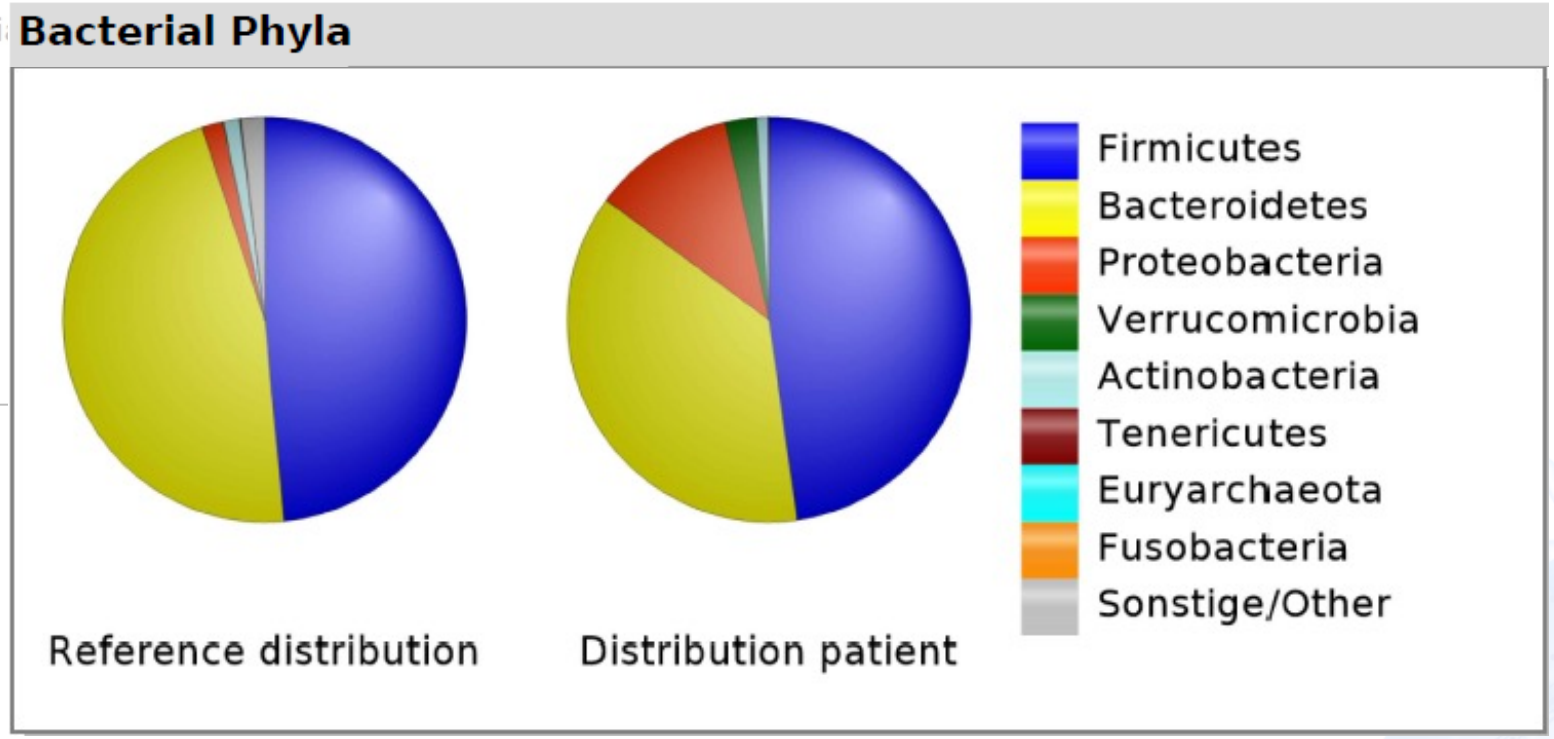
Enterotype

3

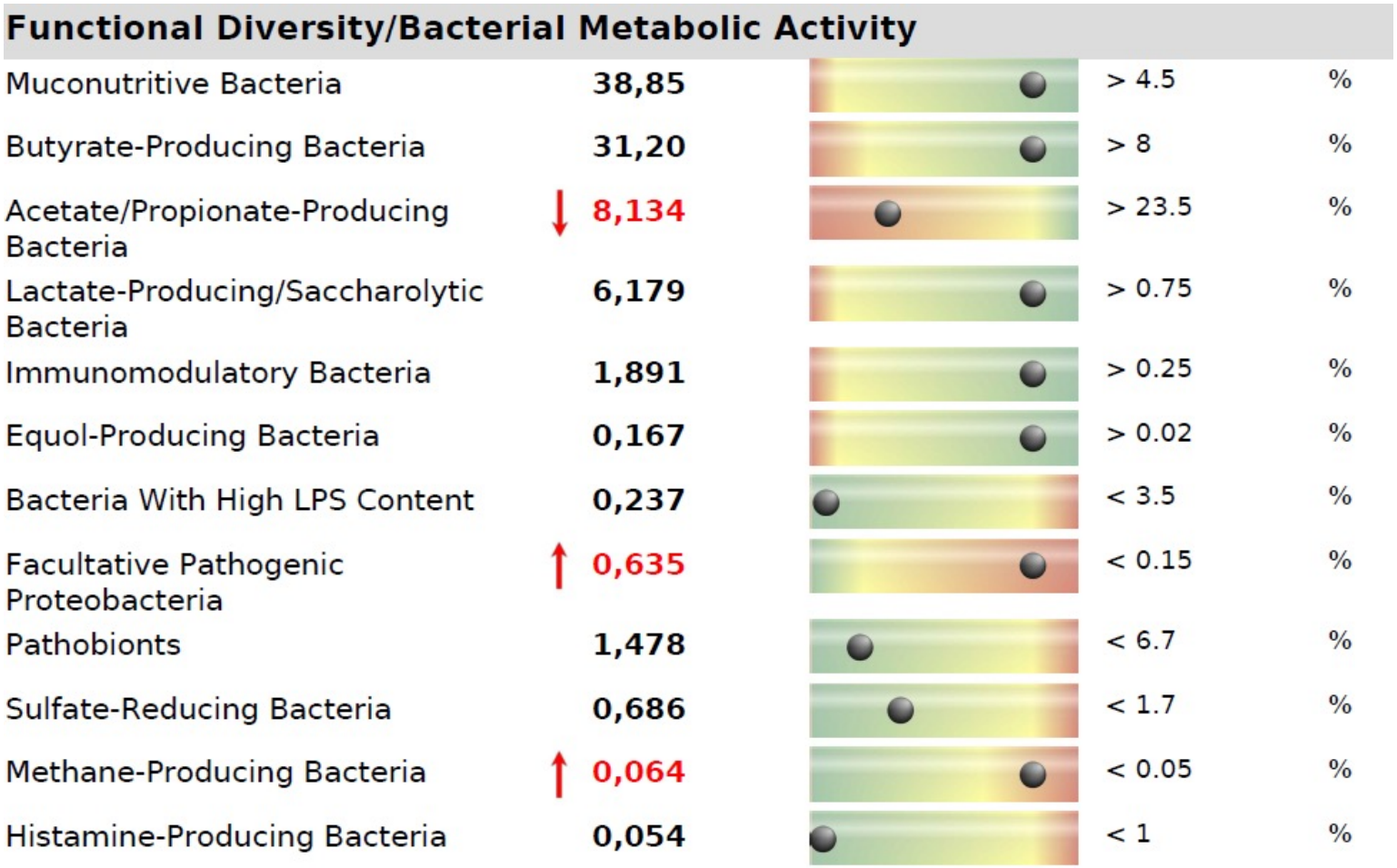


Breakdown of the bacterial groups

Test Name	Result	Ref. Range	Units
Firmicutes	46,9	30 - 53	%
Bacteroidetes	36,6	34 - 61	%
Proteobacteria	↑ 11,2	3.4 - 8.5	%
Verrucomicrobi			
Actinobacteria			
Tenericutes			
Euryarchaeota			
Fusobacteria			
Other			



