VibrantWellness

GUT ZOOMER
INTERPRETATION GUIDE

MC-0093-01

CONTENTS

THE VIBRANT ADVANTAGE

At Vibrant America, we value research and quality. The Gut Zoomer 3.0 is an updated and improved version of prior iterations of this test.

Before offering any test, we validate all markers and assays thoroughly. Those validation reports are always available on request, or can be accessed at any time in our online portal, under the Publications and Resources tab.

Because Vibrant utilizes a state-of-the-art silicon microarray platform to measure genetic material from microorganisms, we are able to achieve significantly higher sensitivity and specificity for all microorganisms and protein-based analytes than traditional testing platforms.

Through regular quality assurance and rigorous quality control standards, we have developed a remarkable test to aid practitioners in clinical practice with the most comprehensive assessment of the entire gastrointestinal environment available.

Thank you for your continued support of Vibrant America and Vibrant Wellness testing!

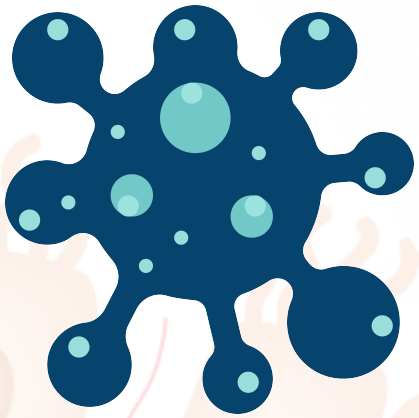
GUT MICROBIOME: *KEY TERMS AND TAXONOMY*

TAXONOMY GUIDE

PHYLUM		*GENUS/SPECIES*
Firmicutes (Gram +)	Clostridia	Anaerococcus; Blautia/ Butyrivibrio; Clostridiales incertae sedis XII; Clostridium; Coprococcus; Dorea; Eubacterium; Faecalibacterium; Fusibacter; Lachnospira; Oscillospira; Papillibacter; Peptostreptococcaceae; Pseudobutyrivibrio; Roseburia; Ruminococcus; Sporobacter
	Bacilli	Bacillus; Enterococcus; Lactobacillus; Staphylococcus; Streptococcus
	Negativicutes	Allisonella; Dialister; Phascolarctobacterium; Veillonella
Verrucomicrobia (Gram -)		Akkermansia
Bacteroidetes (Gram -)		Alistipes; Bacteroides; Porphyromonas; Prevotella; Tannerella
Proteobacteria (Gram -)	Enterobacteriaceae	Edwardsiella; Escherichia; Enterobacterium; Serratia; Klebsiella; Proteus; Providencia; Shigella; Yersinia
Actinobacteria (Gram +)		Bifidobacterium; Gardnerella; Collinsella; Eggerthella; Nocardia; Propionibacterium
Archaea	Methanobacteriales	Methanobrevibacter

Dysbiosis

When assessing the health of the microbiome using a tool like Vibrant Gut Zoomer 3.0, one of the most important considerations is the degree of dysbiosis. Dysbiosis is a term for microbial imbalance or maladaptation. As part of the human microbiome, the gut flora can become imbalanced, in which key beneficial (commensal) species become displaced, by less favorable or more inflammatory bacteria or other microorganisms.



Common Dysbiosis Features

- *Increase in harmful microbes (pathobionts and pathogens)*
- *Low diversity*
- *Decrease in beneficial microbes*
- *Decrease in beneficial bacterial metabolites*
- *Increase in harmful bacterial metabolites/products*
- *Associations with disease conditions*
- *Increased inflammation and intestinal permeability*

GRAM NEGATIVE VS. GRAM POSITIVE

Most bacteria are classified into two broad categories: gram positive (+) and gram negative (-).

The distinction between gram (+) and gram (-) are based on their cell wall composition and reaction to the gram stain test.

Both categories of bacteria have cell walls made of peptidoglycan, however gram (-) bacteria have a lipopolysaccharide (LPS) component on their outer membrane that is not found in gram (+) bacteria.

LPS is a glycolipid complex that protects bacteria from harmful substances. It is also a bacterial toxin (endotoxin).

Overgrowth of LPS-producing bacteria, such as Proteobacteria, can promote inflammation, especially when LPS is able to cross the intestinal epithelium in excessive amounts due to increased intestinal permeability.

IMPORTANT BACTERIA GROUPS

Lactobacillus	• 1% or less of the microbiome • Converts sugar to lactic acid • Contributes to the health of the intestinal lining • Helps to balance the gut immune system • May promote the growth of Clostridia and protect against some pathogens • Feeds preferentially on FOS and pectin fibers • Responds well to red polyphenols (red grapes/wine and pomegranate), curcumin, garlic, apples, cocoa • Intermittent fasting practices can increase abundance
Bifidobacterium	• Makes up 0% to 95% (infants) and 1 to 15% (adults) of the microbiome • One of the first to colonize the guts of newborn infants • Contributes to the health of the intestinal lining • Helps to balance the gut immune system • May promote the growth of Clostridia and protect against some pathogens • Thrives on a wide variety of fibers, oligosaccharides, and phytonutrients • Responds well to blueberries, pomegranate, apples, red wine, coffee, and cocoa
Akkermansia	• <1% to 3% of microbiome • Converts sugars to lactic acid • Contributes to the health of the intestinal lining • Helps to reduce inflammation and may contribute to protection from inflammatory bowel disease • Higher levels are associated with higher diversity of the microbiome
Streptococcus	• Many streptococcal species are not pathogenic, and form part of the commensal human microbiota of the mouth, skin, intestine, and upper respiratory tract

IMPORTANT BACTERIA GROUPS (CONT.)

Clostridia	• 10 to >50% of the microbiome • Critical for health of the intestinal lining • Help to restrain inflammation, autoimmunity, and harmful bacteria • Involved in metabolic regulation • Important producers of butyrate and secondary bile acids • Includes important groups such as Faecalibacterium, Roseburia, and Eubacterium • Thrives on a high-fiber diet • Important phytonutrients to improve levels include berberine, cloves, grape/red wine polyphenols • Probiotics such as S. boulardii, L rhamnosus, B adolescentis and other Bifidobacteria can cross-feed Clostridia • Clostridum is genus of Clostrida that houses several pathogens and opportunistic pathobionts. These Clostridia bacteria metabolites can negatively alter brain and nervous system function, and have been implicated in several disease states including autism, ADD/ADHD, OCD, seizures, psychosis, depression • Consider Organic Acids testing to determine potential elevations in Clostridia metabolites- HPHPA and 4-cresol which inhibit dopamine-beta-hydroxylase (DBH) which converts dopamine to norepinephrine, thus interfering in normal dopamine pathways and metabolism.
Bacillus	• Spore-forming bacteria (also known as spore-based probiotics) • Native microorganisms to soil, and thus have inhabited the human gut since beginning of evolution • Because they are spore-formers, they can survive stomach acid and have a better viability of germination in the small and large intestine • Certain species are vitamin producers (vitamin K2) • May increase butyrate and other SCFAs • Contributes to the health of the intestinal lining and may protect against pathogens • Have anti-inflammatory and immune system supporting benefits • Have shown to be beneficial in the following disease states: intestinal permeability, SIBO, cardiometabolic syndrome

IMPORTANT BACTERIA GROUPS (CONT.)

Desulfovibrio	• A gram (-) sulfate-reducing bacteria, meaning they "breathe" sulfate instead of O_2 and they produce hydrogen sulfide as a byproduct • Hydrogen sulfide is known to inhibit mitochondria and block the oxygen carrying capacity of hemoglobin. It can also increase the toxicity of heavy metals by enhancing their absorption (lowering enzymes that eliminate them) • Elevated levels can be seen with inadequate protein digestion or when abundant dietary protein is consumed without sufficient dietary fiber
Archea	• Single-celled microorganisms with a structure similar to bacteria • Obligate anaerobes; found in the commensal microorganisms of the intestine, Methanobacteriales being the most notable • The role of Archea in GI health remains unclear • One major function of methanogens is the scavenging of various fermentation products produced by other microbes (CO_2, H_2, alcohols, and acetic acid), resulting in the production of methane and CO_2. The reduction of hydrogen favors the growth of polysaccharide fermenting bacteria leading to higher energy utilization of the diet. For example, higher levels of methanogenic archaea have been observed in overweight/obese individuals.
Proteobacteria (Phylum)	• 2% to >10% of the microbiome • Gram negative and typically produce pro-inflammatory LPS • The phylum is mostly beneficial but also contains a large number of pathogens and pathobionts • Tends to thrive in inflammatory conditions

Key Opportunistic Microbes to Note

- **Proteobacteria**
- **Collinsella**
- **Desulfovibrio**
- **Enterobacteriaceae**
- **Helicobacter**
- **Klebsiella**
- **Methanobrevibacter**
- **Proteus**

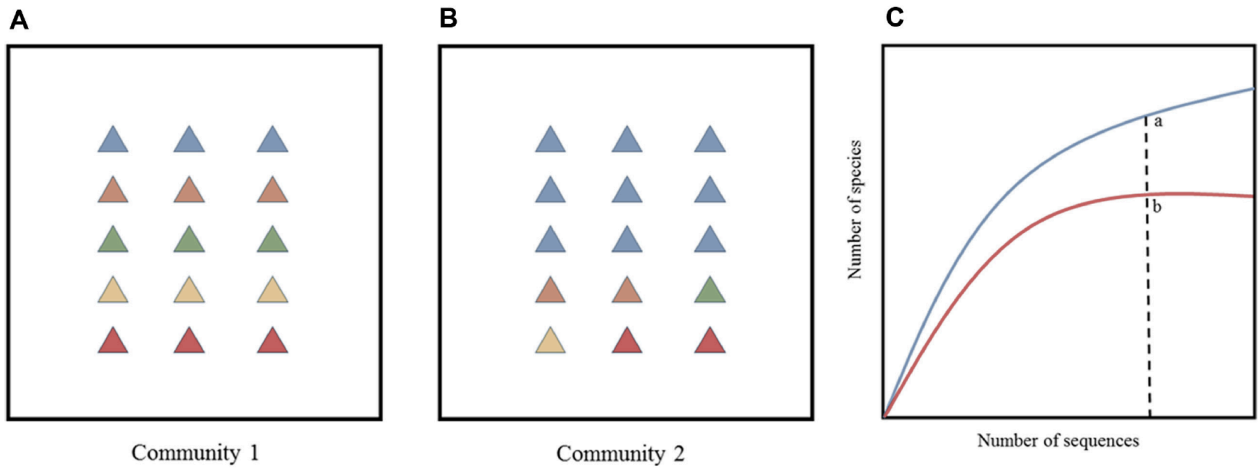


Fig. 1. Species richness, evenness, and rarefaction curve.
Both communities 1 (**A**) and 2 (**B**) have the same species richness, five species each. However, organisms in community 1 (**A**) are more evenly distributed than in community 2 (**B**). With the same sampling efforts, **A** is more diverse than **B** based on the rarefaction curve (**C**). The triangles represent bacterial species, and different species are presented in different colors.

