

Client #: 999999

Doctor: Sample Doctor, MD

Doctors Data Inc 123 Main St.

St. Charles, IL 60174 USA

Patient: Sample Patient

ld:999999

Age: 48 DOB: 01/01/1975

Sex: Male

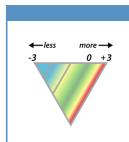
Sample Collection Date Collected 0
Date Received 0
Date Reported 0
Specimens Collected 3

**Date/Time** 04/18/2024 04/24/2024 05/03/2024

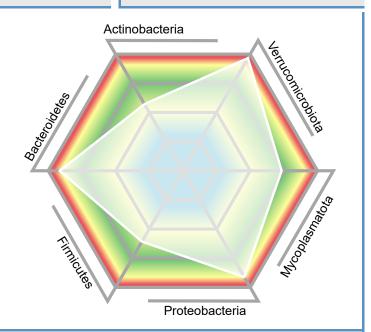
# Microbiome Abundance and Diversity Summary

The abundance and diversity of gastrointestinal bacteria provide an indication of gastrointestinal health, and gut microbial imbalances can contribute to dysbiosis and other chronic disease states. The Gl360  $^{\rm TM}$  Microbiome Profile is a gut microbiota DNA analysis tool that identifies and characterizes more than 45 targeted analytes across six Phyla using PCR and compares the patient results to a characterized normobiotic reference population. The web chart illustrates the degree to which an individual's microbiome profile deviates from normobiosis.

LEGEND



The web image shows the relative diversity and balance among bacteria belonging to the six primary Phyla. The white shaded area represents the patient's results compared to a normobiotic reference population. The center of the web represents less abundance while the outer edges represent more than normobiotic.

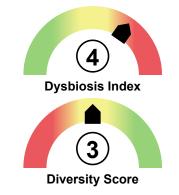


## **Dysbiosis and Diversity Index**

These indexes are calculated from the results of the Microbiome Profile, with scores ranging from 1 to 5, and do not include consideration of dysbiotic and pathogenic bacteria, yeast, parasites and viruses that may be reported in subsequent sections of the Gl360™ test.

The Dysbiosis Index the (DI) is calculated strictly from the results of the Microbiome Profile, with scores from 1 to 5. A DI score above 2 indicates dysbiosis; a microbiota profile that differs from the defined normobiotic reference population. The higher the DI above 2, the more the sample deviates from the normobiotic profile. The dysbiosis test and DI does not include consideration of dysbiotic and pathogenic bacteria, yeast, parasites and viruses that may be reported in subsequent sections of the GI360™ test.

A diversity score of 3 indicates an expected amount of diversity, with 4 & 5 indicating an increased distribution of bacteria based on the number of different species and their abundance in the sample, calculated based on Shannon's diversity index. Scores of 1 or 2 indicate less diversity than the defined normobiotic reference population.



# Butyrate producing bacteria Gut barrier protective bacteria Gut intestinal health marker Pro-inflammatory bacteria Gut barrier protective bacteria Gut barrier protective bacteria Gut barrier protective bacteria Fro-inflammatory bacteria Gut barrier protective bacteria vs. opportunistic bacteria = Expected = Imbalanced



# Microbiome Bacterial Abundance; Multiplex PCR



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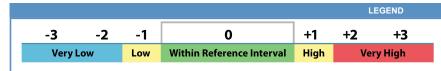
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Results are graphed as deviations from a normobiotic population. Normobiosis or a normobiotic state characterizes a composition of the microbiota profile in which microorganisms with potential health benefits predominate in abundance and diversity over potentially harmful ones.

Actinobacteria	Result								Reference Interval
		-3	-2	-1	0	+1	+2	+3	
Actinobacteria	0				•				0
Actinomycetales	-1			Δ					0
Bifidobacterium family	0				<b>A</b>				0
Bacteroidetes	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Alistipes spp.	-2								0
Alistipes onderdonkii	0				A				0
Bacteroides fragilis	0				<b>A</b>				0
Bacteroides spp. & Prevotella spp.	+1					Δ			0
Bacteroides spp.	+1					Δ			0
Bacteroides pectinophilus	0				<b>A</b>				0
Bacteroides stercoris	+2								0
Bacteroides zoogleoformans	0				A				0
Parabacteroides johnsonii	+1					Δ			0
Parabacteroides spp.	+3								0
Firmicutes	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Firmicutes	0								0
Bacilli Class	0				<b>A</b>				0
Catenibacterium mitsuokai	0								0

#### Notes:

The gray-shaded area of the bar graph represents reference values outside the reporting limits for this test.

