INTUS BIOSCIENCES

REPORT RECIPIENT
**123 Medical**
**Attn:** Dr. James R. Smith, M.D.
808 Disc Street, Suite 100
Somewhere, ID 10011
**Report Date:** 2/22/2023
**Intus Report ID:** 102394-023

SAMPLE
**Jane Doe**
**DOB:** 10/12/1978
**Hospital Number:** 1923-10220

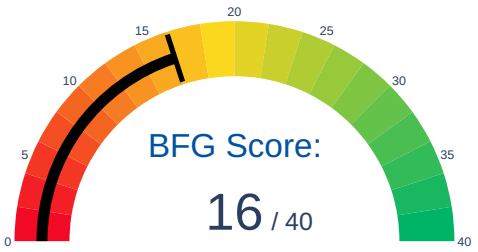
# BFGT - Bacterial Functional Gut Test

FOR CLINICIANS MANAGING PATIENTS WITH IBS AND OTHER FUNCTIONAL INTESTINAL DISORDERS

**Summary:** High risk of bacteria-associated functional intestinal disorders

**Areas to Review:** 9 / 19

**Most Impact:** Bile Acid Metabolizing Bacteria




## Resilience & Biodiversity

- Alpha Diversity
- Richness
- Evenness
- Beta Diversity
- Firmicutes/Bacteroidetes (F/B) Ratio
- Fusobacteria Percentage
- Proteobacteria Percentage
- Pathogens

SUMMARY:

**Imbalance of Bacterial Distribution.**

SUGGESTIONS:

- Impaired diversity, evenness, F/B ratio or richness: Increase fibers, fermented foods, polyphenols, nuts and seeds; decrease simple sugars, fats, artificial sweeteners. Maintain healthy BMI.

## Beneficial Bacteria

- Probiotics
- Mucosa Protective Bacteria
- Short Chain Fatty Acid (SCFA) Producing Bacteria

SUMMARY:

**Various beneficial bacteria out of typical range.**

SUGGESTIONS:

- Low Short Chain Fatty Acid producers: Increase fibers, omega 3. Consider prebiotics, probiotics, symbiotic and postbiotics supplementation.
- Low mucosa protective bacteria: Increase fiber, monounsaturated fatty acids, polyphenols. Decrease fat, alcohol, ultra-processed foods. Consider Prebiotics.
- Reconsider probiotics/postbiotics supplement dosage. Check effects of drugs on microbiome composition.

## Bile Acid Metabolism Associated Bacteria

- Bile Acid Metabolizing Bacteria

SUMMARY:

**Out of typical range: BAs metabolizing bacteria possibly impacting intestinal motility.**

SUGGESTIONS:

- If patient has increased motility: Consider multi-strain probiotics and drugs addressing BAs malabsorption.
- If patient has decreased motility: increase healthy fats such as EVOO. Consider supplements to increase secretion of BAs, probiotics with CORRECT beneficial bacteria profile.

## Colonic SIBO Associated Bacteria (Archaea not included)

- SIBO-Associated Proteobacteria and Pathobionts
- Hydrogen Producing Bacteria
- Hydrogen Sulfide Producing Bacteria

SUMMARY:

**Within typical range.**

## IBS Associated Bacteria

- IBS-Constipation
- IBS-Diarrhea
- IBS-Undefined

SUMMARY:

**Out of typical range.**

SUGGESTIONS:

- See IBS Table for over- or under-abundance of specific bacteria. Look at pathogens, Proteobacteria, Fusobacteria, bile acid metabolizing, short chain fatty acid producers, and probiotic bacteria tables. Potential treatments include targeted antibiotics or supplements.



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## FODMAP Sensitivity

FODMAP Sensitivity Score

SUMMARY:

**Normal response to FODMAP.**

# Resilience & Biodiversity

### Alpha Diversity

Index of a microbiome's resilience and diverse composition, also known as Shannon Index. Higher values are generally associated with better resilience potential.

VALUE: **7.36**

TYPICAL RANGE: **6.53 - 13.06**


### Richness

Index referring to the number of unique species present within a sample.

VALUE: **38.00**

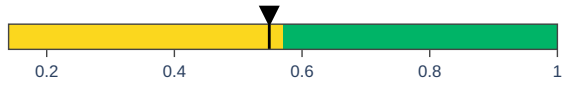
TYPICAL RANGE: **25.00 - 50.00**


### Evenness

Index referring to the distribution of species identified in a microbiome sample. Values closer to 1 indicate a more desirable, even distribution.

VALUE: **0.55**

TYPICAL RANGE: **0.57 - 1.00**




### Beta Diversity

Index quantifying how different a microbial community is to the reference population, with values closer to 0 representing more similarity.

VALUE: **0.87**

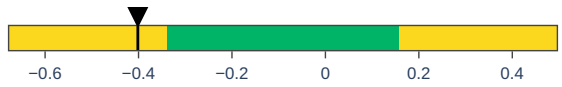
TYPICAL RANGE: **0.70 - 0.85**


### Firmicutes/Bacteroidetes (F/B) Ratio

Negative scores correspond to Bacteroidetes dominance, and positive scores correspond to Firmicutes dominance. A balanced F/B ratio is associated with intestinal homeostasis.

VALUE: **-0.40**

TYPICAL RANGE: **-0.34 - 0.16**




### Fusobacteria Percentage

Bacterial species that may become opportunistic pathogens, most commonly found in the mouth. If detected in the gut, they may be associated with intestinal inflammation, IBS, IBD and chronic diseases.