DIVERSITY

One of the most common features of gut microbial dysbiosis is ***low diversity.***

Ecological diversity is defined as the probability that two randomly selected species in a population will be different.

Two main factors, ***richness*** and ***evenness***, help quantify diversity

SPECIES RICHNESS REFERS TO THE NUMBER OF DIFFERENT SPECIES PRESENT IN A CERTAIN NICHE

If more species are present in "A" than "B", "A" is richer than "B". When it comes to species richness, it does not consider the number of individuals of each species present (Figs. 1A and 1B).

EVENNESS COMPARES THE UNIFORMITY OF THE POPULATION SIZE OF EACH OF THE SPECIES PRESENT.

A measure of the relative abundance of the different species consisting of a community is evenness (Figs. 1A and 1B).

In general, when species richness and evenness increase, diversity does so too. A species diversity index denotes a mathematical measure of species diversity in a community. Shannon-Wiener and Simpson diversity indices have been traditionally used to measure the diversity of microbial communities.

Image source: Kim B-R, Shin J, Guevarra RB, et al. Deciphering diversity indices for a better understanding of microbial communities J. Microbiol Biotechnol. 2017;27(12):2089–2093. https://doi.org/10.4014/jmb.1709.09027

SHANNON AND SIMPSON DIVERSITY INDICES

Shannon's Index

- AKA Shannon-Wiener Index
- Places greater weight on species ***richness***
- Scale of 0-3
- The higher the number, the ***more*** diversity
- <1.5 = extremely low diversity
- >2.5 = good diversity

Simpson's Index

- Places a greater weight on species **evenness**
- Takes into account the number of species present, as well as the relative abundance of each species
- Scale of 0-1
- The lower the number, the **less diversity**
- E.g.: a score of 0.15 means there is a 15% probability that if you pick two species in a sample at random, they will be different....this is low diversity meaning that there is one species dominating the population
- Gives more weight to dominant species (random/rare species won't have as much influence)

GUT HEALTH ASSESSMENT

Many factors can have an impact on the outward symptoms a patient may be having in regard to their gut health. When you decide to order a Gut Zoomer 3.0 on your patients, keep in mind that it is good practice to get the following information from your patients as it can help explain trends or patterns that you see – especially in terms of the commensal bacteria patterns.

OBTAIN THE FOLLOWING INFORMATION FROM THE PATIENT TO COMPLETE THEIR "CLINICAL CASE" :

How many antibiotics has patient taken over their lifetime? Have any been in the last 5 years? Have they had antibiotics before/following surgery or for acne?

Has the patient had their gall bladder removed?

How is the gut health of their parents? Did mom or dad have excess gas, problems having a BM, heartburn, loose stools, abdominal pain, diarrhea?

Do any of their siblings have gut issues?

Have they had a fairly traumatic or largely stressful event (or a series of stressful events) in their lives?

GUT HEALTH ASSESSMENT (CONT.)

"All Disease Begins in The Gut." - ***Hippocrates***

Do they take more than one prescription medication?

Have they taken proton pump inhibitors (e.g. Zantac, Prilosec) for 3 months or longer?

Does the patient have a history of alcohol abuse?

Is there any reason to suspect the presence of excess heavy metals in the system (amalgams, excess tuna/fatty fish consumption, living near an incinerator, coal burning plant, paper mill)?

Has the patient experienced foodborne illness in the U.S. or from traveling abroad?

Please check the symptoms that patient might be experiencing currently or recently:

__Heartburn

__Nausea

__Abdominal pain

__Bloating

__Constipation/diarrhea

__Excess gas

LIFESTYLE FACTORS TO IMPROVE THE MICROBIOME

GENERAL ANTI-INFLAMMATORY PRACTICES FOR OPTIMAL GUT HEALTH

Eat Whole Food

- Minimal ingredients/unprocessed/organic/non-GMO
    - Prebiotics (fibers and herbs)
    - Probiotics / Fermented foods
- Consider intermittent fasting
- Limit refined carbohydrates, no added sugars
- Abundant healthy fats:
    - high omega-3s
    - unrefined plant-based oils
    - organic animal fats in moderation
    - no processed vegetable oils such as corn, peanut, or soybean
    - no trans fats
- Moderate protein: mixed plant and animal sources

Regular physical activity, but not excessive

Support detoxification practices with liver support, exercise, or saunas

Optimize sleep hygiene and **stress management**

Hydrate with clean, filtered water

PREBIOTIC FIBER GUIDE

Food	Source of Benefit
Artichoke	A great source of fiber and high in antioxidants
Asparagus	High in fiber, folate, and other B vitamins
Onion	Has potent anti-inflammatory properties that help to reduce blood pressure; they are also a considerable source of vitamin C, minerals, and potassium
Garlic	Contains active compounds that can reduce blood pressure, lower cholesterol, lower the risk of certain cancers, and protect against heart disease
Bananas	Contain both insoluble and soluble fiber, which provide a prebiotic food source for beneficial bacteria
Chickpeas	Rich in fructo-oligosaccharides, hemicellulose, cellulose, and resistant starch; have been shown to reduce blood lipids
Apples	A rich source of pectin, which makes up nearly half of its fiber content; pectin has been shown to increase levels of the short chain fatty acid butyrate, which increases T cell differentiation and reduces inflammation. Additionally, apples are high in polyphenol antioxidants, which have been linked to improved digestive health and fat metabolism, decreased levels of LDL cholesterol, and a reduced risk of various cancers.
Jicama	Jicama root is a good source of inulin and has been shown to improve digestive health, enhance insulin sensitivity, and lower blood sugar levels
Leeks	They are a great source of vitamin K and, due to their fiber content, help to break down fat during digestion and promote the growth of healthy bacteria in the gut

PREBIOTIC FIBER GUIDE (CONT.)

Food	Source of Benefit
Resistant Starch	Some resistant starch escapes digestion in the small intestine and is consequently fermented in the large intestine, allowing the production of short chain fatty acids Resistant starch has also been shown to lower the severity of insulin resistance
Lentils	Lentils are rich in cellulose, hemi-cellulose, fructo-oligosaccharides, and resistant starch, which can lead to the enhanced production of short chain fatty acids by feeding beneficial bacteria Lentils have also been shown to reduce harmful fats in the blood and induce meaningful weight loss
Oats	Whole oats are a great source of beta-glucan fiber, in addition to resistant starch Beta-glucan from oats has been linked to increased abundance of healthy gut bacteria, lower LDL cholesterol, better blood sugar control and reduced cancer risk. Furthermore, it has been shown to slow digestion and help control appetite
Almonds	Almonds and almond skin are rich in fiber and prebiotic compounds
Flaxseeds	Flaxseeds are a great source of prebiotics, containing 20-40% soluble fiber and 60-80% insoluble fiber These types of fibers help to promote regular bowel movements, feed beneficial bacteria, and can reduce the amount of dietary fat absorbed in the intestinal tract
Yacon Root	Yacon root is rich in prebiotic fructo-oligosaccharides (FOS) and inulin. The inulin in yacon has been shown to improve gut bacteria balance, reduce constipation, enhance immune response, improve mineral absorption, and regulate blood lipids

HERBAL PREBIOTICS

These herbs are often used as part of a multi-combination supplement or alone. They can be in powder, capsule or tincture form and are intended to help increase the frequency of bowel movements or to reduce inflammation in the gut.

Food	Source of Benefit
Triphala	Often used in Ayurvedic medicine to increase the frequency of bowel movements
Aloe	Can be helpful for those with colitis (use under doctor supervision only); reduces inflammation in the gut and has anti-microbial properties
Licorice Root	Used often as a part of Chinese herbal formulas as a tonifier; also known for its antiviral properties; deglycrrhizinated licorice is recommended
Slippery Elm	Contains mucilaginous compounds that help reduce inflammation in the gut as well as urinary tract; also used for sore throats, coughs, heartburn, GERD, and ulcers
Marshmallow Root	Used for the same indications as slippery elm (above) but is also used for enhancing digestion, skin irritations/wound healing
Chicory Root	This root comes from the dandelion plant family and has been used for more than 100 years as a substitute for coffee. It is very high in inulin, which is a type of fiber that feeds bacteria in the gut
Burdock Root	Burdock is very high in inulin and used to clear toxins from the blood, and to heal skin conditions such as eczema, psoriasis, and acne. Burdock root is also high in antioxidants and phenolic acids
Dandelion	Dandelion Root is used to help the gallbladder secrete bile and can enhance digestion. It is used for liver detox and as a diuretic. It can also be useful for chronic urinary tract infections

CAUTION WITH FODMAPs

Many of the prebiotic foods and high polyphenol foods listed in this guide are high in FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) and could cause digestive discomfort.

Some people that have been diagnosed with IBS (Irritable Bowel Syndrome) and/or SIBO (Small Intestinal Bacterial Overgrowth) can experience increased gas, bloating, abdominal pain, and/or heartburn with the consumption of high FODMAP foods and report feeling better by avoiding them.

Because high FODMAP foods also tend to confer the benefits of being "prebiotic" in nature, as well as being high in antioxidants and phytonutrients, the long-term avoidance of FODMAPs is not recommended for everyone.

If a patient experiences adverse gastrointestinal effects following the consumption of a food high in FODMAPs, consider eliminating them from the diet for a specified period of time, while addressing any issues with digestion/inflammation via the use of gut repair modalities (such as zinc, probiotics, omega-3 fatty acids, enzymes, glutamine, curcumin, avoiding inflammatory foods, and/or treatment of pathogens).

Following this treatment period, one can slowly re-integrate high FODMAP foods and monitor their symptoms.

FODMAP CATEGORIES

Fructans		GOS (galacto-oligosaccharides)	Lactose	Fructose	Polyols
Fruits	***Vegetables***	***Legumes***	Condensed milk	Apples	Apples
Apples	Artichoke	Chickpeas	Custard	Boysenberries	Apricot
Nectarines	Asparagus (more than 3 spears)	Dried beans (all), lentils	Dairy desserts	Cherries	Avocado (more than 1/8)
Persimmon	Beets (more than 4 slices)		Evaporated milk	Figs	Blackberries
Pomegranate	Broccoli (more than ½ cup)		Ice cream	Watermelon	Cherries (more than 3)
Tamarillo	Brussel Sprouts (more than ½ cup)		Margarine	Pears	Lychee (more than 5)
Watermelon	Fennel (more than ½ cup)		Milk	Mangoes	Nectarines
White Peaches	Garlic		Milk powder	Canned fruit	Peaches
Cereals/ grains/ starches	Green peas (more than 1/3 cup)		Soft, unripened cheeses		Pears
Wheat, rye and barley	Golden shallots		Yogurt		Plums
	Leek				Prunes

FODMAP CATEGORIES

Fructans		GOS (galacto-oligosaccharides)	Lactose	Fructose	Polyols
Legumes	Onion			***Vegetables***	Watermelon
Nuts	Spring onion			Asparagus	***Vegetables***
Cashews	Savoy cabbage (more than 1 cup)			Artichokes	Cauliflower
Pistachios	Snow peas (more than 10 pods)			Sugar snap peas	Celery (more than 1 stick)
	Sweet corn (more than ½ cob)			***Sweeteners***	Mushrooms
	Drinks			Fructose	Snow peas
	Dandelion tea			Fruit juice concentrate	Sweet potato (more than ½ cup)
	Chicory-based coffee substitutes			Honey	***Additives***
	Additives			High fructose corn syrup	Isomalt
	Inulin				Maltitol
					Xylitol
					Mannitol
					Sorbitol

PROBIOTICS

Fermented Foods

Fermented foods can have their place in helping to repopulate a microbiome, especially after one or more courses of antibiotics. Fortunately, they are now much easier to find, without having to make them from scratch and do not need large portions to experience benefit.

