Gu GutHealth©
by LIFESTYLE GENOMICS

Microbioma Intestinal de Nova Geração

GERMANO DE SOUSA
CENTRO DE MEDICINA LABORATORIAL

LIFESTYLE GENOMICS®

LIFESTYLE GENOMICS®

Lifestyle Genomics is a brand of scientific-based products aimed for personalized health management. We all want to experience fuller and healthier lives therefore, we should look after ourselves in all dimensions that constitute our physical and emotional wellbeing. To achieve this, we must know our own bodies and evaluate, measure and quantify our health. To achieve such goals we must rely on laboratory scientific validated solutions with rigorous quality control measures. This is the LifeStyle Genomics mission.

Gu GutHealth®
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What is measured in this test

The GUT HEALTH(TM) is a gut microbiome test based on a fecal sample. It is an identification and quantification of all bacteria that are a part of the gut flora. It is compreensive due to the use of a molecular sequencing technique called NGS (Next Generation Sequencing) that caracterizes the DNA of the gut bacteria, allowing for identification and quantification of more than 95% of the bacteria present in the sample, in contrast with more traditional approaches that are able to identify less than 30%. It is a relevant test that details results for organisms that can have its population altered by diet, based on the most recent scientifical and clinical research. It is an informative test that details results for global parameters such as diversity and relative frequencies of the organisms in the context of a reference database of healthy individuals. It is a clinically validated test - performed in a certified laboratory and licensed by clinical pathology in its role of molecular and genetics pathology, therefore complying with rigorous quality criteria related to this activity.

NOVA MEDICAL SCHOOL
FACULDADE DE CIÊNCIAS MÉDICAS

How this test was developed

The GUT HEALTH(TM) test is the result of a partnership between Centro de Medicina Laboratorial Germano de Sousa, through its division Lifestyle Genomics, and the group "Nutrição e Metabolismo" from Faculdade de Ciências Médicas from Universidade NOVA de Lisboa. Lifestyle Genomics developed and implemented the laboratory and bioinformatician components of the test, assuring all the quality, safety and rigorous criteria of the results, while the group "Nutrição e Metabolismo" developed the interpretation of the results.

Test Limitations

Gut microbiome analysis, in the context of human health, is a recent scientific and clinical development, only possible due to the current ability to analyse large quantities of DNA with the use of new generation sequencing. This area of expertise is the target of intense study by the scientific community and its results have revealed the importance of a balanced microbiota in human health, as well as the pathological consequences of its alteration. It is important to state that these tests should be analysed by your physician or nutricionist.

GutHealth®
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USER IDENTIFICATION

Name: Teste Tipo

Date of Birth: 01/01/2000

Gender: Feminino

Age: 22

SAMPLE

Sample Identification: Teste

Sample Type: Feces

Collection Date: 01/01/2022

Release Date: 10/2/2022

REQUESTER

Name: N/A

☐ Doctor

☐ Nutricionist

☑ Other

Results Summary

Composition

☑ Statistical analysis of the global composition of this sample identified it as being **NORMAL**, resembling a healthy reference population of individuals.

All other analysed genus and species have similar relative frequencies in relation to the reference population of healthy individuals.

☑ The following pathogenic organisms were not found in the present sample:
Salmonella enterica, Campylobacter, Clostridium difficile, Shigella, Vibrio Cholerae

Global Metrics

☑ The GUT HEALTH test V1.0 indicates that the gut microbiome represented by the analysed sample has an index of **diversity** of **2.094** and resembles a healthy reference population of individuals.

☑ The **richness** of the biologic groups (phylum and class) resembles a healthy reference population of individuals.

☑ **Firmicutes/Bacteroidetes Ratio** de **0.54** resembling healthy reference population of individuals.

Global Analysis

✓ Normal Deviation

! Slight Deviation

✗ Extensive Deviation

✓ Diversity

Disbiosis is a composition and function alteration of the gut microbiota that may serve as basis of several illnesses such as gut inflamatory diseases and obesity. The expansion of pathobionts (comensal microrganisms that may cause pathologies when its growth is unchecked), the decrease of beneficial bacteria and the loss of diversity (abundance and richness of species) are common caracteristics of a dysbiosis state.

The Diversity measure used here is the Shannon Index.

The sample analysis shows a diversity index of **2.094**. This value is **between** the values interval of the healthy reference population of individuals.