Overview – functional groups

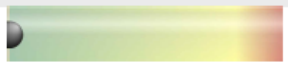


Butyrate – producing bacteria

Butyrate-Producing Bacteria				
Faecalibacterium prausnitzii	↓ 1,505		> 3,5	%
Ruminococcus spp.	1,970		> 1	%
Eubacterium spp.	↓ 0,665		> 0,7	%
Eubacterium rectale	0,471		> 0,3	%
Roseburia intestinalis	0,966		> 0,4	%
Anaerobutyricum hallii	0,200		> 0,03	%
Coprococcus spp.	0,080		> 0,01	%
Butyrivibrio crossotus	↓ 0,000		> 0,01	%
Clostridium butyricum	↓ 0,000		> 0,01	%

Mucosa-nourishing and anti-inflammatory

Pathogenic Bacteria

Pathogenic Proteobacteria				
Acinetobacter spp.	**0,000**		< 0.01	%
Enterobacter spp.	**0,000**		< 0.01	%
Haemophilus spp.	↑ **0,169**		< 0.03	%
Hafnia spp.	**0,000**		< 0.01	%
Klebsiella spp.	↑ **0,016**		< 0.01	%
Morganella spp.	**0,001**		< 0.05	%
Proteus spp.	**0,000**		< 0.01	%
Serratia spp.	**0,001**		< 0.01	%
Pathobionts				
Prevotella copri	**0,010**		< 2.3	%
Clostridium spp.	**2,735**		2 - 4.4	%
Desulfovibrio piger	**0,000**		< 0.01	%
Methanobrevibacter smithii	**0,000**		< 0.01	%
Clostridioides difficile	**0,001**		< 0.01	%