Start slow to make sure the probiotic is well-tolerated. The following is a list of popular fermented foods that can usually be found at a local grocery or health food store.

Kimchi

Sauerkraut

Kefir (dairy and/or coconut water)

Yogurt (dairy or non-dairy)

Lassi

Natto

Miso

Tempeh

Fermented pickles

Kombucha

PROBIOTICS

Supplements

Probiotics can play a vital role in helping to repopulate and maintain balance of one's microbiome ecosystem. There is no one-size-fits-all approach for recommending probiotic supplements. Practitioners should individualize recommendations based on patients' presenting symptoms and degree of dysbiosis.

The Vibrant Gut Zoomer 3.0 provides individualized probiotic recommendations for each disease state association section listed in the report and displayed in the section titled "Your Level of Probiotic Organisms".

YOUR LEVELS OF PROBIOTIC ORGANISMS			
Lactobacillus reuteri	17.1 ↔	≥10.0	
Lactobacillus rhamnosus	3.8 ↓	≥10.0	
Lactobacillus plantarum	4.9 ↓	≥10.0	
Streptococcus thermophilus	4.7 ↓	≥10.0	
Lactobacillus bulgaricus	24.9 ↔	≥10.0	
Lactobacillus acidophilus	7.1 ↓	≥10.0	
Bifidobacterium longum	23.9 ↔	≥10.0	

Vibrant presents a list of commercially available probiotic organisms that clinical literature has demonstrated to be beneficial for the specific disease-state association listed above.

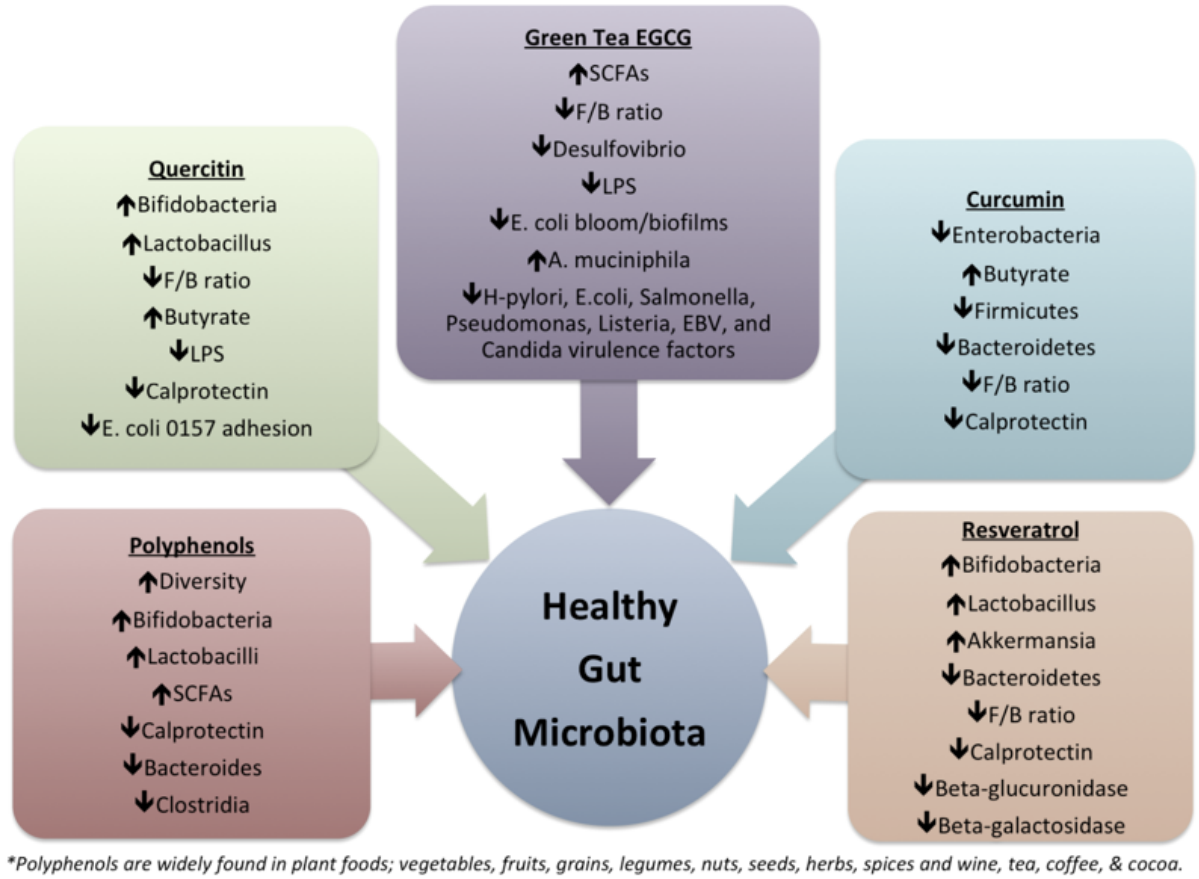
These organisms are also measured on Vibrant Gut Zoomer 3.0 and an individual's abundance score for each organism is given. These recommendations and results can help a practitioner define an individual probiotic recommendation and protocol.

POLYPHENOLS

Polyphenols are a group of chemicals that occur naturally in plants. Currently, there are more than 500 unique polyphenols and collectively, these chemicals are also known as phytochemicals. Polyphenols can be further categorized into the following groups:

- Phenolic acids
- Stilbenes
- Flavonoids
- Lignans

EFFECTS OF POLYPHENOLS ON THE MICROBIOME



**Polyphenols are widely found in plant foods; vegetables, fruits, grains, legumes, nuts, seeds, herbs, spices and wine, tea, coffee, & cocoa.*

POLYPHENOLS BY COLOR: EAT THE RAINBOW

Polyphenols can be found in virtually all plants and are particularly abundant in the pigmented parts of the plant, hence the association with the plant colors.

Color	Vegetable	Fruit	Polyphenol
Red	Cayenne pepper	Apple	Anthocyanins
	Radicchio	Blood Orange	Carotenoids
	Radish	Cherry	Capsaicin
	Red beet	Cranberry	Ellagic acid
	Red bell pepper	Lingonberry	Ellagitannins
	Red cabbage	Nectarine	Fisetin
	Red jalapeno pepper	Pink grapefruit	Flavones
	Red onion	Pomegranate	Lycopene
	Red potato	Raspberry	Phloretin
	Tomato	Red Currant	Quercetin
		Red pear	
		Red plum	
		Strawberry	
		Watermelon	

POLYPHENOLS BY COLOR (CONT.)

Color	Vegetable	Fruit	Polyphenol
Orange	Carrot	Apricot	Alpha-carotene
	Orange bell pepper	Blood orange	Beta-carotene
	Pumpkin	Cantaloupe	Beta-cryptoxanthin
	Sweet potato	Kumquat	Bioflavonoids
	Turmeric root	Mango	Carotenoids
	Yam	Nectarine	Curcuminoids
		Oranges (all)	
		Papaya	
		Passion fruit	
		Peach	
		Persimmon	
		Tangerine	
Yellow	Corn	Yellow apple	Bioflavonoids
	Ginger root	Asian pear	Bromelain
	Yellow potato	Banana	Gingerol
	Yellow squashes (all)	Lemon	Lutein
	Yellow bell pepper	Pineapple	Nobiletin
	Yellow onion	Star fruit	Prebiotic fiber
	Turmeric root		Zeaxanthin

POLYPHENOLS BY COLOR (CONT.)

Color	Vegetable	Fruit	Polyphenol
Green	Artichoke	Avocado	Catechins
	Asparagus	Green apple	Chlorogenic acid
	Bamboo sprout	Lime	Chlorophyll
	Bean sprout	Olive	Epigallocatechin gallate
	Green bell pepper	Green pear	Flavonoids
	Bitter melon		Folate
	Bok choy		Glucosinolates
	Broccoli		Isoflavones
	Broccolini		Isothiocyanates
	Green cabbage		L-theanine
	Celery		Nitrates
	Cucumber		Oleocanthal
	Green bean		Oleuropein
	Green pea		Phytosterols
	Green tea*		Silymarin
	Leafy greens (all)		Sulforaphane
	Okra		Tannins
	Rosemary		Theaflavins
	Snow pea		Tyrosol
	Watercress		Vitexin

POLYPHENOLS BY COLOR (CONT.)

Color	Vegetable	Fruit	Polyphenol
Blue-Purple	Eggplant	Blackberry	Anthocyanidins
	Purple bell pepper	Blueberry	Flavonoids
	Purple cabbage	Boysenberry	Phenolic acids
	Purple carrot	Fig	Proanthocyanidins
	Purple cauliflower	Huckleberry	Pterostilbene
	Purple kale	Plum	Resveratrol
	Purple potato	Prune	Stilbenes
		Purple grape	
		Purple raisin	
White-Tan-Brown	Cauliflower	Banana	Organosulfur compounds
	Ginger root	Citrus pith	Anthoxanthins
	Jicama	White peach	Bioflavonoids
	Mushroom	White pear	Flavonols
	Onion		Gingerol
	Parsnip		Phenolic acids
	Turnip		Sulforaphane
	White potato		Quercetin

DEEP DIVE INTO VIBRANT GUT ZOOMER 3.0

Commensal Bacteria

- The most comprehensive set of normal commensal flora grouped according to disease state associations established from clinical literature
- Two diversity indices
- Phylum distribution wheel

Pathogenic Organisms

- 67 pathogenic gut microorganisms including bacteria, fungi, viruses, parasites, worms
- Antibiotic resistance genes and phenotypes (6)

Inflammatory Markers

- Calprotectin
- Lactoferrin
- Beta defensin 2
- Lysozyme
- S100A12
- MMP 9
- Eosinophil Protein X

Markers of Digestive Insufficiency

- Pancreatic Elastase
- Meat Fiber
- Vegetable Fiber
- Fat Malabsorption
    - Total fecal fat
    - Total fecal triglycerides
    - Long chain fatty acids
    - Total cholesterol
    - Total phospholipids

DEEP DIVE INTO VIBRANT GUT ZOOMER 3.0 (CONT.)