VALUE: **0.00**

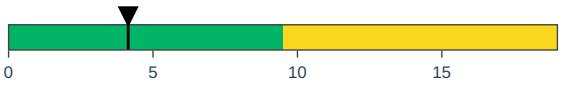
TYPICAL RANGE: **0.00 - 5.00**


### Proteobacteria Percentage

Bacterial species that may exhibit toxic and pathogenic mechanisms of action including lipopolysaccharide (LPS) and endotoxin synthesis and promote gastrointestinal and systemic inflammation. They are strongly associated with IBS, SIBO, IBD and immune dysregulation.

VALUE: **4.14**

TYPICAL RANGE: **0.00 - 9.50**




### Pathogens

Bacterial species that may cause severe gastrointestinal symptoms and be associated with intestinal or systemic chronic illnesses.

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Bacteroides fragilis | 0.04 | 0.04-1.39 | |
| Escherichia coli | 0.43 | 0.12-14.97 | |
| Bilophila wadsworthia | 0.29 | 0.16-1.22 | |



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# Beneficial Bacteria

## Probiotics

Well-characterized bacterial species and strains that can either be ingested via supplements and/or foods or occur naturally in the human gut.

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Bifidobacterium longum | 7.67 | 0.3-18.92 | |
| Bifidobacterium pseudocatenulatum | 0.46 | 0.12-10.77 | |

## Mucosa Protective Bacteria

Bacterial species that support normal gut barrier function. Abnormally low or high levels of these bacteria may lead to alterations in the intestinal mucosa and be associated with inflammation and immune dysregulation.

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Faecalibacterium prausnitzii | 16.33 | 0.19-4.04 | high |

## Short Chain Fatty Acid (SCFA) Producing Bacteria

Anaerobic gut bacteria producing SCFAs such as acetate, propionate, and butyrate, which play a crucial role in maintaining gut and systemic health. A balanced presence of SCFA-producing bacteria is strongly associated with decreased inflammation, reduced risk of disease, and improved immune and metabolic function.

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Bifidobacterium longum | 7.67 | 0.3-18.92 | |
| Anaerostipes hadrus | 0.72 | 0.06-2.49 | |
| Roseburia intestinalis | 0.84 | 0.1-1.37 | |
| Faecalibacterium prausnitzii | 16.33 | 0.19-4.04 | high |
| Ruminococcus bromii | 0.71 | 0.22-3.9 | |

# Bile Acid Metabolism Associated Bacteria

## Bile Acid Metabolizing Bacteria

Bacteria able to metabolize Bile Acids (BAs) and produce several derived metabolites, which can affect intestinal motility, fluid secretion, mucosal permeability, immune responses and microbiome composition.

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Bifidobacterium pseudocatenulatum | 0.46 | 0.12-10.77 | |
| Lachnospira eligens | 0.09 | 0.06-0.97 | |
| Roseburia intestinalis | 0.84 | 0.1-1.37 | |
| Faecalibacterium prausnitzii | 16.33 | 0.19-4.04 | high |
| Thomasclavelia ramosa | 0.13 | 0.07-4.0 | |
| Bacteroides uniformis | 0.13 | 0.12-2.82 | |
| Phocaeicola dorei | 40.98 | 0.2-48.36 | |
| Phocaeicola vulgatus | 0.83 | 5.74-57.82 | |
| Parabacteroides distasonis | 0.06 | 0.15-2.98 | |



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# Colonic SIBO Associated Bacteria (Archaea not included)

## SIBO-Associated Proteobacteria and Pathobionts

Overgrowth of specific Proteobacteria and pathobionts in the small intestine is a significant risk factor for SIBO and can correlate with excessive abundance of these bacteria in the colon.

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Streptococcus | 0.02 | 0.08-13.45 | |
| Bacteroides | 0.17 | 0.25-5.91 | |
| Escherichia | 0.48 | 0.12-15.16 | |

## Hydrogen Producing Bacteria

Intestinal bacteria involved in the breakdown of carbohydrates and other organic compounds through anaerobic fermentation, which produce hydrogen gas as a byproduct. High levels of hydrogen gas in the gut can lead to symptoms such as bloating, flatulence, abdominal pain, and diarrhea. Hydrogen is one the intestinal gases associated with SIBO symptoms

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Roseburia | 0.84 | 0.12-2.43 | |
| Ruminococcus | 0.71 | 0.24-6.38 | |

## Hydrogen Sulfide Producing Bacteria

Intestinal bacteria that reduce sulfate and main producers of hydrogen sulfide. High levels of hydrogen sulfide gas in the gut have been linked to a range of gastrointestinal symptoms, including bloating, abdominal pain, diarrhea and/or constipation. An excessive production of this gas can cause chronic inflammation and increase the risk of chronic diseases. Hydrogen sulfide is one of the intestinal gases associated with SIBO symptoms.

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Escherichia coli | 0.43 | 0.12-14.97 | |
| Bilophila wadsworthia | 0.29 | 0.16-1.22 | |



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# IBS Associated Bacteria

## IBS-Constipation

These are bacteria that large studies have found to be strongly associated with the IBS constipation subtype. A decrease or increase of specific groups of intestinal bacteria may cause, worsen or predispose to constipation by affecting motility, inflammation and pain perception.