✓ Richness

The richness of different microorganisms in the gut microbiota indicates the potential that this has for adaptation to different conditions. A microbiota disbiosis, with prevalence of species with harmful effects or loss of species with protective effects, frequently is accompanied with richness reduction of species.

This sample analysis revealed different **11** phylum and **21** classes, which indicates a **phylum** and **classes** richness **resembling** the values of a healthy reference population of individuals.

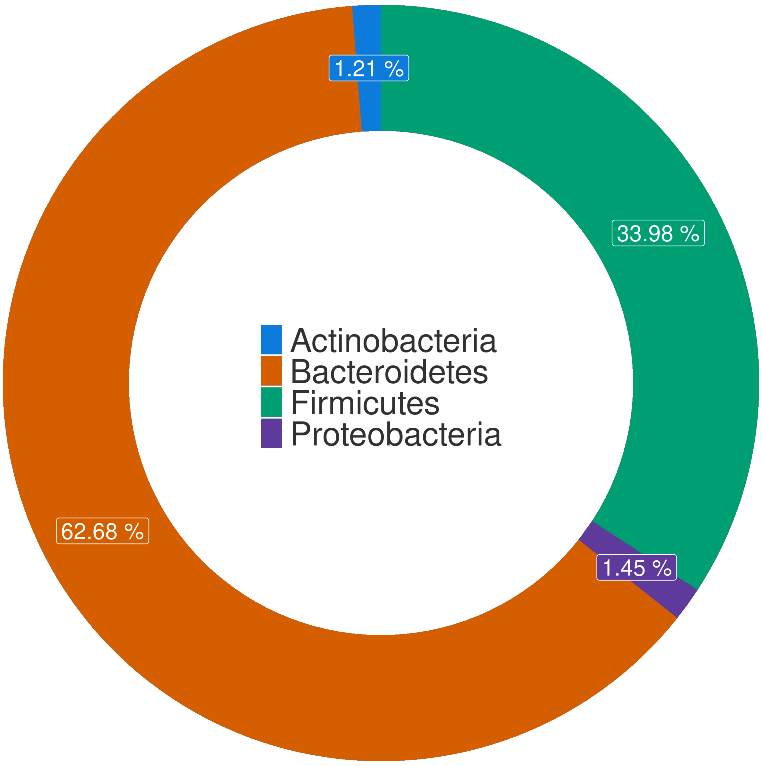
Richness at the taxonomic rank: PHYLUM

Richness at the taxonomic rank: CLASS

☑ Firmicutes/Bacteroidetes Ratio

The gut microbiota is mainly populated by bacteria belonging to the phylum Firmicutes and Bacteroidetes. Firmicutes and Bacteroidetes ratio is increased in obesity.