Metabolites

- Bile Acid Metabolites
  - Cholic acid (CA)
  - Chenodeoxycholic acid (CDCA)
  - Deoxycholic acid (DCA)
  - Lithocholic acid (LCA)
  - LCA/DCA ratio
- Short Chain Fatty Acids
  - Acetate
  - Butyrate
  - Propionate
  - Total SCFA
- B-glucuronidase

Other Markers

- SIgA
- Occult blood
- Fecal pH
- Fecal Zonulin
- Fecal Gliadin

MARKERS OF INTESTINAL INFLAMMATION

The innate immune system of the gut plays a role in maintaining a fine balance between commensal bacteria and immune system response to potential pathogens.

It is a very complex system comprised of multiple components including mechanical barriers, antimicrobial peptides, inflammatory proteins, and microbial products.

Dysfunction of any component can lead to the development of intestinal disease.

Mechanical Barriers	Mucous layer
	Intestinal epithelial cell wall and tight junctions
	Intestinal motility
Antimicrobial Peptides	Defensins
	Lactoferrin
	Lysozyme
Inflammatory Proteins	Calprotectin (S100A/S1009)
	S100A12
Microbes and Microbial Products	Intestinal microflora/microbiome
	Short-chain fatty acids (SCFAS)
Others	Gastric acid
	Biliary secretions
	Digestive enzymes
	Immune cells
	SIgA

Adapted from https://www.frontiersin.org/files/Articles/76335/fped-02-00006-HTML-r1/image_m/fped-02-00006-t001.jpg

CONCEPT: ANTI-MICROBIAL PEPTIDE (AMPS)

Anti-microbial peptides (AMPs) are broad spectrum innate antibiotics that are ***produced by intestinal epithelial cells*** as well as circulating inflammatory cells, particularly neutrophils. They play a primary role is maintaining balance and homeostasis in the GI microbial ecosystem (microbiome).

AMPs have antimicrobial activity. They regulate the number and composition of commensal bacteria, while protecting the ecosystem from pathogens. AMPs are thought to be an ancient defense system that play a significant role in our coevolution with a complex microbiome.

INFLAMMATORY MARKERS MEASURED

CALPROTECTIN

Clinical Utility

Calprotectin is a member of the s100 calcium and zinc-binding protein family. It is a complex of S100A8 and A100A9 and is released by neutrophils. Neutrophils migrate to the site of inflammation and release AMPs there. While calprotectin can be used to determine the degree of inflammation, it cannot determine the source or the exact cause of inflammation.

Elevated fecal calprotectin levels have been seen in IBD, colon cancer, and NSAID enteropathy, thus calprotectin is a sensitive but not specific marker of intestinal inflammation. This marker is often used as part of diagnostic workup in IBD, but it is not definitively diagnostic on its own.

Calprotectin can also be used clinically to differentiate IBS from IBD, or to monitor degree of inflammation in someone already diagnosed with IBD.

Intervention

Follow diet, lifestyle, and phytonutrient recommendations to lower inflammation. Always work to determine root causes of inflammation. *Note calprotectin can be elevated with chronic NSAID use, GI infections (bacterial or parasitic), malignancy, or obesity.*

Additional Workup

Repeat calprotectin levels in 4-8 weeks. Assess next to other makers of inflammation on Vibrant Gut Zoomer. Refer to GI specialist for further workup if calprotectin remains persistently elevated.

LACTOFERRIN

Clinical Utility

Lactoferrin is an iron-binding protein and an anti-microbial peptide (AMP) located within neutrophils that is released in the presence of inflammation and/or dysbiosis. It can sequester iron and, thus, deprive pathogenic bacteria of iron.

Lactoferrin acts on both gram-positive and gram-negative bacteria. It is widely accepted that lactoferrin has antiviral, antifungal, and antiparasitic functions. Lactoferrin is elevated in IBD, but is usually negative in IBS. Therefore, can be used to differentiate the two conditions. It can also be used in treatment monitoring for IBD.

Intervention

Elevated levels have been associated with IBD, diverticulitis, or bacterial/parasitic infection leading to mucosal inflammation. Consider anti-inflammatory supplements such as fish oils, leukotriene inhibitors, N-acetyl glucosamine, and balancing gut diversity with probiotics.

Additional Workup

Consider Iron or Anemia Panel. Consider additional workup for IBD, Celiac, dysbiosis, GI infection (Gut Zoomer). Consider WZ to evaluate LPS antibodies. Evaluate with other inflammatory markers as part of IBD workup.

BETA-DEFENSIN 2

Clinical Utility

Beta-defensin 2 is an antimicrobial peptide (AMP) expressed in epithelial cells. It has potent antimicrobial activity against gram-negative bacteria and Candida, but not gram-positive bacteria.

Intervention

Consider dietary, lifestyle and supplement practices to improve diversity (probiotics, prebiotics, polyphenols).

Additional Workup

Evaluate GI diversity and dysbiosis; rule out infection or yeast overgrowth. Consider Vibrant Fungal Antibodies test if Candida is negative in stool, but symptoms persist.

LYSOZYME

Clinical Utility

Lysozyme is an enzyme secreted by granulocytes. It breaks down the cell wall of certain gram-positive bacteria. It is a marker more specific for inflammation in the colon vs. the small bowel.

Moderate elevations in fecal lysozyme are commonly associated with significant overgrowth of enteropathogens such as yeast or dysbiotic bacteria. Markedly elevated levels of fecal lysozyme have been identified in colonic inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis, as well as other non-IBD gastrointestinal diseases with diarrhea, compared to healthy controls.

Intervention

Lysozyme is helpful in the determination of colonic inflammatory activity rather than small bowel disease. For elevated levels, anti-inflammatory diet along with removal of causative agent such as pathogens or allergens is recommended.

Additional Workup

Seek root causes such as yeast overgrowth or bacterial dysbiosis (Gut Zoomer, Organic Acids, Fungal Antibodies). Consider colonoscopy if markedly elevated and patient has significant symptoms. Ensure proper motility/regular bowel movements to decrease colonic inflammation.

S100-A12

Clinical Utility

S100-A12 is in the S100 protein family, similar to calprotectin. It is produced by neutrophils. S100-A12 tends to be upregulated in IBD compared to healthy controls, particularly in children. It can be used as a marker to distinguish IBS from IBD, to monitor progression of IBD treatment or to predict relapse of IBD flare.

Intervention

Follow diet, lifestyle, and phytonutrient recommendations to lower inflammation. Always work to determine root causes of inflammation. Note S100-A12 can be elevated with chronic NSAID use, GI infections (bacterial or parasitic), malignancy, or obesity.

Additional Workup

Repeat levels in 4-8 weeks. Assess next to other markers of inflammation on Vibrant Gut Zoomer. Refer to GI specialist for further workup if S100A12 remains persistently elevated.

MMP-9

Clinical Utility

Matrix-metalloprotease-9 is significantly increased in the stool of UC (ulcerative colitis) patients compared with healthy controls and patients with IBS. This is a repair enzyme and gets upregulated when there is GI damage.

The marker correlates with the clinical and endoscopic activity of UC (correlates with disease-state severity). MMP-9 is a useful tool in the differential diagnosis of diarrheic disorders and in the noninvasive evaluation of disease activity and mucosal healing in UC.

Intervention

Work to determine root cause of inflammation to lower MMP-9.

Additional Workup

Consider supplements such as curcumin, coumarin, resveratrol and ginger root, which are anti-inflammatory. Calcium supplementation has shown benefit in reducing epithelial permeability and inflammation in the intestine through reduced expression of MMP-9 in some studies. Consider Vibrant Mycotoxins testing as there are some associations with elevated MMP9 and mold toxicity.

EPX

Clinical Utility

Eosinophil Protein X (EPX) is a water-soluble protein that is found in eosinophils. EPX levels in stool are a marker of eosinophil activity in the gastrointestinal system.

Fecal EPX abnormality is suggestive of food allergy, or eosinophil-driven inflammation often caused by parasites. The test has been shown to have higher specificity and positive predictive value for detecting disease activity in inflammatory bowel disease compared to fecal calprotectin.

Intervention

Elevated levels associated with inflammation and tissue damage are likely due to parasitic infections, IBD, or food allergies.

Additional Workup

Consider testing for food allergies and treat based on root cause analysis.

POTENTIAL RISK MITIGATION CHOICES TO LOWER INTESTINAL INFLAMMATION

Diet, Functional Foods, Phytonutrients, and Lifestyle

Always address root causes of inflammation:

- Infection: bacterial pathogens, parasites, or yeast overgrowth
- Chronic NSAID use
- Polyps
- Dietary aggravates, food sensitivities, or allergies
- Lifestyle factors
- Evaluate genetic history for Inflammatory bowel disease (IBD) or colorectal cancer

Any anti-inflammatory lifestyle practices are advised to help reduce intestinal inflammation. This includes an anti-inflammatory diet free from processed foods, especially refined sugar, oils, grains, and food additives.

Food sensitivity testing can be used to determine foods that promote intestinal inflammation on an individual basis. Caffeine, alcohol, and smoking in excess can also promote intestinal inflammation.

Many functional foods can be used for anti-inflammatory support including ***omega-3 fatty acids***, ***aloe***, and ***polyphenols*** such as ***resveratrol, epigallocatechin, curcumin, quercetin, and Boswellia.***

Lifestyle practices such as smoking cessation, adequate sleep, proper sleep hygiene, stress management, and proper hydration with filtered water are also very important for controlling inflammation.

Pancreatic Elastase

Pancreatic elastase is an enzyme produced by exocrine tissue in the pancreas. Fecal Pancreatic elastase is a non-invasive marker of exocrine pancreatic function, as it is not broken down in the digestive tract and can be measured in stool when a person has normal pancreatic function. The level in the stool is decreased when the exocrine tissues of the pancreas are not producing enough elastase and other digestive enzymes.

Stool Assessment for Fat Malabsorption

Chronic diarrhea is often the first symptom prompting one to seek medical evaluation, although diarrhea need not be present for one to have malabsorption. Steatorrhea, or fatty stools, is indicative of malabsorption. Stools will be frothy, foul smelling, and a ring of oil may be left on the toilet water.

Considerations with Fat Malabsorption

Dietary fat is normally absorbed in the duodenum and jejunum portions of the small intestine. Before fat can be absorbed, however, it must first be converted to a water-soluble form. Digested dietary fats combine with bile salts and phospholipids (substances present in bile from the liver) to form a packet called a micelle. The micelle is water-soluble and is easily absorbed in the duodenum and jejunum.

In conditions when insufficient bile reaches the intestine, consider liver or biliary function; fats are not absorbed and, again, diarrhea and weight loss may occur. A person is at risk for deficiency of specifically fat-soluble vitamins: A, D, E, and K.