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Methodology: Multiplex PCR



# Microbiome Bacterial Abundance; Multiplex PCR



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Firmicutes	Result	-3	-2	-1	0	+1 +2	2 +3	F	Reference Interval
Clostridia Class	-1							C	
Clostridium methylpentosum	0				A			C	
Clostridium L2-50	0				A			C	
Coprobacillus cateniformis	0				A			C	
Dialister invisus	0				A			C	
Dialister invisus & Megasphaera micronuciformis	0				A			C	
Dorea spp.	-1			Δ				C	
Holdemanella biformis	0				A			C	
Anaerobutyricum hallii	-2							C	
Agathobacter rectalis	-1							C	
Eubacterium siraeum	0				A			C	
Faecalibacterium prausnitzii	-1							C	
Lachnospiraceae	0				<b>A</b>			C	
Ligilactobacillus ruminis & Pediococcus acidilactici	0				A			C	
Lactobacillus family	0				A			C	
Phascolarctobacterium spp.	0				<b>A</b>			C	
Ruminococcus albus & R. bromii	0				A			C	
Mediterraneibacter gnavus	+1					Δ		C	
Streptococcus agalactiae & Agathobacter rectalis	-1			$\triangle$				C	
Streptococcus salivarius ssp. thermophilus & S. sanguinis	0				<b>A</b>			C	

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Methodology: Multiplex PCR



# Microbiome Bacterial Abundance; Multiplex PCR



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Firmicutes	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Streptococcus salivarius ssp. thermophilus	0				A				0
Streptococcus spp.	0				A				0
Veillonella spp.	+2								0
Proteobacteria	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Proteobacteria	+2								0
Enterobacteriaceae	+1					Δ			0
Escherichia spp.	+1					$\triangle$			0
Acinetobacter junii	0				A				0
Mycoplasmatota	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Metamycoplasma hominis	0				<b>A</b>				0
Verrucomicrobiota	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Akkermansia muciniphila	+3								0



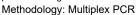
# **Microbiome Abundance Information:**

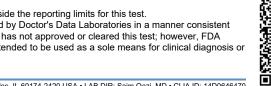
• The GI360™ Microbiome Profile is a focused gut microbiota DNA analysis tool that identifies more than 45 targeted analytes across six phyla using a CE-marked multiplex PCR system. Patient results are compared to a highly defined normobiotic reference population (n > 1,100). The white shadowed web plot within the hexagonal diagram illustrates the degree to which an individual's microbiome profile deviates from normobiosis. The center of the diagram represents less bacterial abundance while the outer edges represent greater than normobiosis. Deviation from a hexagon-shaped plot indicates variant diversity of the microbial community. Key findings for patient's microbiome profile are summarized in the table below the diagram, and detailed results for all of the analytes are presented on the next 3 pages of the report. Detailed results for the specific bacteria are reported as -3 to +3 standard deviations, as compared to the normobiotic reference population.

#### Notes

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Viruses	Result	
Adenovirus F40/41	Negative	
Norovirus GI/GII	Negative	
Rotavirus A	Negative	
Pathogenic Bacteria	Result	
Campylobacter (C. jejuni, C. coli and C. lari)	Negative	
Clostridioides difficile (Toxin A/B)	Negative	
Escherichia coli O157	Negative	
Enterotoxigenic Escherichia coli (ETEC) lt/st	Negative	
Salmonella spp.	Negative	
Shiga-like toxin-producing <i>Escherichia coli</i> (STEC) stx1/stx2	Negative	
Shigella (S. boydii, S. sonnei, S. flexneri & S. dysenteriae)	Negative	
Vibrio cholerae	Negative	
Parasites	Result	
Cryptosporidium (C. parvum and C. hominis)	Negative	
Entamoeba histolytica	Negative	
Giardia duodenalis (AKA intestinalis & lamblia)	Negative	