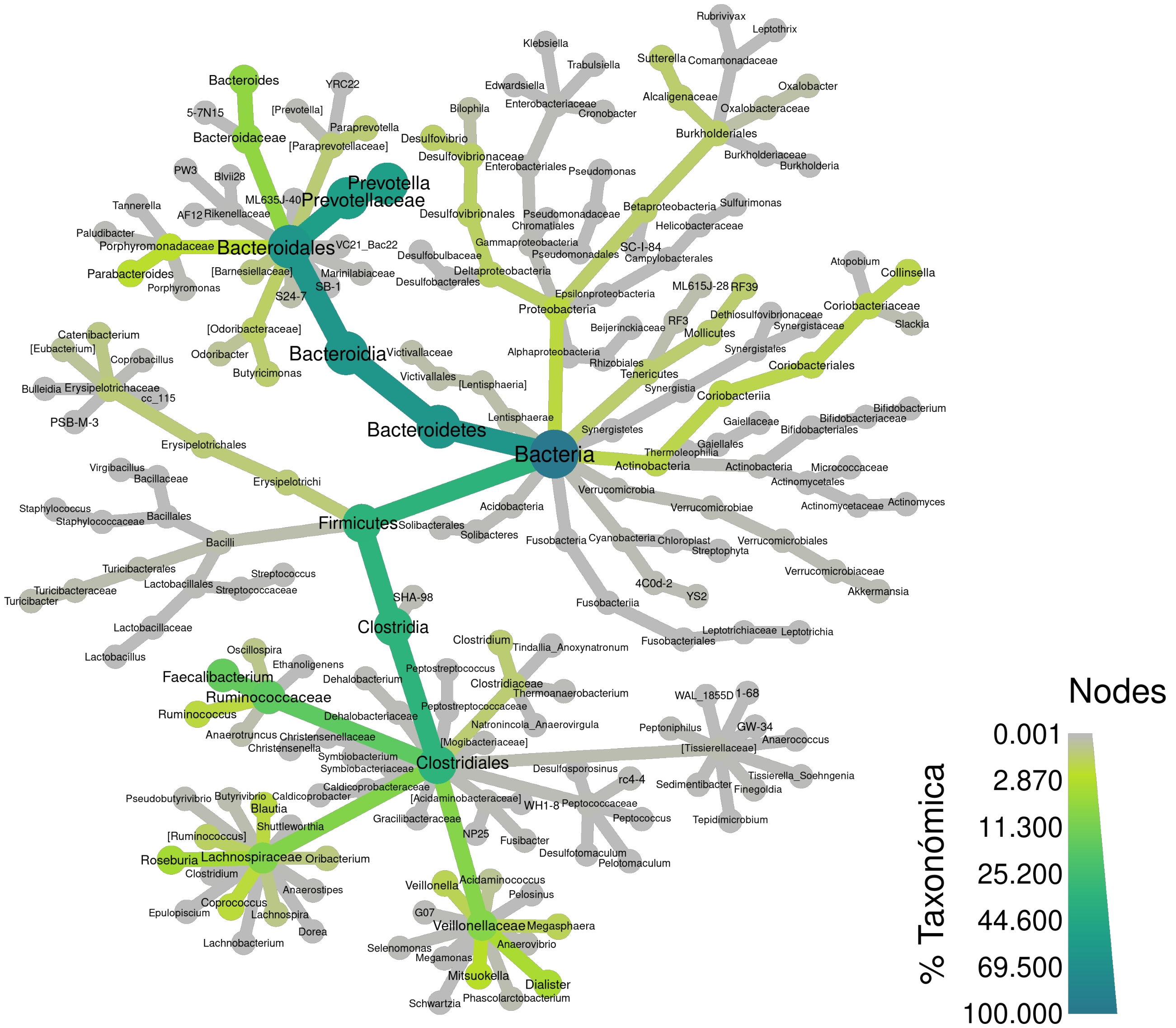
In the sample the value identified was **0.54**. This value is **between** the interval values of a healthy reference population of individuals.



Sample Value (%)	Normal Interval (%)	Comparison
0.54	0.25 - 8.68	Normal

Investigated Genus and Species

We have found **35** genus and **28** species of different bacteria in this sample, globally represented in the phylogenetic tree below.



Global vision of bacteria taxa in this sample. The tree represents the diversity of genus identified in this analysis. The color and dimension represent the frequency (see caption).

☑ **Statistical analysis of the global composition of this sample identified it as being NORMAL, resembling a healthy reference population of individuals.**

Detailed analysis by taxonomic group

Below we outline and describe the relative abundance of bacterial genus and species chosen to integrate this report. These organisms were selected due to clear scientific evidence, clinical relevance and its abundance being responsive to change through diet.

For each genus and species we present its physiological and pathological relevance and compare its relative abundance present in this sample with the relative abundance of a reference population of healthy individuals.

The following graphs represent the sample results in the context of the distribution of reference values.

☑ Blautia (Gram-positive; Obligate Anaerobic)

Sample Value (%)	Normal Interval (%)	Comparison
1.44	0.003 - 4.163	Normal

The *Blautia* genus is important for absorption of nutrients.
These are increased in obesity[3].

☑ Rumminococcus (Gram-positive; Obligate Anaerobic)

Sample Value (%)	Normal Interval (%)	Comparison
1.47	0.003 - 5.896	Normal

The *Rumminococcus* genus has the ability to break down cellulose.
This is linked to a worst cardiometabolic profile[8][20] (elevated LDL cholesterol) and atherosclerosis.

☑ Bifidobacterium (Gram-positive; Obligate Anaerobic)

Sample Value (%)	Normal Interval (%)	Comparison
< 0.01	0 - 2.089	Normal

The *Bifidobacterium* genus produces short-chain fat acids, improves the intestinal barrier and diminishes LPS (Lipopolysaccharide) levels in the gut.
These are diminished in obesity[2] and a diet rich in fibre that estimulates the growth of these bacteria[19].

☑ Bacteroides (Gram-negative; Obligate Anaerobic)

Sample Value (%)	Normal Interval (%)	Comparison
6.54	0.044 - 55.86	Normal

The *Bacteroides* genus ativates the T CD4 + cells and its presence is increased in intestinal inflammatory disease [9,10]. A rich diet in saturated fat and animal origin protein is linked to an increase of these bacteria [19].