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Bifidobacterium | 8.13 | 0.48-25.37 | |
| Eubacteriales Family XIII. Incertae Sedis | 0.06 | 0.07-2.6 | |
| Lachnospiraceae | 1.65 | 0.23-4.48 | |
| Anaerostipes | 0.72 | 0.06-2.49 | |
| Lachnospira | 0.09 | 0.06-0.97 | |
| Lachnospira eligens | 0.09 | 0.06-0.97 | |
| Roseburia | 0.84 | 0.12-2.43 | |
| Oscillospiraceae | 19.99 | 1.21-16.48 | high |
| Faecalibacterium | 19.06 | 0.54-9.02 | high |
| Flavonifractor | 0.19 | 0.02-0.29 | |
| Ruminococcus | 0.71 | 0.24-6.38 | |
| Erysipelotrichaceae | 0.10 | 0.28-17.47 | low |
| Parabacteroides | 0.06 | 0.18-3.53 | |
| Sutterella | 0.80 | 0.22-6.47 | |
| Escherichia | 0.48 | 0.12-15.16 | |
| Akkermansia | 3.57 | 0.27-25.26 | |

## IBS-Diarrhea

These are bacteria that large studies have found to be strongly associated with the IBS diarrhea subtype. A decrease or increase of specific groups of intestinal bacteria may cause, worsen or predispose to loose bowel movements by affecting motility, inflammation and pain perception.

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Bifidobacterium | 8.13 | 0.48-25.37 | |
| Bacilli | 0.02 | 0.08-19.03 | low |
| Clostridia | 22.98 | 4.36-31.7 | |
| Christensenellaceae | 0.34 | 0.09-8.98 | |
| Eubacteriales Family XIII. Incertae Sedis | 0.06 | 0.07-2.6 | low |
| Lachnospiraceae | 1.65 | 0.23-4.48 | |
| Anaerostipes | 0.72 | 0.06-2.49 | |
| Oscillospiraceae | 19.99 | 1.21-16.48 | high |
| Faecalibacterium | 19.06 | 0.54-9.02 | high |
| Flavonifractor | 0.19 | 0.02-0.29 | |
| Ruminococcus | 0.71 | 0.24-6.38 | |
| Erysipelotrichaceae | 0.10 | 0.28-17.47 | low |
| Alistipes | 18.00 | 0.51-8.84 | high |
| Sutterella | 0.80 | 0.22-6.47 | |
| Escherichia | 0.48 | 0.12-15.16 | |
| Akkermansia | 3.57 | 0.27-25.26 | |

## IBS-Undefined

These are bacteria that large studies have found to be strongly associated with the IBS undefined subtype. A decrease or increase of specific groups of intestinal bacteria may cause, worsen or predispose to chronic changes in bowel movements and pain by affecting motility, inflammation and pain perception.

| Species | Relative Abundance (%) | Reference Range (%) | Flag |
|---|---|---|---|
| Bifidobacterium | 8.13 | 0.48-25.37 | |
| Lachnospiraceae | 1.65 | 0.23-4.48 | |
| Anaerostipes | 0.72 | 0.06-2.49 | |
| Faecalibacterium | 19.06 | 0.54-9.02 | high |
| Ruminococcus | 0.71 | 0.24-6.38 | |
| Erysipelotrichaceae | 0.10 | 0.28-17.47 | low |
| Sutterella | 0.80 | 0.22-6.47 | |
| Escherichia | 0.48 | 0.12-15.16 | |

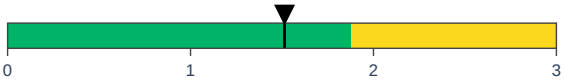
# FODMAP Sensitivity

## FODMAP Sensitivity Score

Score assessing the potential response to foods high in FODMAP (Fermentable oligosaccharides, disaccharides, monosaccharides, and polyols). An excessive representation of certain bacterial taxa and altered population dynamics may cause an increased sensitivity to foods high in FODMAPs as compared to the general population.

VALUE: **1.51**

TYPICAL RANGE: **0.00 - 1.88**



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## Quality Metrics

### Sequencing Quality Control

This sample exceeds the minimum quality control standard of 10,000 sequencing reads per sample.

## Processing Lab Director

**Report Authorized By:**

**John Hancock, Lab Director, AveroDX**



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# Additional Information

## Drug and Supplement Impact Table

| Drug/Supplement | Main effects |
|---|---|
| **Metformin hydrochloride** | **Gut microbiota modulation:** increased abundance of Akkermansia, Bacteroides (especially B. intestinalis, B. vulgatus, and B. acidifaciens), Parabacteroides, Escherichia coli, Bifidobacterium adolescentis, Subdoligranulum. |
| **PPIs** | **Altered microbiota:** increased abundance of Bifidobacterium dentium, Streptococcus (especially S.mutans, S. salivaris, S. parasanguinis, S. vestibularis), Veillonella parvula. Increased risk of SIBO (increased abundance of Streptococcus, Clostridium, Escherichia, Klebsiella in small intestine). Increased risk of C.difficile, Salmonella, Shigella and Campylobacter infection. Increased abundance of oral bacteria in stool (i.e., Fusobacterium nucleatum). |
| **Rifaximin** | **Gut microbiota modulation:** overall eubiotic effect, increased abundance of Bifidobacterium, Faecalibacterium prausnitzii and Lactobacillus, decreased C. difficile |
| **Statins** | **Altered microbiota:** likely decreased diversity. They may cause increased abundance of Akkermansia and F. prausnitzii. Possible disturbances in SCFAs producing and BAs metabolizing bacteria. More human studies are needed. |
| **L-thyroxine** | **Altered microbiota:** likely contributor to SIBO. Possible alterations in abundance of Odoribacter and Enterococcus species (dose-dependent effect: higher abundance with medium dose, lower abundance with high dose of medication). Alistipes, Ruminococcus and Anaerotruncus species may result out of typical ranges. |
| **Metronidazole** | **Altered microbiota:** likely increased abundance of Bifidobacterium (especially B. pseudolongum) and Enterobacteria. |
| **SSRI** | **Altered microbiota:** increased abundance of Eubacterium ramulus. SSRIs in general have an antimicrobial effect. Long-term use may cause dysbiosis. |
| **FOS (Fructooligosaccharides)** | **Gut microbiota modulation:** increased abundance of Bifidobacterium and F. prausnitzii, decreased Proteobacteria. |
| **Resveratrol** | **Gut microbiota modulation:** inhibition of E. coli growth, Enterococcus faecalis. Increased abundance of Bifidobacterium and Lactobacillus. |
| **Berberine chloride** | **Gut microbiota modulation:** possible increased abundance of Akkermansia and SCFAs-producing bacteria in general. Decreased Clostridium spp, inhibition of E. coli growth. Microbiota conversion into dihydroberberine. |

