

Client #: 999999

Doctor: Sample Doctor, MD

Doctors Data Inc 123 Main St.

St. Charles, IL 60174 USA

Patient: Sample Patient

ld:999999

Age: 64 DOB: 00/00/1959

Sex: Female

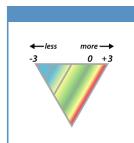
Sample Collection Date Collected Date Received Date Reported Specimens Collected

Date/Time 08/19/2023 08/21/2023 08/31/2023

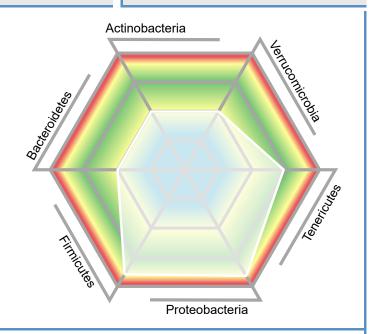
Microbiome Abundance and Diversity Summary

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™ 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

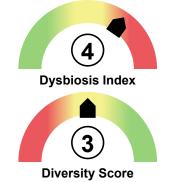


Dysbiosis and Diversity Index

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 GI360™ 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.





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Butyrate producing bacteria		Elastase, Very Low	Enterobacter cloacae complex, Cultured
Gut barrier protective bacteria		Secretory IgA, Very Low	Klebsiella pneumoniae, Cultured
Gut intestinal health marker		Butyrate, High	Candida lambica, Cultured
Pro-inflammatory bacteria		Total SCFA's, Very High	
Gut barrier protective bacteria vs. opportunistic bacteria		pH, Very Low	
		β-glucuronidase, Low	
= Expected = Imi	palanced		



Microbiome Bacterial Abundance; Multiplex PCR



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Specimens Collected 3

					L	EGEND
-3	-2	-1	0	+1	+2	+3
Very l	.ow	Low	Within Reference Interval	High	Vei	ry High
Very l	.ow	Low	Within Reference Interval	High	Vei	ry Hig

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	-3	-2	-1	0	+1	+2	+3	Reference Interval
Actinobacteria	-1			Δ					0
Actinomycetales	-1			Δ					0
Bifidobacterium family	-1			Δ					0
Bacteroidetes	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Alistipes spp.	-2								0
Alistipes onderdonkii	0								0
Bacteroides fragilis	0								0
Bacteroides spp. & Prevotella spp.	0				A				0
Bacteroides spp.	0				A				0
Bacteroides pectinophilus	-1			Δ					0
Bacteroides stercoris	0								0
Bacteroides zoogleoformans	-1			Δ					0
Parabacteroides johnsonii	0				A				0
Parabacteroides spp.	0				A				0
Firmicutes	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Firmicutes	-1			Δ					0
Bacilli Class	0				A				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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08/19/2023

08/21/2023

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Date Reported 08/31/2023 Specimens Collected 3

0

0

Firmicutes	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Clostridia Class	0				A				0
Clostridium methylpentosum	-1			Δ					0
Clostridium L2-50	0				A				0
Coprobacillus cateniformis	0				A				0
Dialister invisus	0				A				0
Dialister invisus & Megasphaera micronuciformis	0				A				0
Dorea spp.	0				A				0
Eubacterium biforme	0				A				0
Eubacterium hallii	0				A				0
Eubacterium rectale	0				A				0
Eubacterium siraeum	0				A				0
Faecalibacterium prausnitzii	0				A				0
Lachnospiraceae	0				A				0
Lactobacillus ruminis & Pediococcus acidilactici	0				A				0
Lactobacillus family	0				A				0
Phascolarctobacterium spp.	0				A				0
Ruminococcus albus & R. bromii	0				A				0
Ruminococcus gnavus	+3								0
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0

0

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Methodology: Multiplex PCR

Streptococcus agalactiae & Eubacterium

Streptococcus salivarius ssp.

thermophilus & S. sanguinis



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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.	-1			Δ					0
Proteobacteria	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Proteobacteria	+1					Δ			0
Enterobacteriaceae	+2								0
Escherichia spp.	0				A				0
Acinetobacter junii	0				A				0
Tenericutes	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Mycoplasma hominis	0				A				0
Verrucomicrobia	Result	-3	-2	-1	0	+1	+2	+3	Reference Interval
Akkermansia muciniphila	-1			Δ					0



Microbiome Abundance Information:

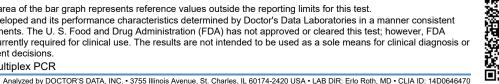
The GI360™ Microbiome Profile is a focused gut microbiota DNA analysis tool that identifies more than 45 targeted analytes across six phyla using a CE-marked multiplex PCR system. Patient results are compared to a highly defined normobiotic reference population (n > 1,100). The white shadowed web plot 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.