Potential Risk Mitigation Choices to Improve Pancreatic Elastase

- Seek primary causes of exocrine pancreatic insufficiency such as chronic pancreatitis, gallstones, diabetes, celiac disease, inflammatory bowel disease (IBD), excessive alcohol consumption
- With moderate deficiencies, digestion can be improved with lifestyle practices such as slowing down when eating and taking the time to chew thoroughly; small/more frequent meals are advised and adopting a diet proportionally lower in fat calories. Medium chain triglycerides (MCTs) may be better tolerated due to decreased reliance of these fats on bile and lipase enzymes for digestion.
- Supplements such as digestive enzymes, digestive herbs, bile salts, taurine, and betaine HCl may be appropriate. Certain foods such as pineapple and papaya are high in natural digestive enzymes.
- With severe pancreatic insufficiency, pancreatic enzyme replacement therapy (PERT) should be considered.

MARKERS OF MALABSORPTION (CONT.)

Fecal Fats

Fecal fats are a measurement of total fats, fatty acids, phospholipids, and cholesterol in the stool. Elevated levels are usually seen in malabsorptive conditions. Assess fecal fat levels next to pancreatic elastase 1 (PE1) and bile acids.

- If PE1 is low and fecal fats are elevated, consider lipase enzyme or higher dose prescription PERT (pancreatic enzyme replacement therapy).
- If fecal fats are elevated and PE1 is robust, consider bile acid insufficiency or hypochlorhydria.
- Always assess along with patient symptoms. Assess if fat malabsorption is present on Gut Zoomer, consider diet composition (fat intake), and supplements with symptoms.

BILE ACID METABOLITES

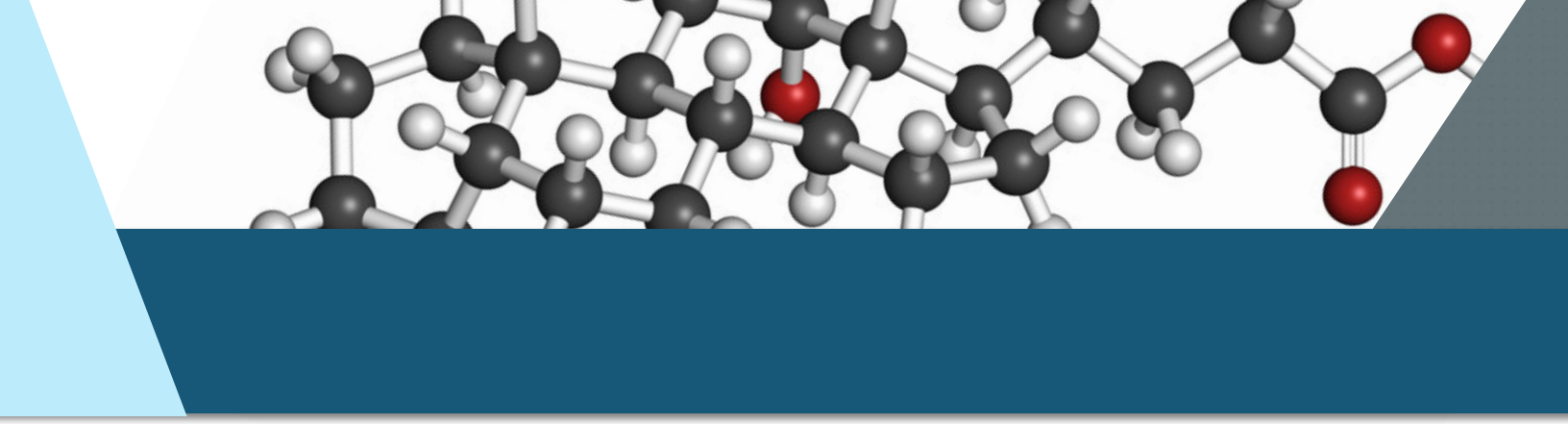
There Are Two Fundamentally Important Functions of Bile in all Species:

- Bile contains bile acids, which are critical for digestion and absorption of fats and fat-soluble vitamins in the small intestine.
- Many waste products, including bilirubin, are eliminated from the body by secretion into bile and elimination in feces.

Bile is synthesized in the liver and stored in the gallbladder where it is ***concentrated*** in the fasted metabolic state. Bile acids are derivatives of cholesterol (either ingested from the diet or synthesized in the liver). Cholesterol is one of the major constituents of bile. There are two important functions of bile acids:

1) **emulsification of lipids:** bile acids have "detergent-like" properties on fat and lipid particles, meaning they help to break down fat globules into tiny droplets, functionally increasing their surface area available to the action of lipases. Without bile acids, dietary fats would not be able to be broken down, digested, or absorbed.

2) **transport of lipids in an aqueous environment:** Bile acids are amphipathic, meaning that they have both a hydrophilic (water loving) and hydrophobic (water hating) side of their molecular structure. This makes them a perfect agent for aiding in transport of dietary fats and fat-soluble vitamins.



BILE ACID METABOLITES (CONT.)

The ***primary*** bile acids are synthesized from cholesterol.

1) Cholic acid (CA)

2) Chenodeoxycholic acid (CDCA)

Bile acids as an assessment of liver health:

Note that liver disease can dramatically influence the process of bile acid recirculation. With liver disease, the hepatocytes have decreased ability to extract bile acids from portal blood and bile acids can escape into circulation (serum).

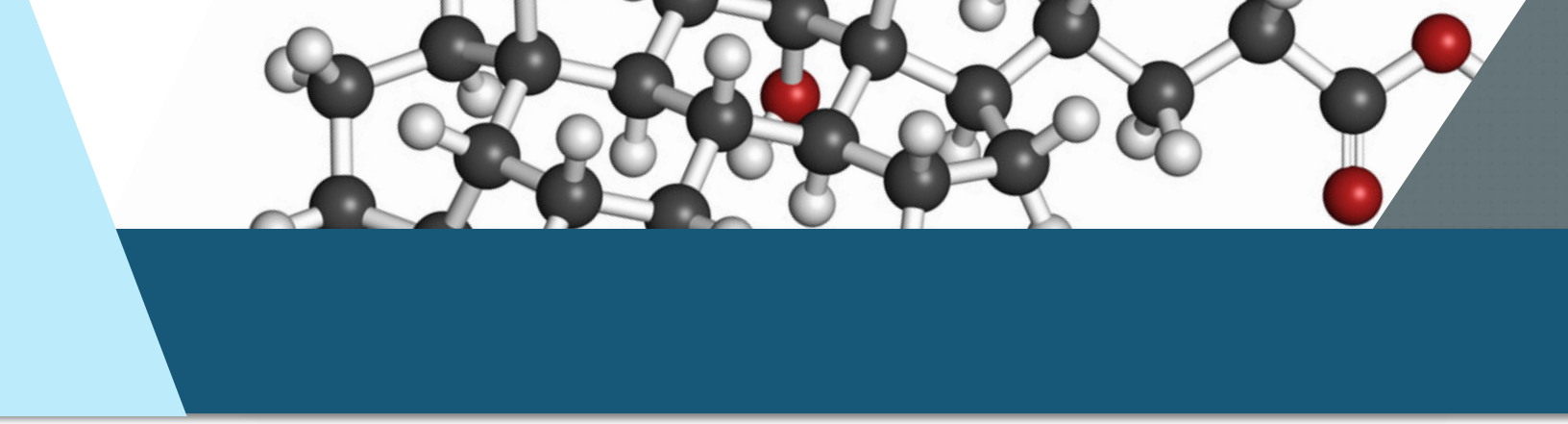
Bile acids in assessment of serum cholesterol and cardiovascular health:

It is important to note that hepatic synthesis of bile acids accounts for the majority of cholesterol breakdown in the body. The ability of bile acids to solubilize cholesterol in bile is the major mechanism of cholesterol elimination from the body to prevent cholesterol accumulation with the attendant risk of atherosclerosis.

Once these primary bile acids enter the colon, they are acted upon by anaerobic bacteria to produce the ***secondary*** **bile acids.** Bacteria genera involved are Clostridium, Enterococcus, Bacteroides, and Lactobacillus.

1) LCA --> Lithocholic acid (LA)

2) CDCA --> Deoxycholic acid (DCA)



BILE ACID METABOLITES (CONT.)

Clinical Considerations Using Fecal Bile Acids

Large amounts of bile acids are secreted into the intestine every day, but normally only small quantities are lost from the body. This is because under normal physiological conditions, the majority of bile acids (~95%) that enter the duodenum are reabsorbed in the ileum of the small intestine and are recycled and reused in the digestion process.

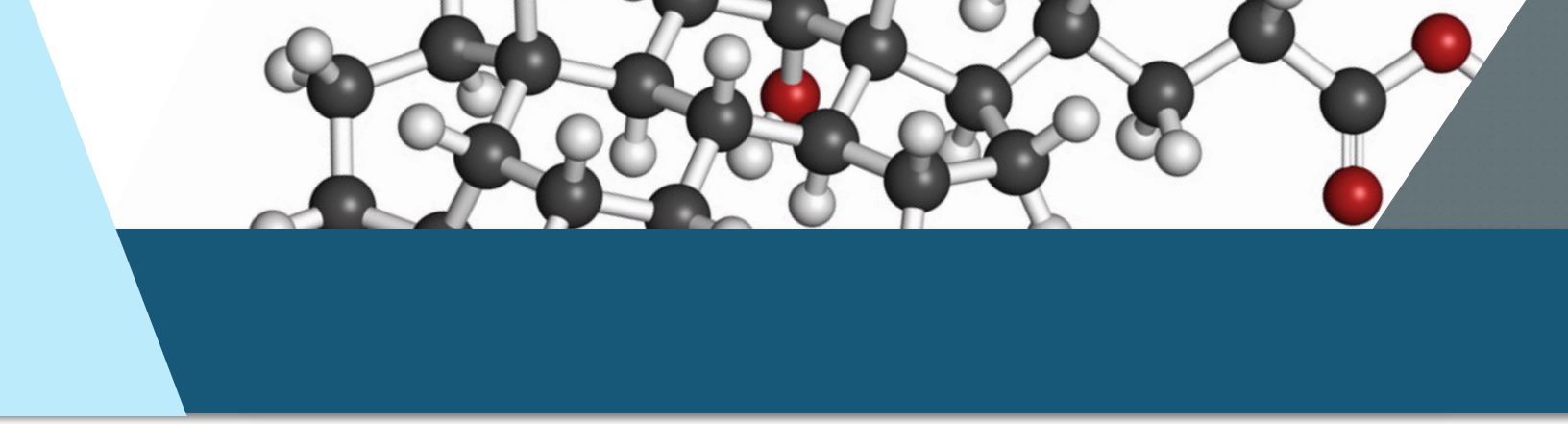
Very few bile acids (~5%) are excreted in stool under normal conditions. Assessing levels of fecal bile acids can yield clinical insight into root cause of digestive symptoms and/or malabsorption disorders.

In a condition referred to as Bile Acid Malabsorption (BAM), excess bile acids build up in the colon. This typically results in symptoms of watery diarrhea and sometimes fecal incontinence.

Quantification of fecal bile acids may be an important screening tool for IBS-D. Studies suggest that up to 1/3 of patients diagnosed with IBS-D may have unidentified BAM.