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**Protozoa** Result Balantidium coli Not Detected Blastocystis spp. Not Detected Chilomastix mesnili Not Detected Dientamoeba fragilis Not Detected Endolimax nana Not Detected Entamoeba coli Not Detected Entamoeba hartmanni Not Detected Entamoeba histolytica/Entamoeba dispar Not Detected Not Detected Entamoeba polecki Enteromonas hominis Not Detected Giardia duodenalis Not Detected Iodamoeba bütschlii Not Detected Not Detected Isospora belli Pentatrichomonas hominis Not Detected Retortamonas intestinalis Not Detected Result **Cestodes - Tapeworms** Diphyllobothrium latum Not Detected Dipylidium caninum Not Detected Not Detected Hymenolepis diminuta Not Detected Hymenolepis nana Taenia Not Detected **Trematodes - Flukes** Result Clonorchis sinensis Not Detected Fasciola hepatica/Fasciolopsis buski Not Detected Not Detected Heterophyes heterophyes Paragonimus westermani Not Detected **Nematodes - Roundworms** Result Not Detected Ascaris lumbricoides Notes:

Methodology: Microscopy



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Nematodes - Roundworms	Result	
Capillaria hepatica	Not Detected	
Capillaria philippinensis	Not Detected	
Enterobius vermicularis	Not Detected	
Hookworm	Not Detected	
Strongyloides stercoralis	Not Detected	
Trichuris trichiura	Not Detected	
Other Markers	Result	Reference Interval
Yeast	Rare	Not Detected – Rare
RBC	Not Detected	Not Detected – Rare
WBC	Not Detected	Not Detected – Rare
Muscle fibers	Not Detected	Not Detected – Rare
Vegetable fibers	Rare	Not Detected – Few
Charcot-Leyden Crystals	Not Detected	Not Detected
Pollen	Not Detected	Not Detected



## Parasitology Information:

- This test is not designed to detect Cyclospora cayetanensis or Microsproridia spp.
- Intestinal parasites are abnormal inhabitants of the gastrointestinal tract that have the potential to cause damage to their host. The presence of any parasite within the intestine generally confirms that the patient has acquired the organism through fecal-oral contamination. Damage to the host includes parasitic burden, migration, blockage and pressure. Immunologic inflammation, hypersensitivity reactions and cytotoxicity also play a large role in the morbidity of these diseases. The infective dose often relates to severity of the disease and repeat encounters can be additive.
- There are two main classes of intestinal parasites, they include protozoa and helminths. The protozoa typically have two stages; the trophozoite stage that is the metabolically active, invasive stage and the cyst stage, which is the vegetative inactive form resistant to unfavorable environmental conditions outside the human host. Helminths are large, multicellular organisms. Like protozoa, helminths can be either free-living or parasitic in nature. In their adult form, helminths cannot multiply in humans.
- In general, acute manifestations of parasitic infection may involve diarrhea with or without mucus and or blood, fever, nausea, or abdominal pain. However these symptoms do not always occur. Consequently, parasitic infections may not be diagnosed or eradicated. If left untreated, chronic parasitic infections can cause damage to the intestinal lining and can be an unsuspected cause of illness and fatigue. Chronic parasitic infections can also be associated with increased intestinal permeability, irritable bowel syndrome, irregular bowel movements, malabsorption, gastritis or indigestion, skin disorders, joint pain, allergic reactions, and decreased immune function.
- In some instances, parasites may enter the circulation and travel to various organs causing severe organ diseases such as liver abscesses and cysticercosis. In addition, some larval migration can cause pneumonia and in rare cases hyper infection syndrome with large numbers of larvae being produced and found in every tissue of the body.

Notes:

Methodology: Microscopy



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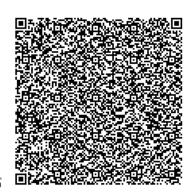
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Sample CollectionDate/TimeDate Collected04/18/2024Date Received04/24/2024Date Reported05/03/2024Specimens Collected3



# Parasitology Information:

- Red Blood Cells (RBC) in the stool may be associated with a parasitic or bacterial infection, or an inflammatory bowel condition such as ulcerative colitis. Colorectal cancer, anal fistulas, and hemorrhoids should also be ruled out.
- White Blood Cells (WBC) and Mucus in the stool can occur with bacterial and parasitic infections, with mucosal irritation, and inflammatory bowel diseases such as Crohn's disease or ulcerative colitis
- **Muscle fibers** in the stool are an indicator of incomplete digestion. Bloating, flatulence, feelings of "fullness" may be associated with increase in muscle fibers.
- Vegetable fibers in the stool may be indicative of inadequate chewing, or eating "on the run".