## Food and Nutrient Impact Table

| Foods, nutrients, diets | Main effects |
|---|---|
| **Fibers** | **Gut microbiota modulation:** in general, microbiota accessible carbohydrates (MAC) may increase microbial diversity and distribution as well as improve short chain fatty acid (SCFA) production by bacterial fermentation. Low abundance of beneficial species such as Akkermansia and/or Bifidobacterium may indicate an inadequate fiber intake. However, excess fiber intake, especially those high in FODMAPs may cause microbiota imbalances and exacerbate gastrointestinal symptoms such as gas, bloating, and abdominal pain. Extremely high fiber intake may decrease the absorption of key nutrients.<br>Additionally, different types of fiber may promote specific modifications to intestinal bacterial composition:<br>**-Inulin** (ex: dandelion greens, asparagus, onions, leeks, bananas, whole wheat) may increase Bifidobacterium spp, especially B. bifidum and F. prausnitzii<br>**-Beta-glucans** (ex: oats, barley) may increase Bifidobacterium, Ruminococcus, Prevotella, Roseburia hominis, and other butyrate-producing bacteria as well as decrease Fusobacteria and Clostridium spp<br>**-Resistant Starch** (ex: green banana, legumes, and potatoes, rice, and pasta that has been cooked and then cooled) may increase Bifidobacterium spp, Ruminococcus bromii, and F. prausnitzii |
| **Polyphenols** | **Gut microbiota modulation:** Polyphenols are metabolized by gut bacteria to positively affect microbiota composition, diversity, and distribution. Sources of polyphenols include berries, dark chocolate and pure cocoa powder, olives, extra virgin olive oil, green tea, black coffee, nuts, peanuts, seeds, and red wine. Adequate polyphenol intake may decrease the abundance of potential pathogens such as Clostridium spp and E. coli, promote LPS-induced inflammation, restore Lactobacillus and Bifidobacterium population, rebalance the F/B ratio, and increase mucosa protective bacteria such as Akkermansia and F. prausnitzii. |
| **Nuts and seeds** | **Gut microbiota modulation**: Nuts contain fiber, polyphenols, and healthy monounsaturated fatty acids (MUFAs) and act as a prebiotic that may promote a beneficial bacterial population. Adequate nut and seed intake is associated with improved bacterial diversity, reduced inflammation, and increased butyrate production. Almonds specifically have been shown to increase alpha diversity. |
| **Fermented foods** | **Gut microbiota modulation:** Consumption of fermented foods may lead to increased microbiome diversity and have a stronger effect than fiber alone. Additionally, adequate intake of fermented foods is associated with increased probiotic abundance, reduced inflammation, and improved microbiota-related immune function. Examples of fermented foods and specific modifications to bacterial composition and gastrointestinal function include:<br>**-Kefir** may increase Lactobacillus spp and improve constipation<br>**-Kombucha** may decrease abundance of pathogens such as E.coli and H.pylori |