If fecal bile acids are elevated, this relates to fat malabsorption and, consequently, malabsorption of fat-soluble vitamins. It is recommended to assess serum markers of fat-soluble vitamins and provide nutritional or supplemental recommendations accordingly.

BAM can also be a symptom of other conditions such as Crohn's disease, celiac disease, pancreatic diseases, and small intestinal bacterial overgrowth (SIBO); this is called secondary BAM.



BILE ACID METABOLITES (CONT.)

Clinical Considerations Using Fecal Bile Acids

Secondary bile acids, when elevated in the colon/feces have been shown to have carcinogenic and inflammatory properties.

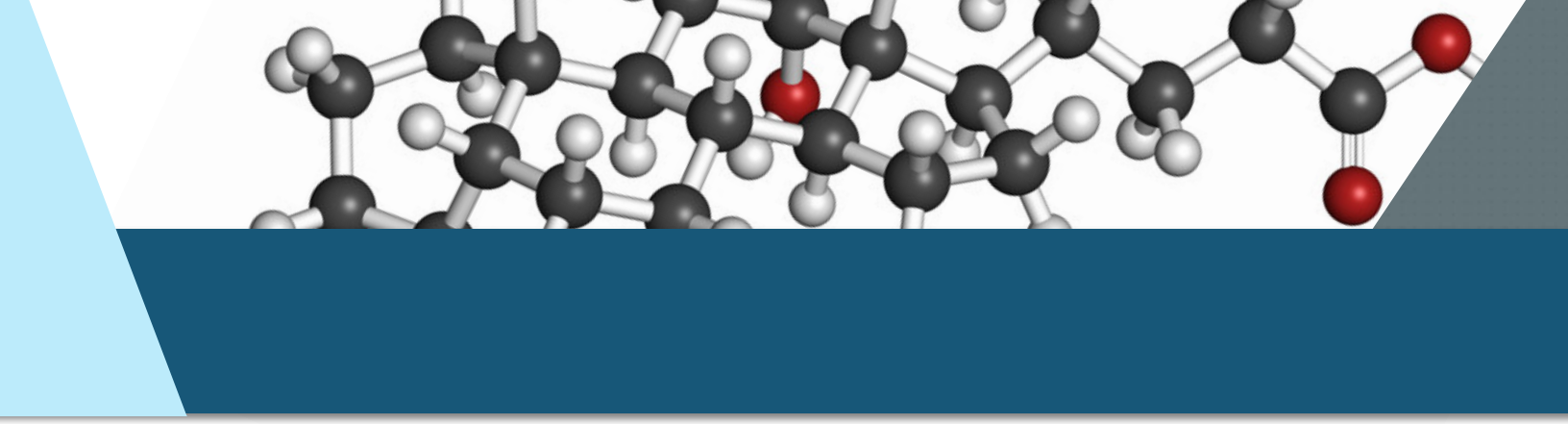
Elevated levels have been associated with:

- increased oxidative stress
- DNA damage
- colorectal cancer
- impaired gallbladder function
- gallstone formation
- colonic inflammation
- increased intestinal permeability

LCA can have inhibitory effects on glutathione-S-transferase in colonocytes.

The LCA:DCA ratio may be an important discriminating marker in colorectal cancer susceptibility. An elevated secondary bile acid ratio is associated with an increased risk of breast and colorectal cancer.

Elevated levels may also occur in patients with gall stones and after cholecystectomy.



BILE ACID METABOLITES (CONT.)

Therapeutic Steps Associated with Fecal Bile Acid Levels

Consider results against functional digestive and stool assessment: analyze Bristol stool index, stool frequency, and indications of fat malabsorption such as oily or frothy stools or next to total fecal fat index.

Correct commensal dysbiosis that may influence the conversion of primary bile acids to secondary bile acids. Consider prebiotic fibers and probiotic supplements/fermented foods accordingly.

Assess fat-soluble vitamin levels. Consider Vibrant's Micronutrient test to determine serum and WBC levels of all fat-soluble vitamins. If low, support with diet and nutraceuticals accordingly.

Assess patient's current macronutrient ratio and consider modifying percent calories from total fat if significant fat malabsorption is present.

Consider medication/supplements such as bile acid sequestrants, betaine HCl, or digestive enzymes/herbs as needed.

Support healthy liver and gallbladder. Consider detoxification practices and functional foods/herbal cholagogues.

SHORT CHAIN FATTY ACIDS

Short-chain fatty acids (SCFAs) are common bacterial metabolites. They are byproducts produced by our gut bacteria when these bacteria ferment insoluble fiber from the diet.

A diet rich in fiber will feed gut bacteria and the gut bacteria produce SCFAs as a byproduct. This is arguably one of the most important features of the symbiotic relationship between humans and the microbiome which inhabits their digestive tract.

SCFAs play several important roles inside the gut lumen

As a preferred fuel source for the cells lining the large intestine, they promote the growth of new cells and repair those cells which have been damaged.

They have a profound anti-inflammatory effect by inducing and selectively expanding T-regulatory cells (T regs) in the large intestine, which in turn, suppress the pro-inflammatory action of Th17.

SCFAs improve and correct (heal and seal) intestinal permeability by stimulating mucus production.

Emerging evidence suggests that they can improve whole body glucose tolerance and insulin sensitivity and may promote satiety.

SHORT CHAIN FATTY ACIDS (CONT.)

The most abundant SCFAs are butyrate, acetate, and propionate which make up 90-95% of SCFAs in the colon.

However, Vibrant's Gut Zoomer 3.0 measures all major short chain fatty acids, including:

- acetate
- butyrate
- propionate
- valerate
- isobutyrate
- isovalerate
- 2-methyl butyrate
- 2,2-dimethylbutyrate
- 2-ethylbutyrate
- 4-methyl valerate
- caproate
- pivalate

Butyrate, acetate, propionate, and valerate are reported as an individual percentage and the total amount of SCFA* in the stool is reported as a direct amount (micromol/gram).

**It is possible to have adequate levels of butyrate, acetate, propionate, and valerate percentages but still have low total SCFAs*

Causes for low SCFA levels:

- Diarrhea (rapid transit leading to decreased SCFA production)
- Constipation (increased SCFA absorption)
- Inflammation (high calprotectin)
- Chronic antibiotic use
- Decreased carbohydrate and fiber consumption
- Severe dysbiosis

SHORT CHAIN FATTY ACIDS (CONT.)

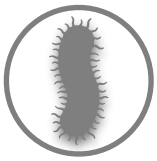
Therapeutic Steps to Increase SCFAs

Dietary composition is critically important. The main bacteria that produce SCFAs are Bacteroides, Ruminococcaceae, Lachnospiraceae, Clostridia, Prevotella, Oscillospira and Verrucomicrobia (Akkermansia muciniphila). Most of these bacteria are anaerobic and cannot be supplemented with probiotics. Remember, your bacteria eat what you eat, thus the goal should be to increase the bacteria that produce SCFAs to indirectly increase SCFAs in the colon. Increasing dietary prebiotic fibers and polyphenols (see previous section) should always be the first consideration if SCFAs are suboptimal.

Increase dietary butyrate and consider butyrate supplementation.

Diet: Butyrate is naturally occurring in high fat dairy such as grass-fed butter or ghee (clarified butter) and raw milk. Butter is about 3-4% butyrate so about 10g (2 Tbsp) of butter daily would equal about 300 mg of butyrate.

Supplemental Butyrate Salts: Most clinical trials, to date, have established a supplemental butyrate dose of 300 mg to be safe and effective at improving digestive function and healing leaky gut symptoms. *Note: It can often take several weeks of using a butyrate supplement to experience symptom improvement*



Some **herbal medicines** have been demonstrated to be able to modulate gut microbiota composition and regulate SCFA production such as berberine, Passiflora edulis, Chinese yam, lotus seed resistant starch, and some species of mushrooms.

ß-GLUCURONIDASE

ß-glucuronidase is an enzyme for which its activity is important for regulating enterohepatic recirculation of compounds, vitamins, and toxins.

Most toxins, hormones, and drugs are excreted from the body after conjugation. ß-glucuronidase can uncouple conjugates, thus "freeing" these compounds, promoting their recirculation and impairing their elimination from the body.

ß-glucuronidase is induced by anaerobic bacteria—its activity is increased by the Clostridia, Ruminococcaceae, Escherichia, and Shigella families of bacteria, which can be over-expressed during dysbiosis.

To date, over 60 bacterial genera and species colonizing the human intestinal tract have been identified that contain genes that encode ß-glucuronidase and ß-galactosidase. The Vibrant Gut Zoomer 3.0 measures all 60 of these species.

Example of ß-glucuronidase action in decoupling bound substances, which releases them back into circulation.

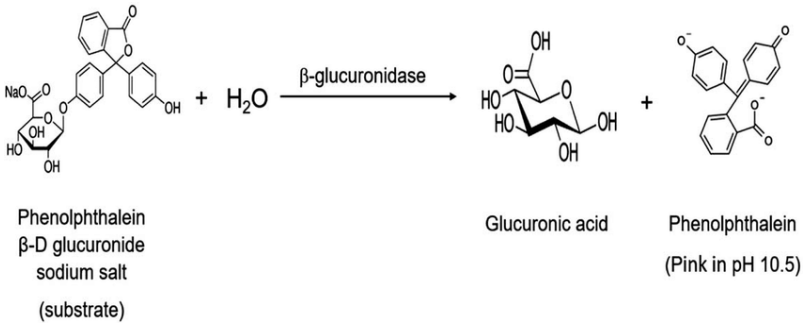


Image from β-Glucuronidase and Its Relationship With Clinical Parameters and Biomarkers of Pesticide Exposure - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Enzymatic-reaction-of-b-glucuronidase-activity_fig1_327913533

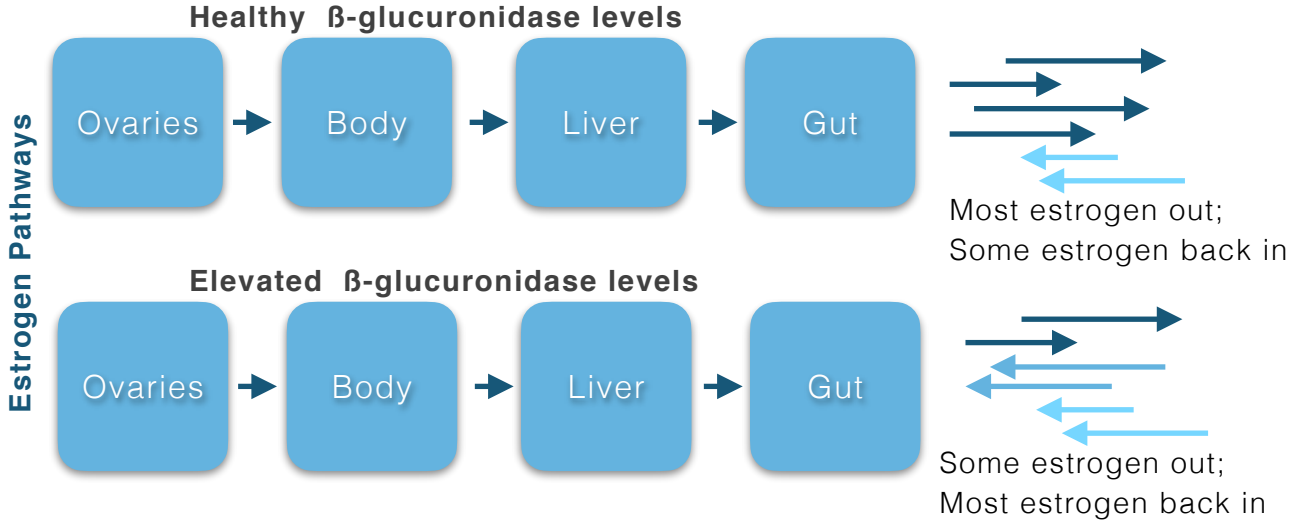
ß-GLUCURONIDASE (CONT.)