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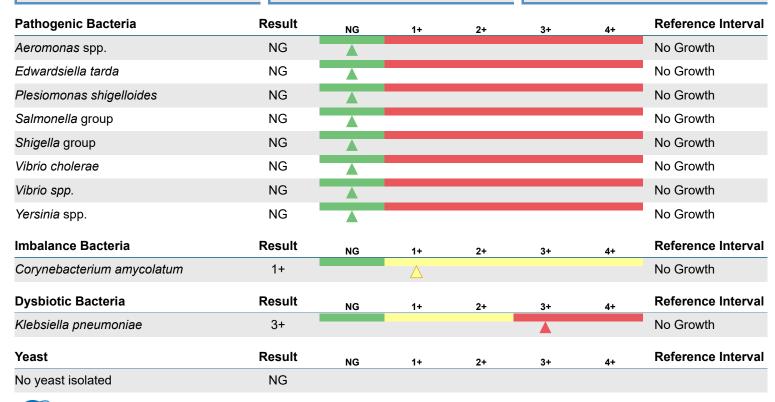
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# Microbiology Information:

- Pathogenic bacteria consist of known pathogenic bacteria that can cause disease in the GI tract. They are present due to the consumption of contaminated food or water, exposure to animals, fish, or amphibians known to harbor the organism. These organisms can be detected by either Multiplex PCR or microbiology culture.
- **Imbalanced bacteria** are usually neither pathogenic nor beneficial to the host GI tract. Imbalances can occur when there are insufficient levels of beneficial bacteria and increased levels of commensal bacteria. Certain commensal bacteria are reported as dysbiotic at higher levels.
- **Dysbiotic bacteria** consist of those bacteria that have the potential to cause disease in the GI tract. They can be present due to a number of factors including: exposure to chemicals that are toxic to beneficial bacteria; the use of antibiotics, oral contraceptives or other medications; poor fiber intake and high stress levels.
- Yeast may normally be present in small quantities on the skin, in the mouth and intestine. While small quantities of yeast may be normal, yeast observed in higher quantities is considered abnormal.









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# Klebsiella pneumoniae

Natural Agents	Low Sensitivity		High Sensitivity	
Black Walnut*				
Caprylic Acid*				
Uva Ursi*				
Olive Leaf Extract*				
Oregano*				
Goldenseal*				
Ionic Silver*				
Colloidal Silver*				
Prescriptive Agents	Resistant	Intermediate	Susceptible	
Amoxicillin-Clavulanic Acid				
Ampicillin				
Cefazolin				
Ceftazidime				
Ciprofloxacin				
Sulfamethoxazole / Trimethoprim				



# Susceptibility Information:

- **Natural antibacterial** agents may be useful for treatment of patients when organisms display in-vitro sensitivity to these agents. The test is performed by using standardized techniques and filter paper disks impregnated with the listed agent. Relative sensitivity is reported for each natural agent based upon the diameter of the zone of inhibition surrounding the disk. Data based on over 5000 individual observations were used to relate the zone size to the activity level of the agent. A scale of relative sensitivity is defined for the natural agents tested.
- Susceptible results imply that an infection due to the bacteria may be appropriately treated when the recommended dosage of the tested antimicrobial agent is used. Intermediate results imply that response rates may be lower than for susceptible bacteria when the tested antimicrobial agent is used. Resistant results imply that the bacteria will not be inhibited by normal dosage levels of the tested antimicrobial agent.



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# Introduction

This analysis of the stool specimen provides fundamental information about the overall gastrointestinal health of the patient. When abnormal microflora or significant aberrations in intestinal health markers are detected, specific commentaries are presented. If no significant abnormalities are found, commentaries are not presented.

The majority of reference intervals are established from adult populations. Results may differ in pediatric populations and care should be taken when interpreting these values.

#### **Microbiome Abundance Information**

## Actinobacteria (phylum)

Actinobacteria is one of the largest bacterial phyla, comprised of Gram-positive bacteria. This phylum includes a wide range of species, with different morphological and physiological characteristics. Significant groups in the human colon include Actinomycetales and Bifidobacteriales. Actinomycetales were inversely associated with clinically significant depression in IBS patients, suggesting these bacteria may be depleted in depressed IBS patients. A strict vegetarian diet may increase the total count of *Actinomyces* spp. compared to following a Western diet.

# 1

#### Actinomycetales (order)

Actinomycetales are considered low abundance colonizers of the gastrointestinal tract with primary residence on the skin. Intake of proton-pump inhibitor drugs has been shown to increase the abundance of Actinomycetales in the gut, possibly by reducing gastric acidity and enabling intestinal colonization by oral microbes. Actinomycetales may be depleted in depressed irritable bowel syndrome patients. The abundance of *Actinomyces* spp. was shown to be higher with a strict vegetarian diet compared to a common Western diet.