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| Foods, nutrients, diets | Main effects |
|---|---|
| | **-Sauerkraut** may improve symptoms in all IBS subtypes<br>**-Kimchi** my increase Lactobacillus<br>**-Natto and Miso** may increase Bifidobacterium and decrease Enterobacteriaceae |
| **Omega 3 and MUFAs** | **Gut microbiota modulation:** Omega-3 fatty acids, found in fatty fish and nuts and seeds, may increase the abundance of butyrate-producing bacteria and probiotics such as Lactobacillus and Bifidobacterium spp. Furthermore, omega-3 may decrease LPS-induced inflammation. MUFAs, found in olive oil, avocado, nuts, and seeds may increase Bifidobacterium spp and favour Bacteroidetes over Firmicutes. |
| **Supplements containing vitamins and minerals** | **Gut microbiota modulation:** Effect of different supplement types depends on dosage, length of intervention, combination of nutrients, and genetic and epigenetic factors and may include:<br>**-Vitamin D** may increase diversity, promote Akkermansia and Bifidobacterium spp, and decrease Proteobacteria abundance<br>**-Iron** may increase Lactobacillus spp, which are dependent on iron availability. However, as excess iron intake may promote inflammation, oxidative stress, and increased abundance of pathogenic bacteria, it is recommended to supplement only when a deficiency is identified. |
| **Mediterranean Diet** | **Gut microbiota modulation:** The MD is high in fiber, polyphenols, nuts and seeds, omega-3 fatty acids, and MUFAs. The MD may decrease the F/B ratio and abundance of Proteobacteria and increase abundance of beneficial bacteria such as probiotics, SCFA-producing bacteria, and mucosa-protective bacteria (as demonstrated in the PREDIMED study). |
| **Ketogenic diet (KD)** | **Gut microbiota modulation/ altered microbiota**: Effects of the KD depend on the health status of the host. KD may increase abundance of Akkermansia and decrease the F/B ratio. Given the very low intake of carbohydrates and fiber, a KD may also decrease probiotics species, particularly Bifidobacterium. KD may increase the abundance of pro-inflammatory Proteobacteria like Bilophila wadsworthia. While specific beneficial effects of a KD on refractory epilepsy and obesity may be mediated by positive changes in the microbiome composition, regular testing may be necessary to avoid negative intestinal bacterial imbalances. |
| **Vegetarian and vegan diets** | **Gut microbiota modulation/ possible alterations in microbiota**: High consumption of plant-based foods and fiber, a characteristic of vegetarian and vegan diets, may increase abundance of SCFA-producing bacteria, improve bacterial diversity, and decrease abundance of potential pathogens. However, sub-optimal intake of essential nutrients such as iron, omega-3, vitamin B12, and protein, associated with unbalanced and strict vegetarian and vegan diets, may negatively affect bacterial distribution and cause an overabundance of some species and should be monitored. |
| **Artificial sweeteners** | **Altered microbiota**: In general, artificial sweeteners may affect microbiome diversity. Acesulfame K, saccharine and sucralose consumption has been linked to decreased abundance of Akkermansia, though these results are controversial. Because research in this area is still early, it is not yet possible to draw conclusions about individual effects of artificial sweeteners on microbiome composition, which are likely dose-dependent and linked to duration of consumption. |
| **Excess of sugars, saturated fats, salt and ultra-processed foods** | **Altered microbiota**: The typical Western Diet (WD) is characterized by high intake of saturated fat, salt, and sugar and inadequate in fiber. The WD can negatively affect microbiome composition and may decrease microbial diversity, increase abundance of Proteobacteria and pathogens, and promote LPS biosynthesis. Specifically, excess saturated fat intake may decrease abundance of F.prausnitzii and increase abundance of species expressing bile acid hydrolases such as Clostridium, Alistipes, Bifidobacterium, and Lactobacillus spp. Furthermore, excessive salt intake may decrease Lactobacillus spp. |
| **Alcohol** | **Altered microbiota**: Chronic excessive alcohol intake may negatively affect bacterial diversity and distribution, increase abundance of Proteobacteria, and promote intestinal inflammation and IBS-related symptoms. However, moderate beer consumption, particularly if unpasteurized, has been demonstrated to exert some prebiotic effects due to polyphenolic compounds and melanoidins, which may increase Bifidobacterium spp and Akkermansia. Moderate consumption of red wine, which is also rich in polyphenols, may increase microbiota diversity. However, as alcohol has several known detrimental effects, non-alcoholic versions of beer and red wines should be consumed, if consumed at all. |

# Bacterial Functional Gut Test FAQs

## What is the Bacterial Functional Gut Test (BFGT)?

The BFGT analyses the presence and abnormal abundance of intestinal bacteria that may be involved in Functional Gastrointestinal Disorder (FGID) symptoms in the lower gastrointestinal (GI) tract, including the small intestine and colon. Although upper GI symptoms such as functional dyspepsia may be triggered or worsened by intestinal imbalances, this test is intended to improve the diagnosis and optimize the treatment of intestinal problems that do not have other pathological causes.

## What are Functional Gastrointestinal Disorders (FGIDs)?

FGIDs are a diverse group of disorders characterized by a combination of unpleasant, chronic, recurrent symptoms such as altered bowel habits (constipation, diarrhea), abdominal pain, excessive gas and bloating, and overall poor digestion. These symptoms are not accompanied by any relevant pathological findings and demonstrable specific disease; therefore, patients affected by FGIDs usually show normal blood, imaging, and histological test results. For this reason, these disorders are said to be "functional" because they occur within the range of normal digestive system functional measurements, but significantly impair quality of life. This group of ailments has been linked to dysfunctions of the so called "gut-brain axis," which includes imbalances in gut microbiota composition (type and number of intestinal micro-organisms and their metabolites) that may influence both GI function and the nervous system's perception of gut functionality.

## Who should use the test?

This test may be used to support patients affected by any form of irritable bowel syndrome (IBS), functional bowel movement issues, and/or abdominal pain and bloating. The test also includes an analysis of the main bacteria found to cause small intestinal bacterial overgrowth (SIBO), as these bacteria may still be detectable in stools. However, while the presence of these bacteria is suggestive of a concern for SIBO, this test is not



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intended to diagnose SIBO and any abnormal results should be followed-up with additional diagnostic testing. Currently, this test does not detect methane-producing Archaea, which are known to cause Intestinal Methanogen Overgrowth (IMO).

### How is the BFG Score calculated?

The overall BFG score is calculated by applying proprietary algorithms that consider both the general features of the microbial population, including bacterial diversity, evenness of distribution, and phyla present in the sample, while also focusing on abundance of potentially pathogenic as well as beneficial bacteria. All measurements in an individual sample are compared to the reference population to generate a quantitative comparison. As a result, scores are designed to indicate where an individual sample differs from the general population, providing guidance for following up with specific actions, rather than providing a diagnosis of disease.

### What is the reference population?

The reference population is made up of a significant and expanding number of individuals who have been screened using the test. The reference population includes all samples tested by Intus Biosciences. The rationale for including all individuals in the reference population, regardless of self-reported health status and symptoms, is to better observe trends associated with health status. Furthermore, as samples are self-collected and health status information is self-reported, inclusion of all data accounts for errors and eliminates scientific assumption bias. As more sample data is compiled, reference population norms will be adjusted to reflect the most updated information, which may result in a slight change to the ranges included in the report. The highest and lowest percentiles are flagged as abnormal values.