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Methodology: Multiplex PCR







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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. dysenteria	ne) Negative	
Vibrio cholerae	Negative	
Parasites	Result	
Cryptosporidium (C. parvum and C. hominis)	Negative	
Entamoeba histolytica	Negative	
Giardia duodenalis (AKA intestinalis & lamblia)	Negative	

Notes: Methodology: Multiplex PCR Page: 5 of 22





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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 Isospora belli Not Detected 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 Ascaris lumbricoides Not Detected 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 Many Not Detected - Rare RBC Not Detected Not Detected - Rare **WBC** Rare Not Detected - Rare Not Detected Muscle fibers Not Detected - Rare Vegetable fibers Rare Not Detected - Few Charcot-Leyden Crystals Not Detected Not Detected Pollen Not Detected Not Detected Result Reference Interval Macroscopic Appearance Color Brown Brown Consistency Soft Soft Negative Negative Mucus



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.

Notes:

Methodology: Microscopy, Macroscopic Observation





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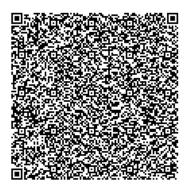
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Sample CollectionDate/TimeDate Collected08/19/2023Date Received08/21/2023Date Reported08/31/2023Specimens Collected3



Parasitology Information:

- 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.
- 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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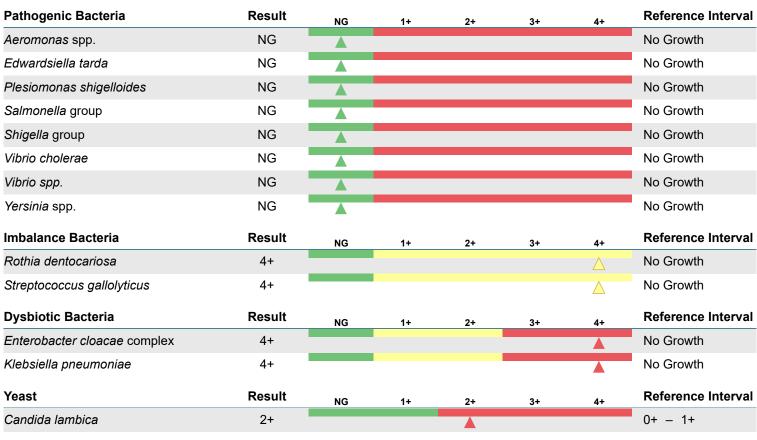
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Specimens Collected 3





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.



NG = No Growth

Methodology: Culture and identification by MALDI-TOF and conventional biochemicals







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Digestion / Absorption	Result	Unit	L	WRI	Н	Reference Interval
Elastase	86	µg/g				> 200
Fat Stain	None					None – Moderate
Carbohydrates [†]	Negative					Negative
Inflammation	Result	Unit	L	WRI	н	Reference Interval
Lactoferrin	3.1	μg/mL		A		<7.3
Lysozyme*	457	ng/mL		<u> </u>		≤500
Calprotectin	17	μg/g				< 80
Immunology	Result	Unit	L	WRI	н	Reference Interval
Secretory IgA*	11.1	mg/dL				30-275
Short Chain Fatty Acids	Result	Unit	L	WRI	н	Reference Interval
% Acetate [‡]	55	%		<u> </u>		50-72
% Propionate [‡]	23	0/				11 – 25
	23	%				11-25
% Butyrate [‡]	23	%		A		11 – 25
•				A		
% Valerate [‡]	22	%	Ξ			11 – 32
% Valerate [‡] Butyrate [‡]	22 0.9	%	Ξ			11 – 32 0.8 – 5.0
% Valerate [‡] Butyrate [‡] Total SCFA's [‡]	22 0.9 4.8	% % mg/mL	=	WRI	A H	11 – 32 0.8 – 5.0 0.8 – 4.0
% Valerate [‡] Butyrate [‡] Total SCFA's [‡] Intestinal Health Markers	22 0.9 4.8 22	% % mg/mL mg/mL	L	WRI	H	11 – 32 0.8 – 5.0 0.8 – 4.0 5.0 – 16.0
% Butyrate [‡] % Valerate [‡] Butyrate [‡] Total SCFA's [‡] Intestinal Health Markers pH β-glucuronidase*	22 0.9 4.8 22 Result	% % mg/mL mg/mL	L	WRI	A H	11 – 32 0.8 – 5.0 0.8 – 4.0 5.0 – 16.0 Reference Interval