# Bacteroidetes (phylum)

Bacteroidetes make up approximately 28% of the gut microbiota in healthy human adults. They are early colonizers of the infant gut and are amongst the most stable, at a species and strain level, in the host. A low preponderance of Bacteroidetes in relation to Firmicutes has been associated with obesity, though this can increase with weight loss and restricted calorie intake.

# 1

## Alistipes (genus)

Alistipes does not contribute significantly to short chain fatty acid production. A diet rich in animal protein and fat increases the abundance of Alistipes. High abundance of Alistipes was identified as a possible predictor of successful weight loss. Increased abundance of Alistipes has been correlated with a greater frequency of pain in pediatric irritable bowel syndrome patients. In contrast, Alistipes onderdonkii was shown to be decreased in patients diagnosed with ulcerative colitis. Lower abundance of the Alistipes genus has been observed in patients with psoriatic arthritis and pediatric Crohn's disease. Alistipes may positively correlate with depression.

# 1

## Prevotella (genus)

*Prevotella*-rich dysbiosis has been associated with insulin-resistance, obesity and hypertension. *Prevotella* have been shown to be significantly decreased in Crohn's disease and Parkinson's disease. High levels of fiber and carbohydrates from fruits and vegetables in a Mediterranean diet have been shown to increase the relative abundance of *Prevotella*.

# \*\*Bacteroides (species)

Species in the genus *Bacteroides* carry out broad metabolic functions, including degradation of complex plant polysaccharides, proteolytic activities, de-conjugation of bile acids, mucosal barrier integrity, short chain fatty acid production, fatty acid storage and glucose metabolism. *Bacteroides* spp. are maintained at a higher abundance in breastfed individuals into adulthood. *Bacteroides fragilis* plays an important role in the prevention of intestinal inflammation. An energy-restricted diet has been shown to increase *B. fragilis* in overweight adolescents. An increase in *B. stercoris* has been associated with higher risk of colon cancer. Decreased levels of *Bacteroides* spp. have been reported in association with multiple sclerosis, rheumatoid arthritis and Parkinson's disease.

# Parabacteroides (genus)

The abundance of *Parabacteroides* spp., major anaerobic producers of acetate and succinate is increased with a high fat diet and is positively correlated with body weight. *Parabacteroides* spp., along with certain *Bacteroides* spp., have been shown to distinguish healthy adults from patients with irritable bowel syndrome or ulcerative colitis. Reduced abundance of this group of bacteria has also been linked to Crohn's disease in children. *Parabacteroides* spp. has been found to be less abundant in patients with multiple sclerosis.



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# Microbiome Abundance Information continued...

# Firmicutes (phylum)

The phylum Firmicutes constitutes the most diverse and abundant group of gastrointestinal microbiota which are grouped into four classes, Bacilli, Clostridia, Erysipelotrichia, and Negativicutes. They constitute about 39% of gut bacteria in healthy adults, but may increase to as high as 80% in an imbalanced microbial community.

# Clostridium (genus)

Clostridium spp. represents an extremely heterogeneous class of organisms that are still actively undergoing taxonomic revision. Clostridium spp. are strict anaerobic, spore-forming bacteria. Decreased abundance of the genus Clostridium was found to be associated with prediabetes. Some Clostridium spp. are transferred to infants from breast milk within the first months of life. Increased levels of some Clostridium spp. were observed in irritable bowel syndrome patients. Many species, some of them related to diarrhea, were decreased after consumption of inulin combined with maltodextrin.

# → Dorea (genus)

Dorea is a genus within the Lachnospiraceae family that is in the Firmicutes phylum. Dorea species are known to produce hydrogen and carbon dioxide as end-products of glucose fermentation and may be associated with bloating. Decreased levels of Dorea spp. were observed in patients with Parkinson's disease. Recent studies have identified increased levels of Dorea spp. in patients diagnosed with IBS, nonalcoholic fatty liver disease and non-alcoholic steatohepatitis, multiple sclerosis and colorectal cancer.

#### Anaerobutyricum hallii (species)

Anaerobutyricum hallii and Agathobacter rectalis (Eubacterium rectale) are both part of the Lachnospiraceae family that is in the Firmicutes family A. hallii and A. rectalis produce butyrate that is a key regulator of mucosal barrier integrity and function. Decreased levels of Anaerobutyricum/Agathobacter spp have been associated with very high protein diets. Anaerobutyricum hallii is capable of metabolizing glucose products with antimicrobial properties.