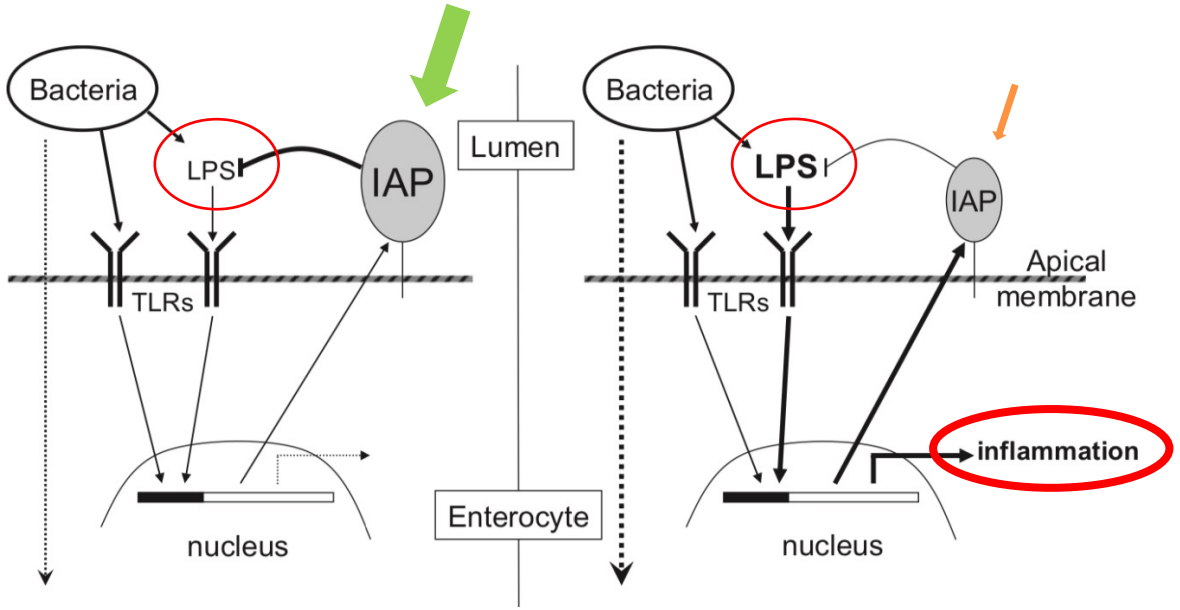
LPS-Positive Bacteria

LPS-Positive Bacteria				
Sutterella spp.	0,006		< 2.5	%
Escherichia spp.	↓ 0,024		0.1 - 1	%
Citrobacter spp.	0,003		< 0.01	%
Enterobacter spp.	0,000		< 0.01	%
Klebsiella spp.	↑ 0,016		< 0.01	%
Providencia spp.	0,000		< 0.01	%
Pseudomonas spp.	0,006		< 0.01	%

Endotoxin Index

Endotoxin Index		
Endotoxin Index	**4,1**	1 - 20

Normal Endotoxin Index
(sufficient IAP)



High Endotoxin Index
(insufficient IAP)

Lallès, J. (2010). *Nutrition reviews, 68 6*, 323-32.

Microbiome Analysis Interpretation

How can the results help the patient?

Inflammation – Leaky Gut

Patient history: Dysbiosis, **Leaky-Gut-Syndrome**, Meteorism

Muconutritive Bacteria				
Faecalibacterium prausnitzii	↓ 1,505	> 3,5	%
Akkermansia muciniphila	24,243	> 0,1	%
Roseburia intestinalis	0,966	> 0,4	%
Bacteroides thetaiotaomicron	↓ 0,333	> 0,4	%
Bifidobacterium longum	↓ 0,004	> 0,01	%
Ruminococcus gnavus	0,041	> 0,002	%
Prevotella spp.	4,144	0,1 - 6,8	%

Permeability			
Zonulin (Stool)	↑ 260,0	< 120	ng/ml

Anti-inflammatory therapy

- Curcuma
- Boswellia
- Devil‘s claw

Mucous membrane integrity

- L-Glutamine
- Choline
- GABA Capsules

Reduced Fermentation

Patient history: Dysbiosis, Leaky-Gut-Syndrome, **Meteorism**

Butyrate-Producing Bacteria				
Faecalibacterium prausnitzii	↓ 1,505	> 3,5	%	
Ruminococcus spp.	1,970	> 1	%	
Eubacterium spp.	↓ 0,665	> 0,7	%	
Eubacterium rectale	0,471	> 0,3	%	
Roseburia intestinalis	0,966	> 0,4	%	
Anaerobutyricum hallii	0,200	> 0,03	%	
Coprococcus spp.	0,080	> 0,01	%	
Butyrivibrio crossotus	↓ 0,000	> 0,01	%	
Clostridium butyricum	↓ 0,000	> 0,01	%	

Improvement of fermentation

- Resistent starch type-3
- Fructooligosaccharides
- Inulin

Prebiotics

- Acacia fiber
- Oat/wheat bran
- Pectin

Dysbiosis - Bacterial Imbalance

Patient history: **Dysbiosis**, Leaky-Gut-Syndrome, Meteorism

Lactate-Producing / Saccharolytic / Fiber-Degrading Bacteria				
Lactobacillus spp.	2,315		> 0,2	%
Bifidobacterium spp.	↓ 0,008		> 0,1	%
Bifidobacterium adolescentis	↓ 0,001		> 0,01	%
Enterococcus spp.	↓ 0,001		> 0,01	%
Ruminococcus bromii	↑ 0,824		0,1 - 0,8	%
Immunomodulating Bacteria				
Lactobacillus spp.	2,315		> 0,2	%
Escherichia coli	↓ 0,030		0,1 - 1	%
Enterococcus spp.	↓ 0,001		0,01 - 0,04	%