### What is more important: The score, or individual sections of the report?

It is advisable to act in accordance with any specific imbalances detected, rather than focusing on the individual score alone.

### What does 'Typical Range' mean and may the range change?

Typical ranges represent the findings in the majority of the reference population. As the reference population expands, the typical ranges will change to become more refined and precise.

### Who provides the test?

The test is provided by AveroDX, using technology under license from Intus Biosciences, LLC.

### What technology generates the results?

The test is powered by the patented, high resolution and high throughput Intus Bio Titan-1™ platform. Titan-1™ uses the latest Next Generation Sequencing (NGS) Technology, with a 'long' target sequence of 16S-ITS and partial 23S. Visit intusbio.com for more information.

### What makes the test unique?

Side by side analysis of different approaches has demonstrated that the Intus Bio technology is the most effective and accurate method for strain level identification of bacteria - see https://doi.org/10.1099/mgen.0.000794. The BFG Test is the only test of its type benefiting from the power of the technology.

### Are there any drugs, supplements or foods that drastically interfere with the test's results?

Antibiotics and probiotics can significantly change the microbiota composition, although not necessarily in the long-term. We suggest waiting for a couple of weeks after completing a course of antibiotics before taking this test. If you are interested in monitoring the effects of a probiotic intervention, the patient may take the test while on the supplement or after completing treatment. We also recommend that patients follow their typical diet in the weeks preceding the test so that the results reflect average and normal food intake (no drastic changes in the diet or new foods should be introduced prior the test).

### How many times should a patient take this test?

It is strongly recommended to take a test before and after any intervention aiming at improving a functional intestinal disorder like a pharmaceutical, dietary supplement, and/or dietary intervention. Repeated tests will allow the provider to monitor and evaluate the efficacy of treatment(s) over time.

## Test Category Definitions

### Resilience & Biodiversity

#### Alpha Diversity

Calculated using the Shannon Index, the alpha diversity score incorporates measures of richness and evenness and is an indicator of microbiome resilience. Resilience can be defined as how resistant the bacterial community is to changes that may push it out of its current state, such as after an infection, course of antibiotics, a long period of ill-health, or another stressor. In general, a healthy and well-balanced gut bacterial community should have many different species that are well-distributed, such that no one species is dominant. Therefore, highest resilience is achieved when richness and evenness are in balance.

#### Richness

The number of total bacterial species detected in the sample, higher richness values generally are generally associated with a healthier microbiome. In rare cases, however, the presence of several pathogenic species may result in a higher richness score, making it important to evaluate the types and abundance of bacterial species present, including potentially harmful pathogenic species.

#### Evenness

A measure of how well different bacterial species are distributed throughout the microbiome. Scores closer to 1 indicate a more desirable, even distribution while scores closer to 0 suggest one or more species may be dominant.



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### Beta diversity

Calculated using the Bray-Curtis dissimilarity, beta diversity compares how similar or different the sample is from the reference population. In general, it is better to have a bacterial composition closer to that of the reference population. A high beta diversity score may be an indicator of an unusual microbial profile, typically dominated by a single species. Although a sample may be different due to a high abundance of beneficial bacteria, gut-related symptoms are frequently associated with higher beta diversity scores.

### Firmicutes/Bacteroidetes (F/B) ratio

These two bacterial phyla are the dominant types of bacteria present in the healthy adult human gut. The phyla are usually present at about equal amounts, and together they typically represent more than 90% of the entire bacterial community. The F/B ratio is a well-recognized marker of microbiome health and balance, and an unusual ratio can indicate a predisposition to certain diseases. For example, higher abundance of Firmicutes tends to be associated with obesity, while higher abundance of Bacteroidetes is more common in individuals suffering from Inflammatory Bowel Disease (IBD). However, this result should be considered within the context of overall health rather than used as a primary indicator.

### What do pathogen and pathobiont mean?

Some bacteria are known to have a negative impact on health. Pathogens are bacteria known to cause infections while pathobionts are bacteria that are potentially pathogenic under specific circumstances such as during immune system dysfunction. This test will flag these bacteria when they appear in quantities higher than those observed in the reference population.

### Fusobacteria Percentage

Typically absent or in very low abundance in the lower digestive system, Fusobacteria are naturally occurring bacteria that colonize mucosal surfaces, especially in the oral cavity. Fusobacteria are mostly associated with periodontal disease and formation of biofilm. When found in the gut, however, it may indicate that an insufficient immune surveillance and/or low gastric acidity have allowed these bacteria to translocate from the mouth to the intestine, or that disrupted conditions in the gut are allowing Fusobacteria to thrive. High abundance of Fusobacteria in the gut may be an indicator of chronic inflammation and an increased risk of disease and some cancers, such as is the case with Fusobacterium nucleatum.

### Proteobacteria Percentage

Proteobacteria, gram-negative bacteria characterized by the presence of lipopolysaccharide (LPS) on the outer membrane, activate the immune system response causing systemic inflammation. Common examples of Proteobacteria include Shigella, E. coli, Salmonella, Enterobacter, and Klebsiella. As emerging research suggests that not all proteobacteria negatively affect health, this score takes into consideration both the overall abundance of Proteobacteria as well as the presence of highly-pathogenic species.