Microbiome Estrobolome Axis

The gut microbiome secretes bioactive metabolites: reactivated estrogens, short chain fatty acids, amino acid metabolites, and secondary bile acids that modulate estrogen levels.

Estrogens are primarily produced in the ovaries, adrenal glands, and adipose tissue and circulate in the bloodstream (both in free and protein-bound form) before undergoing metabolism and conjugation in the liver.

Conjugation of estrogens: Conjugated estrogens are eliminated from the body after being converted to water-soluble molecules (during Phase 2 hepatic detoxification), that are then excreted in urine (assuming optimal liver and kidney function) or in bile in the feces (assuming optimal intestinal health).



ß-GLUCURONIDASE (CONT.)

Microbial Deconjugation of Estrogens

The conjugated estrogens excreted in bile can be deconjugated by bacterial species in the gut that have ß-glucuronidase and ß-glucosidase activity, which leads to estrogen reabsorption into circulation (a.k.a enterohepatic circulation of estrogens).

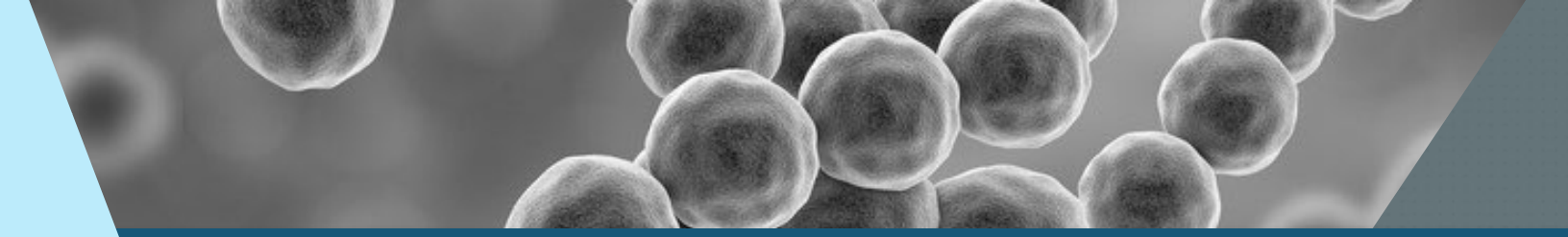
ß-glucuronidase activity is up-regulated by the Clostridia and Ruminococcacea families, which can be over-expressed during dysbiosis, as well as Escherichia and Shigella bacteria.

Over 60 bacterial genera and species colonizing the human intestinal tract have been identified that contain genes that encode ß-glucuronidase and ß-glucosidase.

Modulating enterohepatic circulation of estrogens by optimizing gut health can have a significant effect on hormone health.

Host and Environmental Factors

The composition of the microbiome and the estrobolome can be impacted by host factors such as age, ethnicity, genetics, and adiposity/obesity, as well as lifetime exposure (the ***exposome***) and environmental influences such as diet, alcohol, antibiotics, xenobiotics, and pollutants, all of which exert selective pressure on gut microbiota that modulate estrogen levels.



ß-GLUCURONIDASE (CONT.)

Microbial Deconjugation of Estrogens

Bidirectional relationship

Estrogen-like compounds may negatively impact proliferation of certain species of bacteria. Thus, the gut microbiome-estrobolome relationship is a bi-directional relationship where crosstalk between microbiota and endogenous hormones may provide protection from disease or alternatively, increase disease risk.

Intestinal permeability

Intestinal epithelial cells possess the ability to metabolize sex steroids, notably estrogen. Estrogen and estrogen receptor signaling play a role in maintaining intestinal epithelial barrier function, tight junction expression and permeability, and gastrointestinal motility.

Inflammation and oxidative stress

Estrogen-like metabolites can be also produced by oxidative and reductive reactions in the gut and induce synthesis of estrogen-inducible growth factors, which might have carcinogenic potential. Decreasing intestinal inflammation and oxidative stress is critical to gut microbiome and estrobolome health.

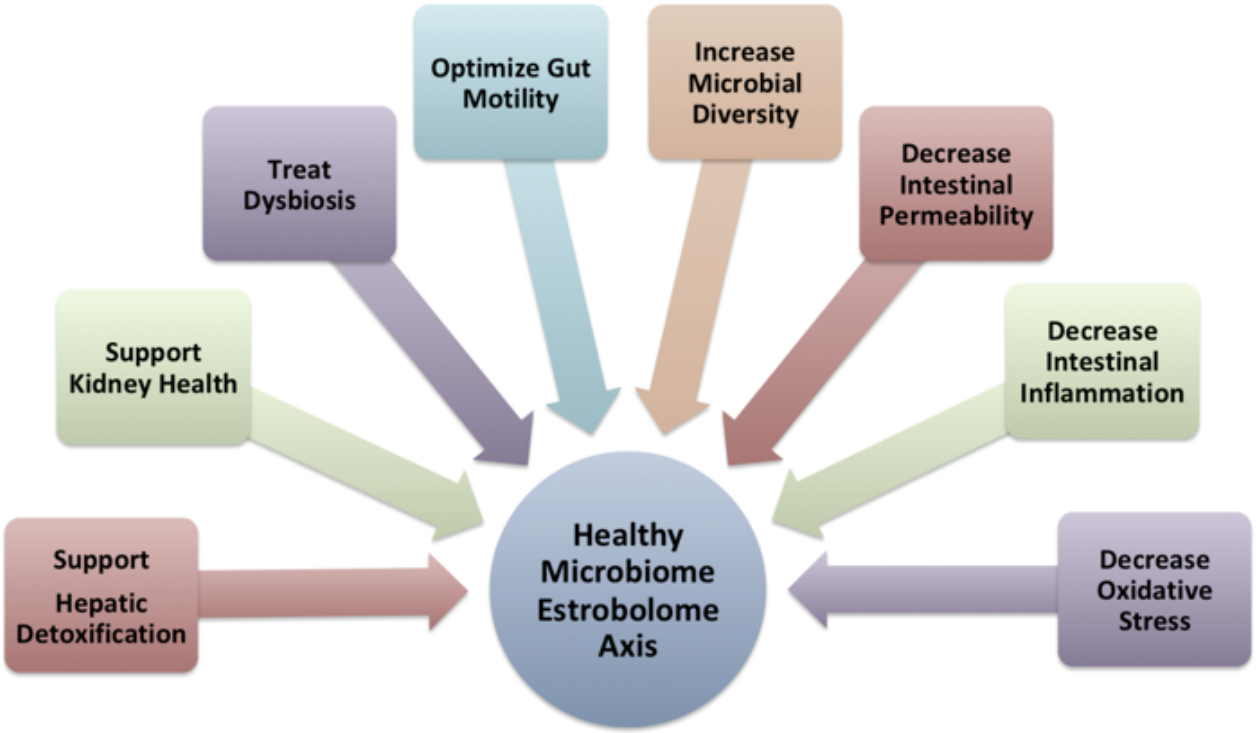
Microbial diversity

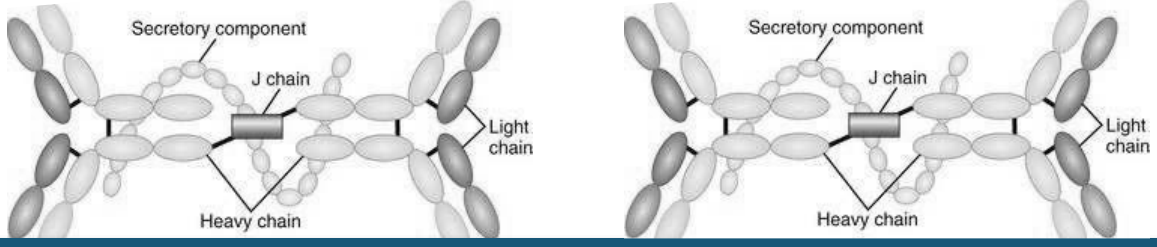
A relationship has also been found between gut microbial richness, as measured by the Shannon Index, systemic and fecal estrogens, and ß-glucuronidase activity in healthy men and postmenopausal women.

ß-GLUCURONIDASE (CONT.)

Factors that Affect ß-Glucuronidase

In summary, there are many influences on the ß-glucuronidase activity of the microbiome, and there will likely need to be a systems approach to optimizing levels of this important bacterial metabolite.





SIgA (Secretory IgA)

Secretory IgA is the primary antibody that protects us from pathogens and from toxins penetrating mucosal surfaces. The mucus layer that lines the intestinal wall is rich in SIgA, and there SIgA plays a crucial role in protecting the integrity of the intestinal epithelium.

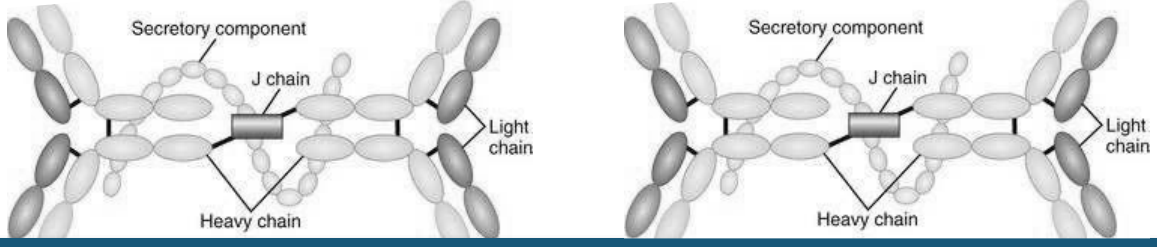
The antibody blocks access to the epithelial receptors and traps pathogens and toxins in the mucus, which are then excreted by peristaltic movements.

Multiple cytokines, including IL-4, TGF-β, IL-5, IL-6, IL-10 are instrumental in stimulating intestinal SIgA production. A subset of these cytokines, notably TGF-β and IL-10, are also required for maintaining mucosal tolerance, thus establishing one of the many links between SIgA production, immunity, and intestinal homeostasis.

Too Much of a Good Thing?

Like all aspects of the gut microbiome and immunity, balance is critical. Marked elevations in SIgA are indicative of immune upregulation in the gut. Causes could be due to food sensitivities, intestinal permeability, inflammation, or infections.