☑ Roseburia (Gram-variable; Obligate Anaerobic)

Sample Value (%)	Normal Interval (%)	Comparison
4.07	0 - 6.091	Normal

The *Roseburia* genus produces short-chain fat acids and it is diminished in inflamatory intestinal diseases[7].

☑ Butyrivibrio (Gram-positive; Obligate Anaerobic)

Sample Value (%)	Normal Interval (%)	Comparison
0.02	0 - 0.073	Normal

The *Butyrivibrio* genus produces butyrate.
These prevent weight gain[4].

☑ Prevotella (Gram-negative; Obligate Anaerobic)

Sample Value (%)	Normal Interval (%)	Comparison
52.56	0.004 - 53.865	Normal

The *Prevotella* genus produces short-chain fat acids and is linked to a better cardiometabolic profile (lower LDL cholesterol LDL)[8].
A fiber rich diet promotes the growth of these bacteria[19].

☑ Lactobacillus (Gram-positive; Aerotolerant Anaerobes)

Sample Value (%)	Normal Interval (%)	Comparison
< 0.01	0 - 0.15	Normal

The *Lactobacillus* genus produces short-chain fat acids and have anti-inflammatory and anti-carcinogenic activities.
These restrict inflammatory intestinal disease[6]. And are present in fermented foods like yogurt, cheese and kefir[19].

☑ **Bilophila** (Gram-negative; Obligate Anaerobic)

Sample Value (%)	Normal Interval (%)	Comparison
0.06	0 - 0.269	Normal

The *Bilophila* genus is resistant to bile acids and has pro-inflammatory activity.
Bilophila wadsworthia is linked to colitis and cholecystitis[13].
A diet rich in saturated fats and protein of animal origin is linked to an increase of these bacteria[19].

☑ **Akkermansia muciniphila** (Gram-negative; Obligate Anaerobic)

Sample Value (%)	Normal Interval (%)	Comparison
0.01	0 - 29.122	Normal

The *Akkermansia muciniphila* species has anti-inflamatory activity.
These are diminished in intestinal inflamatory disease in obesity[15].

☑ **Faecalibacterium Prausnitzii** (Gram-positive; Obligate Anaerobic)

Sample Value (%)	Normal Interval (%)	Comparison
14.38	2.246 - 58.49	Normal

The *Faecalibacterium Prausnitzii* species produces short-chain fat acids and has anti-inflamatory activity.
These are diminished in intestinal inflamatory disease and in obesity[5].

Methodologies and Limitations

This test was performed in concordance with all quality recommendations, by a licensed laboratory in Clinical Analysis, Molecular Pathology and Medical Genetics, therefore it can be interpreted in a clinical context.

A database of 482 healthy samples was used as control. These were selected from the American Gut Project (www.americangut.org), filtered to present healthy values of relative abundance in concordance with its BMI (Body Mass Index) and the absence of pathologies such as SIBO (Small Intestinal Bacterial Overgrowth), IBS (Irritable Bowel Syndrome), IBD (Inflammatory Bowel Disease), Diabetes, Intolerance to Gluten/Lactose and autoimmune diseases.

The studied sample was caracterized through the V3 and V4 regions that encode the "16S rRNA" gene of each bacteria and its taxonomy was identified through a database "16S rRNA" + "GreenGenes 13_8" named GutHealth_DB.

To attain the sample classification of NORMAL/ABNORMAL a *machine learning* classification algorithm was used (Random Forests)[18], based on healthy reference values described above.

For this analysis only organisms that can be altered by diet[1] were selected:

At the Genus taxonomic level: *Bacteroides*, *Bifidobacterium*, *Bilophila*, *Blautia*, *Butyrivibrio*, *Lactobacillus*, *Prevotella*, *Ruminococcus*, *Roseburia*.

At the Species taxonomic level: *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*.

The sample was sequenced by NGS (Next Generation Sequencing), using the IonTorrent S5 platform and amplified with the Ion 16S™ Metagenomics kit. The taxonomy was identified resorting to Kraken2[16] and improved with Bracken[17] resorting to the classification database 'GutHealth_DB'.

In GUT HEALTH we report relative frequencies present in the sample, as well as some derived metrics. **It is recommended that these results be discussed in the presence of your physician or nutricionist or another health professional that ordered this test.**

We have filtered out from the test organisms with a number of sequencing *reads* less or equal than 10, therefore the absence of an organism cannot be interpreted as a true negative as it might be present with values not detected by the technique; not correctly represented in the reference databases; or others.