Probiotics
- Bifidobacterium
- Lactobacillus

Enterococcus
- Omega-3-fatty acids
- Glucans

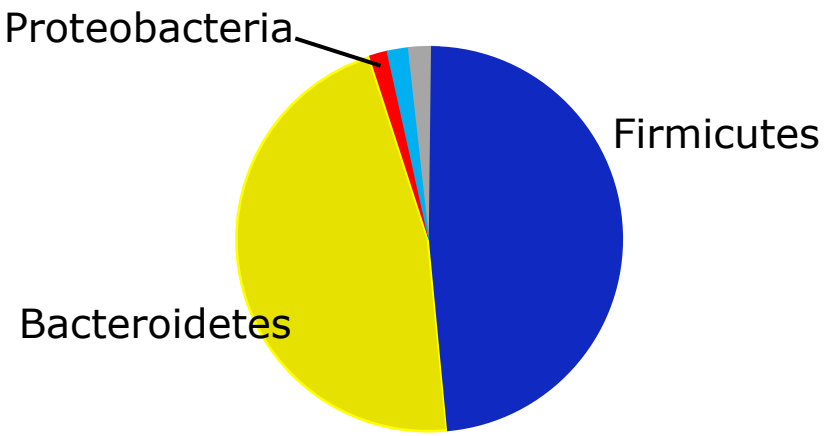
Examples: before/after

Changes in the microbiome

Example 1: Antibiotic Treatment

Healthy Patient

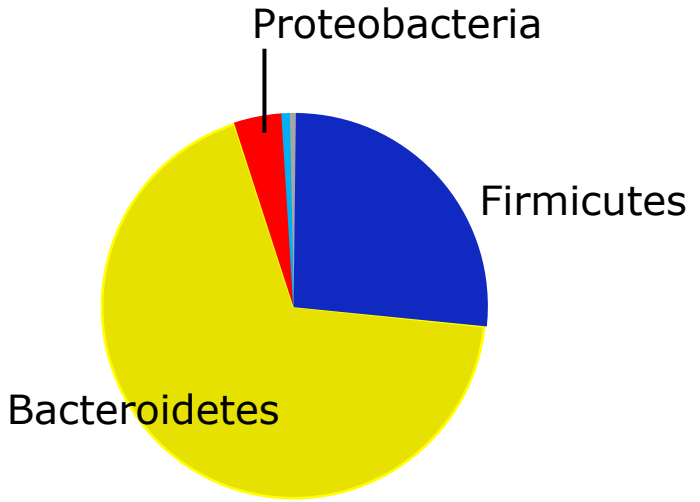
Diversity	
Shannon Index	4,3



before

Patient after 2 weeks of antibiotics

Diversity	
Shannon Index	3,4



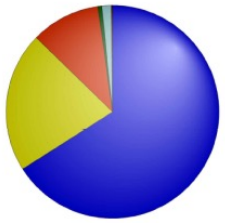
after

Example 2: pre/post-Therapy (9 Months)

Symptoms: Pain, bloating, diarrhea

IMP (Intestinal Microbial Phenotype)