# Agathobacter rectalis (Eubacterium rectale)

Agathobacter rectalis (Eubacterium rectale) is part of the Lachnospiraceae family and produce butyrate. Agathobacter rectalis was found to be in lower abundance in patients with type 2 diabetes, colorectal cancer, and chronic idiopathic diarrhea. There is a negative correlation between Agathobacter rectalis and the symptomology of irritable bowel syndrome (IBS). Decreased levels of Anaerobutyricum/Agathobacter spp. have been associated with very high protein diets.

# Faecalibacterium prausnitzii (species)

Faecalibacterium prausnitzii is one of the most abundant butyrate producing bacteria in a healthy gastrointestinal tract. As such, F. prausnitzii is a protective factor for the intestinal mucosa and supports very important intestinal barrier functions. F. prausnitzii exerts anti-inflammatory effects via metabolites such as short-chain fatty acids. F. prausnitzii is reduced in inflammatory bowel disease, irritable bowel syndrome, celiac disease and gastrointestinal inflammation in general. It is reduced in patients diagnosed with Parkinson's disease, bipolar disorder, colorectal cancer, diabetes and chronic idiopathic diarrhea. Diminished levels of F. prausnitzii were found in patients with major depressive disorder. The abundance of F. prausnitzii together with E. coli has been proposed as a discrimination tool between ulcerative colitis and Crohn's disease. F. prausnitzii has been correlated with pediatric obesity in instances of high consumption of foods that are rich in unabsorbed carbohydrate (banana, maize, rice). The prebiotic inulin has been shown to increase the proportion of F. prausnitzii in the human intestinal microbiota. Low FODMAP diets are associated with diminished F. prausnitzii and butyrate production.

#### Ruminococcus/Mediterraneibacter (genus)

Members of the Ruminococcus and the new genus Mediterraneibacter sensu produce acetate, but not butyrate. Mediterraneibacter (Ruminococcus) gnavus, like Akkermansia muciniphila is a mucin degrading specialist. Higher levels of Ruminococcus/Mediterraneibacter were associated with non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Reduced levels of Ruminococcus bromii were observed in patients with primary biliary cirrhosis. Increased abundance of Ruminococcus/Mediterraneibacter spp. has been reported in irritable bowel syndrome (IBS), whereas Ruminococcus/Mediterraneibacter spp. are reportedly decreased in abundance with Chrohn's disease and ulcerative colitis. Mediterraneibacter gnavus has been found to be in higher abundance in diarrhea predominant IBS. Intake of resistant starch has been associated with increased levels of R. bromii, whereas a diet rich in animal protein and fat was found to reduce the abundance of this species in the human gut.



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# Microbiome Abundance Information continued...

# 1

## Veillonella (genus)

Veillonella (Firmicutes phylum) are known for their ability to ferment lactate, producing the short chain fatty acids propionate and acetate. Veillonella spp. were shown to be significantly increased in patients with Crohn's disease, type 1 diabetes, and patients diagnosed with liver cirrhosis. Increased amounts of Veillonella have been found in patients with constipation dominant irritable bowel syndrome (IBS-C). It is hypothesized that the relationship between Veillonella strains and IBS stems from its robust production of organic acids (propionate and acetate) which contribute to bloating, anxiety and abdominal pain. Higher levels of Veillonella were found in formula-fed infants compared to breast-fed infants.

#### Proteobacteria (phylum)

Proteobacteria include a wide variety of pathogens, including species within the *Escherichia*, *Shigella Salmonella*, *Vibrio*, and *Helicobacter* genera. The phylum includes a number of species that are permanent residents of the microbiota and capable of inducing nonspecific inflammation and diarrhea when their presence is increased. Proteobacteria make up approximately 2% of the gut microbiota in healthy adults.

# **↑** i

# Proteobacteria

A high-fat diet is positively associated with an abundance of Proteobacteria. Slightly increased abundance of Proteobacteria may be associated with low-grade inflammation. Proteobacteria are increased in inflammatory bowel disease and irritable bowel syndrome. Higher abundance of Proteobacteria has been associated with a moderate to severe disease course in newly discovered ulcerative colitis patients. They are associated with diarrhea in IBS.