Bacterial organisms found

In this sample we identified **8** phylum, **12** classes, **13** orders, **20** families, **35** genus and **28** species with relative frequencies greater than **0,01%**.

Next we list all these bacterial taxa, grouped by taxonomic hierarchy, its relative frequency, its relation to the standard-deviation and the normal values interval in the healthy reference population of individuals.

Organisms (Phylum)	%	Deviation	Normal Interval (IQR) %
Bacteroidetes	62.68	3.0x	16.795 - 45.809
Firmicutes	33.98	1.5x	18.609 - 55.956
Proteobacteria	1.45	0.1x	0.904 - 39.272
Actinobacteria	1.21	0.4x	0.044 - 0.495
Tenericutes	0.61	0.6x	0.002 - 0.594
Lentisphaerae	0.04	0.4x	0.000 - 0.010
Cyanobacteria	0.01	=	0.000 - 0.037
Verrucomicrobia	0.01	=	0.017 - 5.148

Organisms (Class)	%	Deviation	Normal Interval (IQR) %
Bacteroidia	62.68	3.0x	16.788 - 45.809
Clostridia	33.5	1.5x	17.033 - 54.803
Coriobacteriia	1.21	1.0x	0.012 - 0.066
Deltaproteobacteria	0.77	3.4x	0.025 - 0.172
Betaproteobacteria	0.67	0.1x	0.116 - 0.887
Mollicutes	0.58	0.6x	0.000 - 0.548
Erysipelotrichi	0.45	0.6x	0.019 - 0.353
[lentisphaeria]	0.04	0.4x	0.000 - 0.010
Rf3	0.03	0.1x	0.000 - 0.007
Bacilli	0.03	=	0.025 - 0.104
Verrucomicrobiae	0.01	=	0.015 - 5.148
4c0d-2	0.01	=	0.000 - 0.023

Organisms (Order)	%	Deviation	Normal Interval (IQR) %
Bacteroidales	62.68	3.0x	16.788 - 45.809
Clostridiales	33.49	1.5x	17.029 - 54.803
Coriobacteriales	1.21	1.0x	0.012 - 0.066

Organisms (Order)	%	Deviation	Normal Interval (IQR) %
Desulfovibrionales	0.77	3.4x	0.018 - 0.169
Burkholderiales	0.67	0.1x	0.114 - 0.879
Rf39	0.58	0.6x	0.000 - 0.500
Erysipelotrichales	0.45	0.6x	0.019 - 0.353
Victivallales	0.04	0.4x	0.000 - 0.010
Ml615j-28	0.03	0.1x	0.000 - 0.007
Turicibacterales	0.02	0.2x	0.000 - 0.003
Verrucomicrobiales	0.01	=	0.015 - 5.148
Ys2	0.01	=	0.000 - 0.023
Sha-98	0.01	1.2x	0.000 - 0.003

Organisms (Family)	%	Deviation	Normal Interval (IQR) %
Prevotellaceae	52.56	3.1x	0.010 - 4.493
Ruminococcaceae	16.12	0.8x	12.796 - 44.119
Lachnospiraceae	8.61	1.4x	0.490 - 8.646
Veillonellaceae	8.17	5.0x	0.101 - 0.986
Bacteroidaceae	6.54	0.4x	7.816 - 30.974
Porphyromonadaceae	1.96	0.8x	0.247 - 1.997
Coriobacteriaceae	1.21	1.0x	0.012 - 0.066
Desulfovibrionaceae	0.77	3.4x	0.018 - 0.169
[odoribacteraceae]	0.73	3.7x	0.046 - 0.239
Alcaligenaceae	0.62	0.9x	0.032 - 0.434
Clostridiaceae	0.54	1.1x	0.005 - 0.024
[paraprevotellaceae]	0.5	0.4x	0.000 - 0.304
Erysipelotrichaceae	0.45	0.6x	0.019 - 0.353
[barnesiellaceae]	0.35	0.7x	0.005 - 0.165
Oxalobacteraceae	0.05	0.1x	0.000 - 0.063
Victivallaceae	0.04	0.4x	0.000 - 0.010
S24-7	0.04	=	0.000 - 0.037
Turicibacteraceae	0.02	0.2x	0.000 - 0.003
[tissierellaceae]	0.02	=	0.003 - 0.037
Verrucomicrobiaceae	0.01	=	0.015 - 5.148