Firmicutes / Bacteroidetes Ratio **3,1**

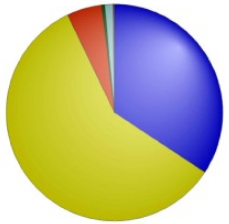


Potential for inflammation indicated by elevated Firmicutes/Bacteroidetes ratio

Anti-inflammatory treatment: Boswellia, Curcuma, Devil‘s claw

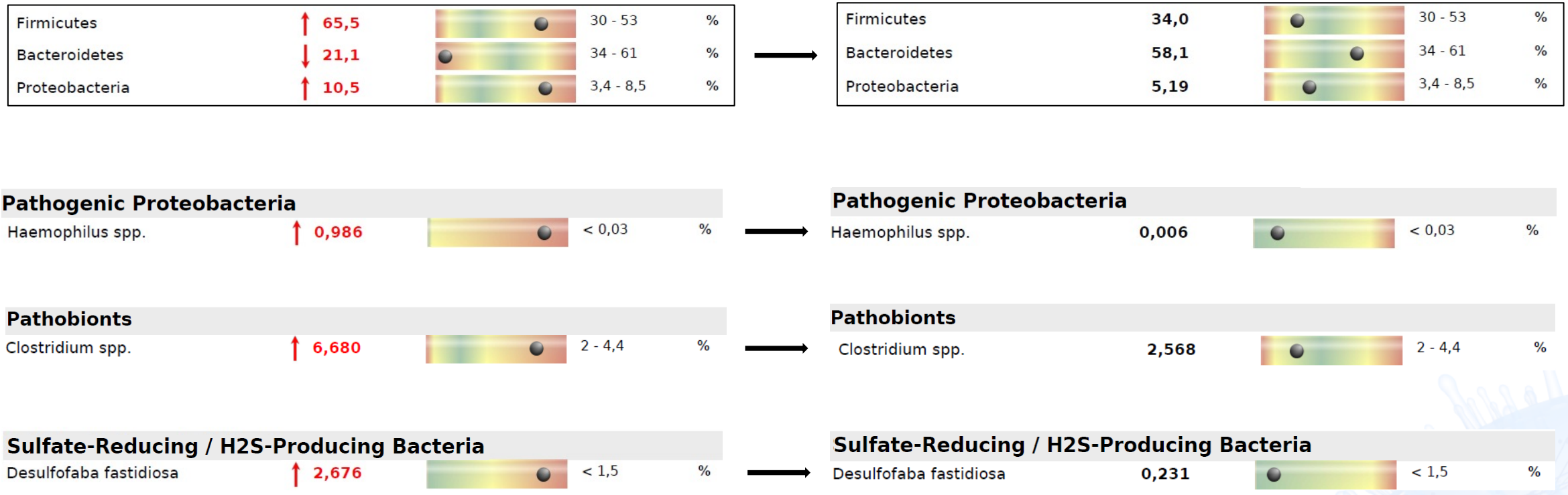
IMP (Intestinal Microbial Phenotype)