### Pathogens

Although many common human pathogenic bacteria belong to the Proteobacteria phylum, there are species belonging to other phyla, such as Streptococcus, Staphylococcus and Clostridium from the Firmicutes phylum. While Firmicutes do not have LPS, they are often associated with GI conditions. It is important to note that a microbiome test is not intended to diagnose a bacterial infection. While detection of small amounts of pathogenic bacteria in the absence of symptoms may not be a cause for concern, chronic presence of a high pathogen abundance may indicate that the microbiome is providing a favorable environment for pathogens to thrive. Abundance of pathogenic bacteria may increase after a course of antibiotics, surgery, serious illness, and/or elevated stress levels. Additionally, unhealthy dietary patterns, such as diets high in saturated fats and sugars, may favor increased levels of pathogenic bacteria in the gut.

## Beneficial bacteria

Several intestinal bacterial species and strains can confer protection and health benefits to the host by supporting gut mucosa permeability and barrier function, producing anti-microbial molecules such as bacteriocins, regulating GI motility, optimizing metabolic health, metabolizing xenobiotics, and modulating the local and systemic immune system. Contrary to common perception, probiotic bacteria are not the only beneficial gut micro-organisms, as other common commensal bacteria can protect the host from opportunistic pathogens and sustain overall health. However, all bacteria, even those deemed as beneficial, may become pathobionts by negatively affecting diversity if present in high abundance. This report analyses the presence and abundance of three distinct categories of beneficial bacteria: probiotics, mucosa protective bacteria, and short chain fatty acid (SCFA) producing bacteria.

### Probiotics

According to the definition provided by the International Scientific Association for Probiotics and Prebiotics (ISAPP), the term probiotic should be applied only to "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Although certain gut commensals can be considered probiotic strains, these must be well-characterized and clearly demonstrated to be beneficial. If well-recognized probiotic species such as Lactobacillus and Bifidobacterium are detected in the sample, relative abundance is reported in the probiotic table. Akkermansia muciniphila is a fairly newly recognized probiotic that may confer metabolic health benefits and be imbalanced in individuals with type 2 diabetes, obesity and/or metabolic syndrome. However, as abnormally high levels of Akkermansia muciniphilia have been observed in those with autoimmune and neurodegenerative disorders, careful evaluation of probiotic abundance is important.

### Probiotic Supplementation

Supplements containing specific strains of probiotics are increasingly being used by patients and providers. As probiotic strain(s), dose, and duration are individualized, supplementation should be overseen by a trained practitioner. If abnormally high levels of Lactobacilli, Bifidobacterium, and/or Akkermansia are detected, current pre- and probiotic supplements may need to be reevaluated. Probiotic abundance may also be affected by pharmaceutical drugs, as is seen with metformin, which may promote intestinal barrier function and the production of beneficial short chain fatty acid (SCFA) producing bacteria.

### Mucosa Protective Bacteria

Mucosa protective bacteria, including Akkermansia muciniphila and Faecalibacterium prausnitzii, have been demonstrated to support a normal intestinal barrier function, regulate mucosal inflammation and act as "sentinels of the gut". While FGIDs are associated with a low abundance of these species, abnormally high levels may also be harmful. In fact, a recent study showed that those with constipation-predominant irritable bowel syndrome (IBS-C) had a higher abundance of Akkermansia, while those with diarrhea-predominant irritable bowel syndrome (IBS-D) were more likely to be Akkermansia-depleted.



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### Short chain Fatty Acid (SCFA) Producing Bacteria

Dietary fiber is metabolized by SCFA-producing bacteria to generate acetate, propionate, and butyrate, which play an important role in metabolic health and regulation of the immune system. For example butyrate is the main energy supply for colonocytes and has been extensively studied for its antiproliferative effects due to epigenetic regulation via histone deacetylase activity. Moreover, SCFAs can influence bacterial gene expression and reduce virulence of intestinal pathogens. A very high level of a single species, although not necessarily cause for concern, should be evaluated as dietary patterns, pharmaceuticals and supplements may cause alterations in bacterial distribution.

## Bile Acid (BA) Metabolizing Bacteria

BAs are synthesized from cholesterol in the liver and enter the intestine as conjugated BA where they are converted into secondary BAs by intestinal bacteria through 7α dehydroxylation and deconjugation. Several types of bacteria express BA metabolizing enzymes, including Clostridium, Bacteroides and Listeria species as well as some Lactobacilli and Bifidobacterium. Secondary BA affect enterochromaffin cells (EC) and levels of 5-hydroxytryptamine (5-HT, serotonin) causing increased visceral sensitivity and intestinal motility. Upregulation of intestinal serotonin availability leads to increased bowel peristalsis and may cause diarrhea in individuals predisposed to IBS-D. Moreover, Clostridium species can promote a higher liver biosynthesis of BAs and increase their secretion. There is evidence that almost 70% of patients with IBS-D have high fecal BAs or some form of BA malabsorption. The association between certain bacterial species metabolizing BAs and IBS symptoms is stronger for the diarrhea subtype, with increased BA production and secretion and corresponding increased intestinal serotonin. However, a decreased abundance or impaired distribution of BA metabolizing bacteria may cause constipation. Detection of an abnormal abundance in the main bacteria involved in BA metabolism may help personalize treatment by addressing underling dysbiosis with possible use of antibiotics and/or herbal supplements as well as evaluate other factors affecting BA production and absorption.