Chemistry Information:

Elastase findings can be used for assessing pancreatic exocrine function and insufficiency.

Notes:

RI= Reference Interval, L (blue)= Low (below RI), WRI (green)= within RI, Yellow= moderately outside RI, L or H, H (red)= High (above RI)

[‡]This test was developed and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements. The U.S. Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA clearance is not currently required for clinical use.

Methodology: Turbidimetric immunoassay, Microscopy, Colormetric, Elisa, Gas Chromotography, ph Electrode, Enzymatic, Guaiac

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[†]This test has been modified from the manufacturer's instructions and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements.





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Chemistry Information:

- Fat Stain: Microscopic determination of fecal fat using Sudan IV staining is a qualitative procedure utilized to assess fat absorption and to detect steatorrhea.
- Carbohydrates: The presence of reducing substances in stool specimens can indicate carbohydrate malabsorption.
- Lactoferrin and Calprotectin are reliable markers for differentiating organic inflammation (IBD) from function symptoms (IBS) and for management of IBD. Monitoring levels of fecal lactoferrin and calprotectin can play an essential role in determining the effectiveness of therapy, are good predictors of IBD remission, and can indicate a low risk of relapse.
- Lysozyme is an enzyme secreted at the site of inflammation in the GI tract and elevated levels have been identified in IBD patients.
- Secretory IgA (sIgA) is secreted by mucosal tissue and represents the first line of defense of the GI mucosa and is central to the normal function of the GI tract as an immune barrier. Elevated levels of sIgA have been associated with an upregulated immune response.
- Short chain fatty acids (SCFAs): SCFAs are the end product of the bacterial fermentation process of dietary fiber by beneficial flora in the gut and play an important role in the health of the GI as well as protecting against intestinal dysbiosis. Lactobacilli and bifidobacteria produce large amounts of short chain fatty acids, which decrease the pH of the intestines and therefore make the environment unsuitable for pathogens, including bacteria and yeast. Studies have shown that SCFAs have numerous implications in maintaining gut physiology. SCFAs decrease inflammation, stimulate healing, and contribute to normal cell metabolism and differentiation. Levels of **Butyrate** and **Total SCFA** in mg/mL are important for assessing overall SCFA production, and are reflective of beneficial flora levels and/or adequate fiber intake.
- pH: Fecal pH is largely dependent on the fermentation of fiber by the beneficial flora of the gut.
- Occult blood: A positive occult blood indicates the presence of free hemoglobin found in the stool, which is released when red blood cells are lysed.
- **β-glucuronidase** is an enzyme that breaks the tight bond between glucuronic acid and toxins in the intestines. The binding of toxins in the gut is protective by way of blocking their absorption and facilitating excretion.