# ← Enterobacteriaceae (family)

Enterobacteriaceae is a large family of bacteria within the Proteobacteria phyla. Enterobacteriaceae is inclusive of normal commensal species, harmless opportunists, and many of the more familiar pathogens, such as Salmonella, Escherichia coli, Klebsiella, Shigella and Proteus. Other potential disease-causing bacteria in this family include Enterobacter and Citrobacter species. The abundance of Proteobacteria, which are generally pro-inflammatory, is presented on the white shadowed web plot within the hexagonal diagram. The presence of specific dysbiotic and pathogenic Enterobacteriaceae bacteria, if detected by PCR or culture, are reported in the Gastrointestinal Pathogens and Microbiology sections of this report.

Overall, *Enterobacteriaceae* were found at higher levels in patients with NAFLD and PD. Diets rich in in complex carbohydrates are associated with lower levels of *Enterobacteriaceae*, in comparison to diets rich in fat and/or protein.

# Escherichia (genus)

Clinically, *Escherichia* has been reported to contribute to irritable bowel syndrome. *Escherichia* spp. are commonly recovered from inflamed tissues of both Crohn's disease and ulcerative colitis patients. Untreated inflammatory bowel disease patients were shown to have higher abundance of *Escherichia* and lower abundance of *Faecalibacterium prausnitzii*. Increased levels of *Escherichia* were observed in colorectal cancer patients. Patients diagnosed with nonalcoholic steatohepatitis have higher abundance of *Escherichia*. Consumption of a Western diet is positively associated with *Escherichia* levels. Increased levels of *E. coli* were observed in people on a gluten-free diet. A non-pathogenic strain of *Escherichia*, *Escherichia nissle*, is a widely used probiotic for treating gut related diseases such as chronic constipation.

# Mycoplasmatota (Tenericutes) (phylum)

Mycoplasmatota are cell wall-less bacteria that do not synthesize precursors of peptidoglycan. Mycoplasmatota consist of four main clades designated as the *Acholeplasma*, *Spiroplasma*, *Pneumoniae* and *Hominis* clusters. Mycoplasmatotas are typically parasites or commensals of eukaryotic hosts.

# Verrucomicrobiota (Verrucomicrobia) (phylum)

Verrucomicrobiota is a less common phylum in the human microbiota, but one with increasing recognition with regards to health. Verrucomicrobiota includes *Akkermansia muciniphila*. The obligate anaerobe *A. muciniphila* constitutes 3-5% of total bacteria in a healthy microbiome, and has a protective or anti-inflammatory role in the intestinal mucosa.



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#### Microbiome Abundance Information continued...



## Akkermansia muciniphila (genus)

Higher abundance of *Akkermansia muciniphila* has been associated with a milder disease course in newly discovered ulcerative colitis patients. Archaea and *Akkermansia* were significantly more prevalent after weight reduction. A Low FODMAP diet has been shown to decrease the abundance of *A. muciniphila* leading to recommendations against long-term use of such a diet. *A. muciniphila* is a mucolytic specialist that has potent anti-inflammatory effects in part associated with a specific surface coat protein (Amuc- 1100).

# **GI Pathogens**

#### Introduction

The GI Pathogen profile is performed using an FDA-cleared multiplex PCR system. It should be noted that PCR testing is much more sensitive than traditional techniques and allows for the detection of extremely low numbers of pathogens. PCR testing does not differentiate between viable and non-viable pathogens and should not be repeated until 21 days after completion of treatment or resolution to prevent false positives due to lingering traces of DNA. PCR testing can detect multiple pathogens in the patient's stool but does not differentiate the causative pathogen. All decisions regarding the need for treatment should take the patient's complete clinical history and presentation into account.

# **Microbiology**

# Pathogenic/Dysbiotic Flora

In a healthy balanced state of intestinal flora, the beneficial bacteria make up a significant proportion of the total microflora. However, in many individuals there is an imbalance or deficiency of beneficial flora (insufficiency dysbiosis) and an overgrowth of non-beneficial (imbalance) or even pathogenic microorganisms. This can be due to a number of factors including: consumption of contaminated water or food; daily exposure of chemicals that are toxic to beneficial bacteria; the use of antibiotics, oral contraceptives or other medications; poor fiber intake and high stress levels.