Firmicutes / Bacteroidetes Ratio **0,6**

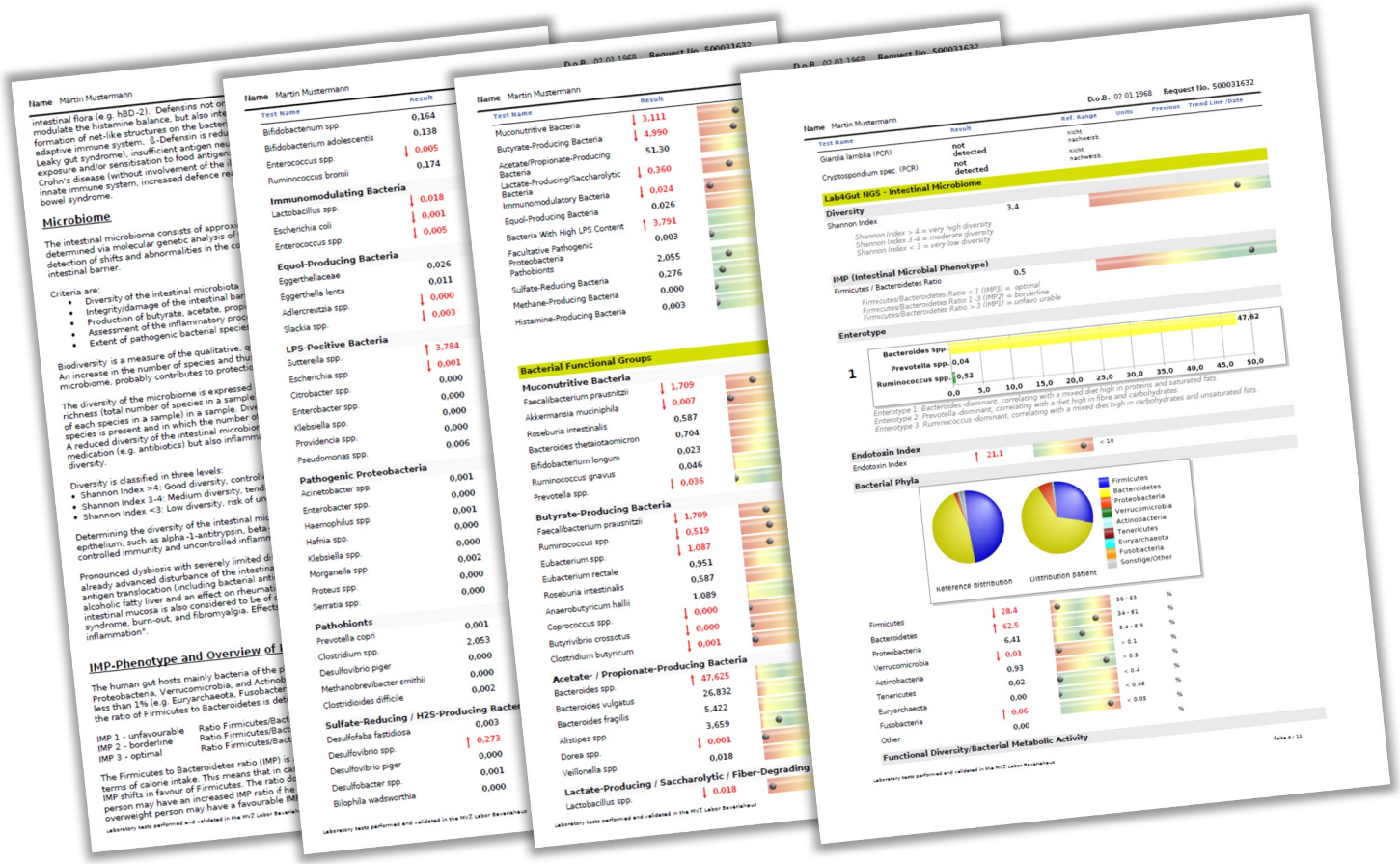


Example 2: pre/post-Therapy (9 Months)

Probiotic treatment: Lactobacillus, Bifidobacterium, Streptococcus



NGS Report



MVZ Labor Bavariahaus
Karlstrasse 16
D-80333 München
Tel.: 0049 89 54 32 17 0

Lab4more
Mehr als nur ein Labor

Muster Arzt	**Name**	Martin Mustermann			
Mustergasse 12	**D.o.B.**	02.01.1968	**Sex**	M	**Request No.** 500031632
80999 München	**Address**	Musterstrasse 67			**Received** 14.05.2021
Fax		D 822969 Musterstadt			**Output Date** 26.05.2021
Client No. 4070	**Patient No.**	359965			**Archive No.** 992165

Height [ ] cm **Weight** [ ] kg **Body Mass Index** [ ]

Medical History:
Keine Angaben

Interpretation:
1. The pH-value is high.
2. Biliary acids and pancreatic elastase are unremarkable.
3. The inflammatory markers are raised.
4. The intestinal alkaline phosphatase is below normal range.
5. Zonulin levels within normal range, no evidence of abnormal intestinal permeability (Leaky Gut) due to loss of functionality of the tight junctions.
6. No parasites were detected.
7. Lab4gut NGS
- The diversity is reduced.
- The Firmicutes/Bacteroidetes ratio is optimal.
- The Endotoxin Index is high (LPS Bacteria raised).
- Verrucomicrobia are decreased; Fusobacteria are increased.
- Muconutritive bacteria are low.
- Butyrate-producing bacteria are below the norm.
- Lactate-producing/saccharolytic bacteria are low.
- Immunomodulating bacteria are low.
- Bacterial species with high LPS structural content are high.