## Irritable Bowel Syndrome (IBS)

IBS risk factors include genetic predisposition, microbiome composition, dietary intake, age, gender, and lifestyle factors such as smoking, high levels of emotional stress, and prior GI infection. IBS subtypes, including IBS-C, IBS-D, and mixed or undefined IBS (IBS-U) may present with gut microbiome dysbiosis, where microbiota composition differs significantly from the reference population, and may be involved in triggering, worsening and/or sustaining IBS symptoms. Results of this report can help practitioners monitor and optimize treatment, which may include using specific antibiotics, supplements, and pre- pro- and/or postbiotics. Additionally, several herbal supplements, pharmaceuticals, vitamins, minerals and omega-3 fatty acids may also be useful in modifying the microbiota composition.

## Small Intestinal Bacterial Overgrowth (SIBO)

SIBO is a specific form of dysbiosis caused by high levels of specific bacteria in the small intestine. A variety of symptoms have been attributed to SIBO which may depend on the specific bacteria involved and may include excessive bloating, diarrhea, macronutrient malabsorption, and less commonly, constipation. Bacteria categories associated with SIBO include Proteobacteria, hydrogen-producing bacteria, and hydrogen-sulfite-producing bacteria. Proteobacteria and hydrogen-producing bacteria may cause or exacerbate diarrhea in both IBS and SIBO, while hydrogen-sulfide-producing bacteria may be associated with constipation and/or diarrhea, foul smelling stools, excessive gas and bloating, and may negatively affect gut barrier function and inflammation. This test measures the abundance of species that are commonly present in duodenal and/or jejunal aspirates of patients with SIBO (as demonstrated by the REIMAGINE study, 2019)and indicates if relative abundance of these species is higher than in the reference population. This test does not diagnose SIBO and detection of SIBO-associated bacteria may need to be followed-up with additional diagnostic testing, such as a breath test. Note, SIBO and other FGIDs, such as IBS, are often comorbid and tend to influence each other.

## FODMAP Sensitivity

FODMAPs are short-chain carbohydrates that are usually poorly absorbed in the small intestine and are fermented by bacteria in the colon. Over-abundance of FODMAP-fermenting bacteria in the presence of FODMAP-containing foods may cause GI symptoms such as gas, bloating, abdominal pain, and diarrhea and/or constipation. Those affected by IBS and/or SIBO may be particularly sensitive to FODMAP-containing foods. Being aware of a potential FODMAP sensitivity can assist the practitioner in recommending and personalizing a low-FODMAP diet trial that has been shown to improve symptoms in those with FGIDs. However, as a low-FODMAP diets is highly restrictive, it should only be followed for a short period of time and overseen by a knowledgeable practitioner who can work to determine specific intolerances and liberalize the diet as much as is tolerated.

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# Disclaimers

This test is not intended to diagnose, treat, or cure any medical condition, and is offered for information and guidance only. The results and associated information should be interpreted exclusively by certified practitioners such as physicians, nutritionists, dietitians, clinicians, or similar professional figures who are, therefore, able to evaluate the report and implement changes in patients' diet, drugs, or supplements regime, if considered necessary by said practitioner. The companies and organizations providing this test and its affiliates shall not be held responsible for any misinterpretation or misapplication of the results by the patient or any third party.

This report is not derived from a culture-based microbiological test. Therefore, it is not intended to diagnose any bacterial infection. The presence of pathogens included in the report is calculated based on sequencing methods and expressed as percents of relative abundance. Beneficial commensal or probiotics are characterized and expressed in the same way.

This report ONLY characterizes and analyses the bacterial species/strains that have been reported in the scientific literature to be strongly associated with functional gastrointestinal disorders. Consequently, if a patient suffers from disorders/diseases that are not influenced by these groups of bacteria, the score and the summary of results may not show any abnormalities. Conversely, patients affected by functional GI disorders may still have a normal or almost normal bacterial gut composition. In fact, the impact of bacterial dysbiosis may present with different degrees of severity in individuals. If no abnormalities are detected, the etiology of the patient's symptoms may not be linked to gut dysbiosis but some other causes (i.e., psychological distress).

The gut bacterial microbiome composition is highly dynamic and tends to be significantly affected by changes in diet, drugs, and supplements. Patients may have a normal intestinal score if they are already taking medication or supplementations that alter the gut microbiota. We strongly recommend taking this test before and after any significant intervention such as dietary changes and drugs/supplements introduction or discontinuation. The companies and organizations providing this test and its affiliates shall not be held responsible for any adverse reactions or consequences resulting from any changes made to the patient's diet, drugs, or supplements regime based on the results of this test.

This test is subject to regular revision and updates based on the most recently published scientific literature. It may, therefore, be possible that the scoring methodology, guidance and/or selection of bacterial species and strains that have been included in this report are not included in future reports. This is because bacteria relevant for Functional GI disorders change in accordance with the most recent evidence-based research and/or improvements in microbiome sequencing methods, resolution, and interpretation of results.

By using this test, the patient consents to the collection, use, and analysis of their anonymized data for scientific research and development purposes by the company providing the test and its affiliates. The company guarantees that any such data will be anonymized and will not be used for any other commercial purposes, nor will it be shared with any third parties without the explicit consent of the patient. The patient agrees to indemnify and hold harmless the company providing the test and its affiliates from any and all claims, liabilities, damages, expenses, and costs, including reasonable attorneys' fees, arising from the collection, storage, use, and analysis of their anonymized data for scientific research and development purposes.

The patient acknowledges that they have been provided with a disclaimer and the patient fully understands and accepts the risks associated with a misinterpretation and/or any limitations of this test. The patient agrees to release all the companies and organizations providing the test from any and all claims, liabilities, damages, expenses, and other losses arising out of or in connection with the use of this test or the results of this test or the storage and use of their data.



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