A number of toxic substances can be produced by the dysbiotic bacteria including amines, ammonia, hydrogen sulfide, phenols, and secondary bile acids which may cause inflammation or damage to the brush border of the intestinal lining. If left unchecked, long-term damage to the intestinal lining may result in leaky gut syndrome, allergies, autoimmune disease (e.g. rheumatoid arthritis), irritable bowel syndrome, fatigue, chronic headaches, and sensitivities to a variety of foods. In addition, pathogenic bacteria can cause acute symptoms such as abdominal pain, nausea, diarrhea, vomiting, and fever in cases of food poisoning.

Bacterial sensitivities to a variety of prescriptive and natural agents have been provided for the pathogenic bacteria that were cultured from this patient's specimen. This provides the practitioner with useful information to help plan an appropriate treatment regimen. Supplementation with probiotics or consumption of foods (yogurt, kefir, miso, tempeh, tamari sauce) containing strains of lactobacilli, bifidobacteria, and enterococci may help restore healthy flora levels. Soluble fiber and polyphenols derived from chocolate, green tea, blackcurrant, red wine and grape seed extracts have been found to increase the numbers of beneficial bacteria. Hypochlorhydria may also predispose an individual to bacterial overgrowth, particularly in the small intestine. Nutritional anti-inflammatories can aid in reversing irritation to the GI lining. These include quercetin, vitamin C, curcumin, gamma-linoleic acid, omega-3 fatty acids (EPA, DHA), and aloe vera. Other nutrients such as zinc, beta-carotene, pantothenic acid, and L-glutamine provide support for regeneration of the GI mucosa. A comprehensive program may be helpful in individuals in whom a dysbiotic condition has caused extensive GI damage.

# Klebsiella spp

Klebsiella spp. are gram-negative bacilli belonging to the Enterobacteriaceae family and closely related to the genera Enterobacter and Serratia. Klebsiella spp. are considered dysbiotic in the amount of 3 - 4 +. Klebsiella spp. are widely distributed in nature and in the gastrointestinal tract of humans. In humans, they may colonize the skin, oral cavity, pharynx, or gastrointestinal tract. Regarded as normal flora in many parts of the colon, intestinal tract and biliary tract, the gut is the main reservoir of opportunistic strains. This bacteria has the potential to cause intestinal, lung, urinary tract, and wound infections, but overgrowth of Klebsiella spp. is commonly asymptomatic. K. pneumoniae, in particular, may cause diarrhea and some strains are enterotoxigenic. Infection has been linked to ankylosing spondylitis as well as myasthenia gravis (antigenic cross-reactivity), and these patients usually carry larger numbers of the organism in their intestines than healthy individuals. Klebsiella oxytoca causes antibiotic associated hemorrhagic colitis. These strains have been shown to produce a cytotoxin that is capable of inducing cell death in various epithelial-cell cultures.



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# Microbiology continued...

Klebsiella is a significant nosocomial infectious agent, partially due to the ability of organisms to spread rapidly. Klebsiella accounts for approximately 3-7% of all hospital-acquired infections, placing it among the top eight pathogens in hospitals. Extraintestinal infection typically involves the respiratory or urinary tracts, but may infect other areas such as the biliary tract and surgical wound sites. K. pneumoniae and K. oxytoca are the two members of this genus responsible for most extraintestinal human infections.

Treatment of these organisms has become a major problem because of resistance to multiple antibiotics and potential transfer of plasmids to other organisms. Proper hand washing is crucial to prevent transmission from patient to patient via medical personnel. Contact isolation should be used for patients colonized or infected with highly antibiotic-resistant *Klebsiella* strains. *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis* are infrequent isolates that are subspecies of *K. pneumoniae*; however, each is associated with at unique spectrum of disease. *K. ozaenae* is associated with atrophic rhinitis, a condition called ozena, and purulent infections of the nasal mucous membranes. *K. rhinoscleromatis* causes the granulomatous disease rhinoscleroma, an infection of the respiratory mucosa, oropharynx, nose, and paranasal sinuses.

Antibiotics may be indicated if symptoms are prolonged and in systemic infections. Refer to the antimicrobial susceptibilities for treatment.

## **Imbalanced Flora**

Imbalanced flora are those bacteria that reside in the host gastrointestinal tract and neither injure nor benefit the host. Certain dysbiotic bacteria may appear under the imbalanced category if found at low levels because they are not likely pathogenic at the levels detected. Imbalanced bacteria are commonly more abundant in association with insufficiency dysbiosis, and/or a fecal pH more towards the alkaline end of the reference range (5.8 - 7.0). Treatment with antimicrobial agents is unnecessary unless bacteria appear under the dysbiotic category.