Organisms (Genus)	%	Deviation	Normal Interval (IQR) %
Prevotella	52.56	3.1x	0.010 - 4.493

Organisms (Genus)	%	Deviation	Normal Interval (IQR) %
Faecalibacterium	14.38	0.8x	11.732 - 41.788
Bacteroides	6.54	0.4x	7.816 - 30.974
Roseburia	4.08	1.6x	0.004 - 2.049
Dialister	3.67	3.4x	0.004 - 0.294
Mitsuokella	2.33	194.5x	0.000 - 0.000*
Parabacteroides	1.95	0.8x	0.200 - 1.977
Coprococcus	1.61	1.7x	0.006 - 0.114
Ruminococcus	1.47	0.6x	0.010 - 1.643
Blautia	1.44	0.7x	0.009 - 1.730
Collinsella	1.19	1.0x	0.004 - 0.024
Veillonella	1.02	1.1x	0.002 - 0.009
Megasphaera	0.95	3.4x	0.000 - 0.002
[ruminococcus]	0.77	0.2x	0.011 - 2.984
Desulfovibrio	0.72	4.7x	0.000 - 0.044
Sutterella	0.62	1.3x	0.028 - 0.417
Butyricimonas	0.59	5.2x	0.004 - 0.092
Clostridium	0.54	2.4x	0.000 - 0.000*
Paraprevotella	0.49	0.5x	0.000 - 0.260
Oribacterium	0.37	6.6x	0.000 - 0.007
Catenibacterium	0.31	1.2x	0.000 - 0.002
Lachnospira	0.3	0.3x	0.081 - 0.793
Oscillospira	0.24	0.8x	0.034 - 0.346
Acidaminococcus	0.15	9.0x	0.000 - 0.000*
Odoribacter	0.14	1.0x	0.018 - 0.128
[eubacterium]	0.13	0.2x	0.000 - 0.257
Bilophila	0.06	0.4x	0.009 - 0.101
Oxalobacter	0.05	0.2x	0.000 - 0.039
Anaerotruncus	0.03	1.5x	0.000 - 0.006
Turicibacter	0.02	0.2x	0.000 - 0.003
Butyrivibrio	0.02	1.0x	0.004 - 0.030
Slackia	0.02	0.6x	0.000 - 0.003
Phascolarctobacterium	0.02	=	0.004 - 0.328
Akkermansia	0.01	=	0.015 - 5.148
Anaerovibrio	0.01	0.6x	0.000 - 0.000*

Organisms (Species)	%	Deviation	Normal Interval (IQR) %
Copri	51.36	3.3x	0.005 - 3.234
Prausnitzii	14.38	0.8x	11.732 - 41.788
Faecis	4.08	1.6x	0.000 - 2.049
Ovatus	3.71	0.6x	0.023 - 8.017
Multacida	2.33	-	0.000 - 0.000*
Distasonis	1.95	0.8x	0.174 - 1.942
Eutactus	1.61	1.7x	0.000 - 0.012
Caccae	1.38	0.2x	0.772 - 9.589
Obeum	1.16	0.6x	0.002 - 1.264
Uniformis	1.06	0.3x	0.009 - 2.421
Dispar	1.02	1.6x	0.000 - 0.005
Bromii	0.99	0.6x	0.000 - 0.890
Melaninogenica	0.84	0.4x	0.000 - 0.005
Aerofaciens	0.8	0.7x	0.000 - 0.010
Gnavus	0.77	0.5x	0.004 - 1.407
D168	0.72	15.2x	0.000 - 0.000*
Perfringens	0.54	1.1x	0.000 - 0.000*
Flavefaciens	0.47	0.4x	0.000 - 0.188
Stercoris	0.39	1.6x	0.000 - 0.000*
Fragilis	0.37	0.1x	1.082 - 7.937
Producta	0.28	0.3x	0.000 - 0.008
Nigrescens	0.15	2.2x	0.000 - 0.000*
Biforme	0.12	0.2x	0.000 - 0.090
Stercorea	0.1	0.1x	0.000 - 0.007
Pallens	0.09	1.7x	0.000 - 0.000*
Formigenes	0.05	0.2x	0.000 - 0.039
Muciniphila	0.01	=	0.015 - 5.148
Nanceiensis	0.01	0.9x	0.000 - 0.000*

* The value interval that limit IQR, identified in the healthy reference population of individuals, are found below the technique detection limits or have relative frequencies below **0,01%**.

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