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Enterobacter cloacae complex

Natural Agents	Low Sensitivity		High Sensitivity	
Black Walnut*				
Caprylic Acid*				
Uva Ursi*				L
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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Klebsiella pneumoniae

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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			



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- 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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Age: 64 DOB: 00/00/1959

Sex: Female

Sample Collection
Date Collected
Date Received
Date Reported
Specimens Collected

Date/Time 08/19/2023 08/21/2023 08/31/2023

Candida lambica

Natural Agents	Low Sensitivity		High Sensitivity	
Caprylic Acid*		Δ		
Allicin (garlic)*				
Uva Ursi*				
Oregano*				
Undecylenic Acid*				
Goldenseal*				
Pau d'arco*				
Propolis*				
Non-Absorbed Antifungals	Low Sensitivity		High Sensitivity	
Nystatin				
Azole Antifungals	Resistant	S-DD	Susceptible	
Fluconazole				
Itraconazole				
Ketoconazole				



Susceptibility Information:

- **Natural antifungal** agents may be useful for treatment of patients when organisms display in-vitro susceptibility to these agents. The test is performed by using standardized techniques and filter paper disks impregnated with the listed agent. Relative activity is reported for each natural agent based upon the diameter of the zone of inhibition or no growth zone 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 activity is defined for the natural agents tested.
- Non-absorbed antifungals may be useful for treatment of patients when organisms display in-vitro susceptibility to these agents. The test is performed using standardized commercially prepared disks impregnated with Nystatin. Relative activity is reported based upon the diameter of the zone of inhibition or no growth zone surrounding the disk.
- Susceptible results imply that an infection due to the fungus may be appropriately treated when the recommended dosage of the tested antifungal agent is used. Susceptible Dose Dependent (S-DD) results imply that an infection due to the fungus may be treated when the highest recommended dosage of the tested antifungal agent is used. Resistant results imply that the fungus will not be inhibited by normal dosage levels of the tested antifungal agent.



*This test was developed and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements.
The U. S. Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA clearance is not currently required for clinical use. The results are not intended to be used as a sole means for clinical diagnosis or patient management decisions.





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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.

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.

4

Bifidobacterium (genus)

Considered amongst the most beneficial commensal bacteria in the human gut, Bifidobacterium spp. are able to degrade monosaccharides, galacto-, manno-, and fructo-oligosaccharides, as well as some complex carbohydrates. Many of the nondigestible oligosaccharides, found as natural components in mother's milk, select for colonization of these species which dominate the infant gut shortly after birth. Bifidobacteria may provide health benefits directly through interactions with the host, and indirectly through interactions with other microorganisms. Bifidobacterium spp. take part in production and adsorption of vitamins, such as vitamins K and B12, biotin, folate, thiamine, riboflavin, and pyridoxine. They are also involved in lipid absorption and metabolism, glucose and energy homeostasis, and regulating intestinal barrier function. Although Bifidobacterium produce acetate over butyrate, healthy levels of Bifidobacterium spp. facilitate colonization of Faecalibacterium. prausnitzii. Polyphenols derived from chocolate, green tea, blackcurrant, red wine and grape seed extracts have been shown to increase Bifidobacterium species. The increased abundance of Bifidobacterium species has been associated with amelioration of inflammation. Multiple published studies have suggested that there is an association between obesity and a lower abundance of bifidobacteria. They may also be less abundant in elderly populations, patients with rheumatoid arthritis, and in individuals diagnosed with Alzheimer's disease. Patients with active inflammatory bowel disease (IBD) have a lower abundance of Bifidobacterium spp. than patients whose IBD is in remission. Taking a probiotic containing bifidobacteria, lactobacilli, and streptococci might help in controlling ulcerative colitis symptoms and preventing their recurrence. Some Bifidobacterium strains have been shown to have beneficial effects in irritable bowel syndrome (IBS). Bifidobacterium spp. abundance has been shown to be diminished with IBD and with long term use of macrolide antibiotics. Luminal bifidobacteria is reduced with restriction of fermentable carbohydrates, i.e. a low FODMAP diet. High fat dietary feeding is also associated with reduced abundance of bifidobacteria. Consumption of maize and barley-based whole grain products and red berries, which are comprised of anthocyans, are known to increase levels of bifidobacteria.

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.

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.



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Microbiome Abundance Information continued...