INTESTINAL MICROBIOME / SUMMARY OF RESULTS

Diversity
The diversity is reduced (3-4). In the long term, a reduction in diversity can impair the physiological regulation of the intestinal immune system and lead to an uncontrolled inflammatory reaction of the intestine, resulting in a dysfunction of the intestinal barrier, which can also have systemic effects.

Firmicutes / Bacteroidetes (IMP)
Intestinal microbial phenotype (IMP) type 3, the Firmicutes/Bacteroidetes ratio is optimal (<=1).

Enterotype
Bacterial strains of the genus Bacteroides (enterotype 1) were dominantly detected. Bacteroides mainly metabolise proteins, but also carbohydrates. Bacteroides species are primarily found when a mixed diet (meat, carbohydrates, fibre, fat) is preferred.

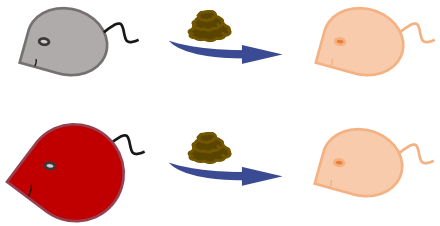
Endotoxin Index
The elevated Endotoxin Index indicates that the high number of bacterial species with high LPS structural content can no longer be sufficiently neutralized by the IAP. This increases the bacterial translocation at the intestinal mucosa and thus the risk of an inflammatory reaction.

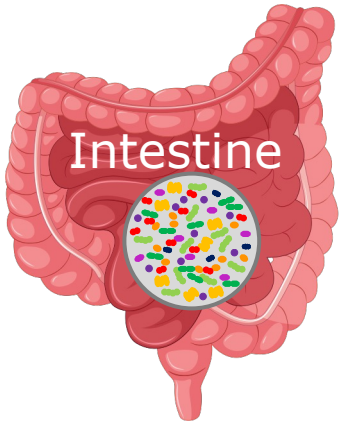
Bacterial Phyla
Firmicutes are reduced, *Bacteroidetes* increased, which is reflected in the very low IMP.
Verrucomicrobia (esp. *Akkermansia muciniphila*) were detected at levels below the norm.
Fusobacteria (esp. *Fusobacterium nucleatum*) were detected at elevated levels.

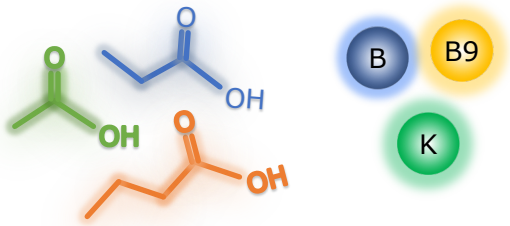
Bacterial Protection of the Mucus Layer / Intestinal Barrier Integrity
A healthy and diverse composition of beneficial gut bacteria ensures that the intestinal epithelial cells (enterocytes) are nourished and that the mucosa provides a sufficient barrier. Butyrate is the main energy source of these enterocytes. Acetate and propionate are used as nutrients by other bacteria. Propionate is also an important

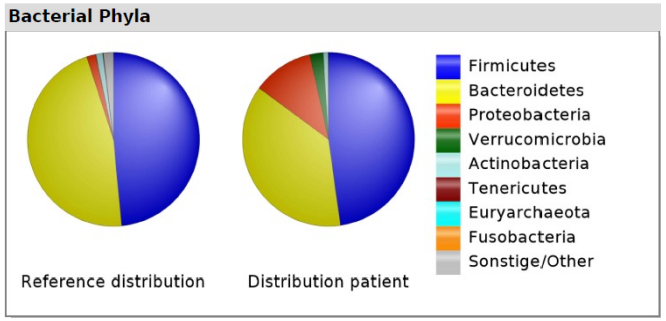
Laboratory tests performed and validated in the MVZ Labor Bavariahaus — Seite 1 / 11

Summary and Review









Thank you for your Attention

Dr. Sibylle Blumenthal

blumenthal@lab4more.de