Bacteroides pectinophilus (species)

Bacteroides pectinophilus contributes to breakdown of dietary pectins which are prebiotics. Pectins are complex, plant-derived carbohydrates that are indigestible by human enzymes, but can be easily degraded by certain commensal bacteria in the gut. Subsequent microbial fermentation of constituent sugar moieties yields important short chain fatty acids and other metabolites. The pectin-derived microbial fermentation products have important functions including reduction of ammonia, delay of gastric emptying and postprandial glucose regulation, induction of gut immunity, and maintenance of the mucosal barrier. Adequate intake and microbial metabolism of pectins appears to stimulate growth of various beneficial bacteria, including Lachnospiraceae, Dorea species, Bifidobacterium, Lactobacillus species, Faecalibacterium prausnitzii, and Eubacterium rectale. The abundance of B. pectinophilus has been positively correlated with a healthy fasting serum lipid profile, and negatively correlated with biomarkers of for insulin resistance and dyslipidemia. B. pectinophilus was less abundant for IBS patients compared to healthy controls. High consumption of kimchi (fermented cabbage) may be associated with lower than normal levels of B. pectinophilus.

1

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.

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.



Firmicutes

High Firmicutes and low Bacteroidetes abundances have been equivocally associated with obesity. A high-fat diet is associated higher abundance of both Firmicutes and Proteobacteria, and lower abundance of Bacteroidetes in mice. Low abundance of Firmicutes and greater abundance of *Akkermansia muciniphila* have been reported in lean individuals. Increased levels of Firmicutes have been associated with Crohn's disease and ulcerative colitis.



Clostridium methylpentosum (species)

Appropriate digestion and metabolism of complex dietary carbohydrates from plants drives healthy diversity in the gut microbiota. *Clostridium methylpentosum* ferments the naturally occurring sugar L-rhamnose that is released by microbial breakdown of plant-derived pectin. Rhamnose is fermented to propionate and acetate which are short chain fatty acids that have pivotal regulatory roles in the maintenance of mucosal barrier integrity, gut microbiota balance, post-prandial appetite suppression and normoglycemia. Lower levels of *C. methylpentosum* were reported for children with autism and pervasive developmental disorder compared to neurotypicals controls. Consumption of probiotic yogurt LKM512 containing *Bifidobacterium animalis* (subspecies lactis LKM512) increased the levels of *C. methylpentosum*.



Ruminococcus (genus)

Members of *Ruminococcus* sensu produce acetate, but not butyrate. *Ruminococcus gnavus*, like *Akkermansia muciniphila* is a mucin degrading specialist. Higher levels of *Ruminococcus* spp. were associated with non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Reduced levels of *R. bromii* were observed in patients with primary biliary cirrhosis. Increased abundance of *Ruminococcus* spp. has been reported in irritable bowel syndrome (IBS), whereas *Ruminococcus* spp. are reportedly decreased in abundance with Crohn's disease and ulcerative colitis. *Ruminococcus 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 human gut.



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Microbiome Abundance Information continued...



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.

1

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.

1

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.

Tenericutes (phylum)

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

Verrucomicrobia (phylum)

Verrucomicrobia is a less common phylum in the human gut microbiota, but one with increasing recognition with regards to health. Verrucomicrobia 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.



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).





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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.

Parasitology

Microscopic yeast

Microscopic examination has revealed more yeast in this sample than normal. While small quantities of yeast (reported as rare) may be normal, yeast observed in higher amounts (moderate to many) is considered abnormal. Yeast does not appear to be dispersed uniformly throughout the stool. Yeast may therefore be observed microscopically, but not grow out on culture even when collected from the same bowel movement. Further, some yeast may not survive transit through the intestines rendering it unviable for culturing. Therefore, both microscopic examination and culture are helpful in determining if abnormally high levels of yeast are present. If significant yeast are reported by microscopy, but not by culture, consider the presentation of patient symptoms.

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.

Enterobacter cloacae complex

Enterobacter cloacae complex is part of the Enterobacteriaceae family. E cloacae complex is a group of six closely related species with similar resistance patterns: E. cloacae, E. asburiae, E. hormaechei, E. kobei, E. ludwigii, and E. nimipressuralis. This gram-negative bacterium is considered dysbiotic at levels of 3+ or greater. E. cloacae complex is considered an opportunistic pathogen associated with diarrhea in children. A Shiga-like toxin-producing E. cloacae was isolated from the feces of an infant with hemolytic-uremic syndrome. However, E. cloacae complex is most often involved in extraintestinal infections including the urinary tract, respiratory tract, and cutaneous wounds.

Widely distributed in the environment, *Enterobacter* spp. is commonly isolated from both human and animal feces. Environmental strains of *Enterobacter* spp. are capable of growth in foods at refrigeration temperature.





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Microbiology continued...

E. cloacae complex is known to possess inducible β-lactamases. Isolates may become resistant to all cephalosporins after initiation of therapy. Avoid β-lactam-inhibitor drugs such as amoxicillin/ clavulanate, ampicillin/sulbactam, and piperacillin/tazobactam.

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

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.

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.

Cultured Yeast

Small amounts of yeast (+1) may be present in a healthy GI tract. However higher levels of yeast (> +1) are considered to be dysbiotic. A positive yeast culture and sensitivity to prescriptive and natural agents may help guide decisions regarding potential therapeutic intervention for yeast overgrowth. When investigating the presence of yeast, disparity may exist between culturing and microscopic examination. Yeast grows in colonies and is typically not uniformly dispersed throughout the stool. Further, some yeast may not survive transit through the intestines rendering it unviable for culturing. This may lead to undetectable or low levels of yeast identified by culture, despite a significant amount of yeast visualized microscopically. Therefore, both microscopic examination and culture are helpful in determining if abnormally high levels of yeast are present.





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Microbiology continued...

Dysbiotic Yeast

Yeast was cultured from this stool specimen at a level that is considered to be dysbiotic. A positive yeast culture and sensitivity to prescriptive and natural agents may help guide decisions regarding potential therapeutic intervention for chronic yeast syndrome. When investigating the presence of yeast, disparity may exist between culturing and microscopic examination. Yeast grows in colonies and is typically not uniformly dispersed throughout the stool. This may lead to undetectable or low levels of yeast identified by culture, despite a significant amount of yeast visualized microscopically.

Stool Chemistries

Elastase

Elastase is a pancreatic enzyme that digests and degrades a number of proteins. A finding of low elastase in a formed stool specimen is an indicator of pancreatic exocrine insufficiency. Moderate pancreatic insufficiency is defined at $100-200 \,\mu\text{g/mL}$, and severe pancreatic insufficiency as < $100 \,\mu\text{g/mL}$. Fecal elastase can be artefactually low due to fluid dilution effects in a loose/watery stool sample. Check the reported consistency of the stool specimen. Fecal elastase measured by a sensitive immunoassay is a specific marker for pancreatic function and maintains a high diagnostic accuracy among patients with small intestinal diseases. This elastase marker allows for the diagnosis or exclusion of pancreatic exocrine insufficiency and degree of severity, which can be caused by chronic pancreatitis, cystic fibrosis, pancreatic tumor, cholelithiasis or diabetes mellitus. This test does not differentiate between pancreatic insufficiency due to chronic pancreatitis and that due to pancreatic cancer. Immunoreactive elastase concentrations are similar for children and adults.

In cases of severe exocrine pancreatic insufficiencies, fecal fat stain may also be elevated. Supplementation with pancreatic enzymes, minerals, and vitamins may be warranted.

Secretory IgA (slgA) Low

The concentration of sIgA is abnormally low in this fecal specimen. Secretory IgA represents the first line of defense of the gastrointestinal (GI) mucosa and is central to the normal function of the GI tract as an immune barrier. Immunological activity in the gastrointestinal tract can be accessed via fecal sIgA levels in a formed stool sample. However, sIgA may be artefactually low due to fluid dilution effects in a watery or loose/watery stool sample.

Chronic mental and physical stress as well as inadequate nutrition have been associated with low fecal slgA concentrations. This includes dietary restrictions, excessive alcohol intake, body mass loss, negative moods, and anxiety. One study found decreased levels of slgA in malnourished children, particularly protein malnourishment, which responded well to nutritional rehabilitation with a significant increase in slgA. A possible explanation for this may be the synthesis and expression of slgA requires adequate intake of the amino acid L-glutamine. An increase of dietary L-glutamine may restore GI immune function by protection of cells that synthesize slgA. Saccharomyces boulardii is a nonpathogenic yeast that has been used for the treatment of acute infectious enteritis and antibiotic-associated diarrhea. Restored levels of slgA and subsequent enhanced host immune response have been found following S. boulardii administration (animal models). With low slgA one might consider a salivary cortisol test.

Short Chain Fatty Acids (SCFAs)

The total concentration and/or percentage distribution of the primary short chain fatty acids (SCFAs) are abnormal in this specimen. Beneficial bacteria that ferment non-digestible soluble fiber produce SCFAs that are pivotal in the regulation of intestinal health and function. Restoration of microbial abundance and diversity, and adequate daily consumption of soluble fiber and polyphenols can improve SCFA status.

The primary SCFAs butyrate, propionate and acetate are produced by predominant commensal bacteria via fermentation of soluble dietary fiber and intestinal mucus glycans. Key producers of SCFAs include *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Bacteroides fragilis*, *Bifidobacterium*, *Clostridium* and *Lactobacillus* spp. The SCFAs provide energy for intestinal cells, and regulate the actions of specialized mucosal cells that produce anti-inflammatory and antimicrobial factors, mucins that constitute the mucus barriers, and gut active peptides that facilitate appetite regulation and euglycemia. The SCFAs also contribute to a more acidic and anaerobic microenvironment that disfavors dysbiotic bacteria and yeast. Abnormal SCFAs may be associated with dysbiosis (including insufficiency dysbiosis), compromised intestinal barrier function (intestinal permeability) and inappropriate immune and inflammatory conditions.

"Seeding" with supplemental probiotics may contribute to improved production and status of SCFAs, but it is imperative to "feed" the beneficial microbes. Sources of soluble fiber that are available to the microbes include chick peas, beans, lentils, oat and rice bran, fructo- and galacto- oligosaccharides, and inulin.



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Stool Chemistries continued...

pH low

The pH of this stool sample is more acidic (<6.0) than expected. The pH of the stool, reflective of colonic pH, is normally slightly acidic. An acidic pH is commonly associated with rapid transit time, e.g. diarrhea or loose stools, more than three bowel movements per day. Check stool consistency. Further investigation of the cause of rapid transit such as food intolerance, and viral, bacterial, parasitic infection, may be warranted. An acidic pH is common in individuals with lactose malabsorption/intolerance. Unabsorbed lactose in the gut can be hydrolyzed by colonic bacteria forming volatile fatty acids which cause the stool to become acidic, often times accompanied by a sweet, sickly stool odor.

β-glucuronidase

 β -glucuronidase (β -G) is an enzyme that breaks the tight bond between glucuronic acid and toxins in the intestines. The liver and intestine bind toxins, steroid hormones and some dietary components to glucuronic acid. That is a protective process that limits absorption and enterohepatic resorption of toxins, and enhances excretion. A high level of activity of β -G in the gut is not desirable. A low level of β -G activity is not known to be of any direct clinical consequence.

 β -glucuronidase is produced by the intestinal epithelium and many species of intestinal bacteria. Observational studies have indicated a correlation between high β -G activity and certain cancers, but a definitive causal relationship has not been established. Higher levels of β -G have been associated with higher circulating estrogens and lower fecal excretion of estrogens in premenopausal women. A potential dietary carcinogen derived from grilled/smoked meat and fish induces high β -G activity and prolongs internal exposure to the toxin in an experimental animal model.

Diet and intestinal bacterial imbalance modulate β -G activity. High fat, high protein and low fiber diets are associated with higher β -G activity compared to vegetarian or high soluble fiber diets. Higher β -G may be associated with an imbalanced intestinal microbiota profile. Some major bacterial producers of fecal β -G include *Bifidobacterium*, *Lactobacillus*, *Escherichia coli*, *Clostridium*, *Bacteroides fragilis* and other *Bacteroides* spp., *Ruminococcus gnavus*, and species that belong to the genera Staphylococcus *and* Eubacterium.

Low β -G activity is an indicator of abnormal metabolic activity among the intestinal microbiota that may be influenced by dietary extremes, diminished abundance and diversity of the intestinal microbiota, or heavy probiotic and/or prebiotic supplementation. A low fat, low meat and high fiber diet, such as consumed by strict vegetarians, may be benignly associated with lower β -G activity compared to a typical "Western diet." High-end consumption of soluble fiber (e.g. inulin) and supplementation with *Lactobacillus acidophilus* may be inconsequentially associated with lower fecal β -G.