Consider Vibrant Food Zoomers or other food sensitivity tests to determine reactive foods and modify diet accordingly. Follow anti-inflammatory evaluation and practices.



SIgA (Secretory IgA)

How to Increase SIgA levels

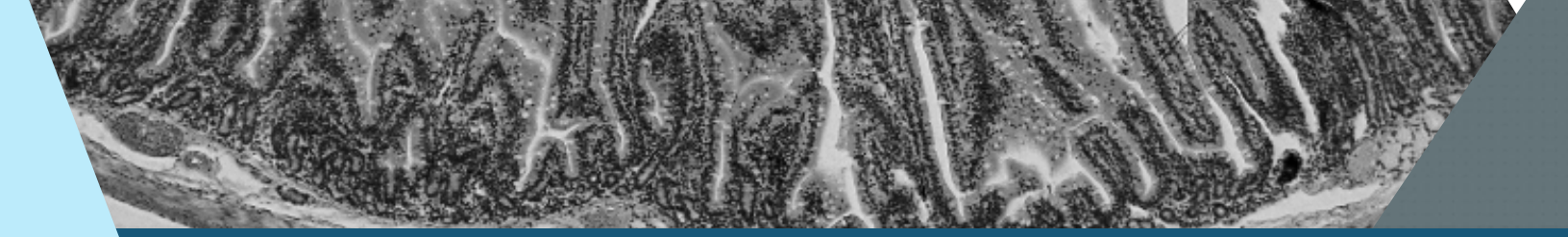
- **Address the underlying cause of low SIgA.**
  - Low SIgA is often seen with chronic stress, adrenal fatigue, chronic use of corticosteroids, chronic viral, bacterial, or parasitic infection.
- Increase bacterial diversity
- Assess status of fat-soluble vitamins A, D, E, K, and supplement as needed
- Evaluate short-chain fatty acids and increase through supplementation or increased fiber diversity
- Glutathione support (such as precursors, liposomal, s-acetyl glutathione, IV, etc.)
- Adrenal adaptogens and phosphatidylserine supplementation may be therapeutic to raise SIgA levels

How to Decrease SIgA Levels

- Assess food sensitivities and modify diet accordingly
- Assess inflammation levels and follow anti-inflammatory practices

SIgA vs. IgA

IgA and SIgA are not entirely the same. The main reason there is confusion is that the terms SIgA and IgA are often used interchangeably in the literature. There is a direct connection between the two biomarkers in that SIgA is produced from IgA, but low/high levels of serum IgA cannot necessarily accurately predict or determine SIgA levels.



Other Markers

Occult Blood

Fecal occult blood indicates blood loss from anywhere along the GI tract (occult = hidden).

Detected occult blood is not diagnostic for colon cancer but is often used in clinical screening. This marker is also used in early diagnosis/workup for anemia or other gastrointestinal disorders (celiac, IBD, etc.)

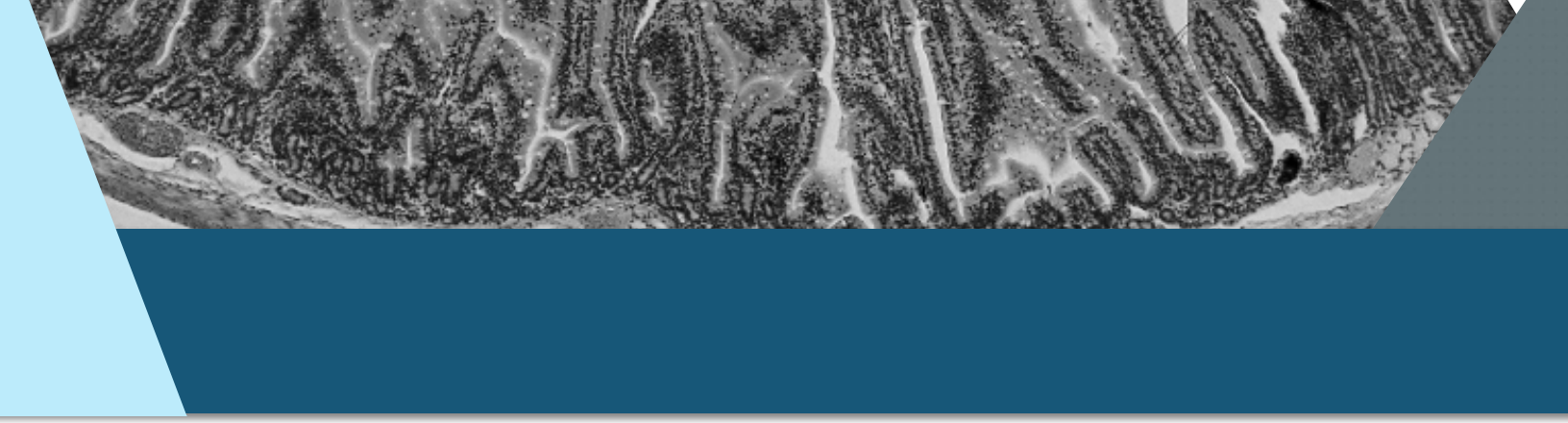
Fecal pH

Fecal pH tests for acidity in human feces. Healthy human stool has an average pH of 6.6.

- An acidic stool is suggestive of a digestive problem such as lactose intolerance, a pathogen such as E. coli or rotavirus, or overgrowth of the acid producing bacteria (such as lactic acid bacteria).
- A high alkaline pH rating is associated with the body's inability to create enough acid along with undigested food

Factors Which Influence Fecal pH

- Bacterial dysbiosis
- PPIs (cause dysbiosis which alters pH); hypochlorhydria
- H. pylori
- Maldigestion/malabsorption
- SIBO (small intestinal bacterial overgrowth)
- Transit time (rapid transit lowers pH; slow transit raises pH)
- Osmotic laxatives
- Excess dietary fat or protein / insufficient dietary plant-based fibers



Other Markers

Fecal Zonulin

Zonulin is a protein that regulates the reversible permeability of intestinal tight junctions. Toxins, dietary proteins (I.e. gliadin), or inflammatory signaling cytokines can activate a zonulin signaling cascade, which results in the ***disassembly of paracellular tight junctions*** in the epithelial cell wall, resulting in increased intestinal permeability in both the duodenum and ileum.

Fecal zonulin measurement may be advantageous, compared to serum zonulin, when assessing intestinal permeability, as serum zonulin may constitute secretion not only from intestinal cells, but also from extraintestinal tissues such as the ***liver, heart and brain.*** Stool may therefore present a more appropriate specimen for analyzing only intestinal production of zonulin. Serum zonulin and zonulin antibodies are measured on the Vibrant Wellness Wheat Zoomer.

Fecal Gliadin

Gliadin is a protein unique to wheat gluten that is a known autoimmune trigger in celiac disease and has a direct interaction with intestinal zonulin to drive the zonulin signaling cascade and, thus, permeability. The fecal anti-gliadin antibody tests for immune system reaction, IgA and IgG, to gluten in the diet. It enables ***direct and quantitative assessment of gluten exposure*** early after ingestion and could aid in the diagnosis and clinical management of non-responsive CD and refractory CD.

Fecal anti-gliadin is a less sensitive marker of wheat sensitivity in comparison to serum antibodies to peptide fragments of wheat (measured on Vibrant Wellness Wheat Zoomer). Individuals with elevated fecal anti-gliadin should consider a gluten/wheat-free diet. Elevated fecal anti-gliadin for someone that is already gluten free likely indicates accidental exposure.

FAQS FOR GZ 3.0

Can this test tell me If I have SIBO?

No. A stool test is never determinant for SIBO, as stool testing evaluates large bowel microorganism ecosystem and SIBO is a manifestation in the small bowel (SIBO = Small Intestinal Bacterial Overgrowth). However, there is a correlation with an overabundance of certain bacteria that are associated with SIBO. The Gut Zoomer 3.0 has a specific section for these bacteria which includes both hydrogen and methane producing bacteria (below).

If SIBO is suspected, the provider should complete an extensive intake and review of symptoms and consider running Vibrant's IBSSure, which includes anti-CdTB and anti-vinculin antibodies that have a strong positive correlation with positive SIBO diagnosis.

Hydrogen Producing

- Streptococcus: primary colonizers of the human oral cavity
- Escherichia coli: primary colonizers of the lower intestine
- Staphylococcus species: primary colonizers of the upper respiratory tract and on the skin
- Micrococcus: primary colonizers of the oral cavities, mucous membranes, and skin
- Bacteroides: most abundant phylum of the GI tract
- Clostridium: primary colonizers of the intestinal tract, playing a crucial role in gut homeostasis by interacting with the other resident microbe populations
- Peptostreptococcus: normal inhabitant of the healthy female reproductive tract, normal flora of the mouth, upper respiratory tract, intestinal tract and skin
- Enterococcus species: primary colonizers of the gastrointestinal tract (formerly considered part of the Streptococcus system)

Methane producing

Methanobrevibacter smithii: methane oxidizing and producing

FAQS FOR GZ 3.0

What should I do if my calprotectin is significantly elevated?

Calprotectin is a marker of inflammation in the gut. Although it is not diagnostic of inflammatory bowel disease, calprotectin can indicate the possibility of Crohn's disease, chronic ulcerative colitis, and/or the overuse of NSAID medication. If your calprotectin has been found to be elevated on the GZ 3.0 test, you may want to schedule a visit with a gastroenterologist.

What should I do if my pancreatic elastase is low?

Pancreatic elastase is low when the tissues of the exocrine pancreas are not producing enough pancreatic elastase and digestive enzymes. This condition is called exocrine pancreatic insufficiency and can occur when the pancreatic ducts are blocked or the pancreatic enzyme producing cells are blocked. It is more often seen in conditions such as pancreatitis and sometimes pancreatic cancer. It is recommended you consider taking a pancreatic enzyme supplement with your regular meals, as well as scheduling a visit with your primary care provider or gastroenterologist.

**Note: if you did the GZ 3.0 test with a sample of loose stools/diarrhea, your pancreatic elastase value may be reduced.*

Should I stop probiotic supplements before taking a Vibrant Gut Zoomer sample?

The Vibrant Gut Zoomer measures microorganism 16sRNA from a person's stool sample. The results reflect the relative abundance of these microorganisms (bacteria, candida yeast, parasites, worms) compared to a reference range at the time of the sample. Thus, if a person is using a probiotic supplement the days/weeks leading up to the sample collection, their sample will reflect the ecosystem as influenced by the probiotics.

If you plan to have the patient remove or discontinue the probiotic prior to testing, it is recommend to discontinue for ~2 weeks before sample collection. The sample will then represent a person's "baseline" microbiome ecosystem and a practitioner can individually recommend probiotic supplementation from baseline.

An alternative strategy would be to run a Gut Zoomer about one month into a probiotic supplement to determine if that particular product is affective for that individual.

FAQS FOR GZ 3.0

Will this test tell me what probiotic supplement I should use?

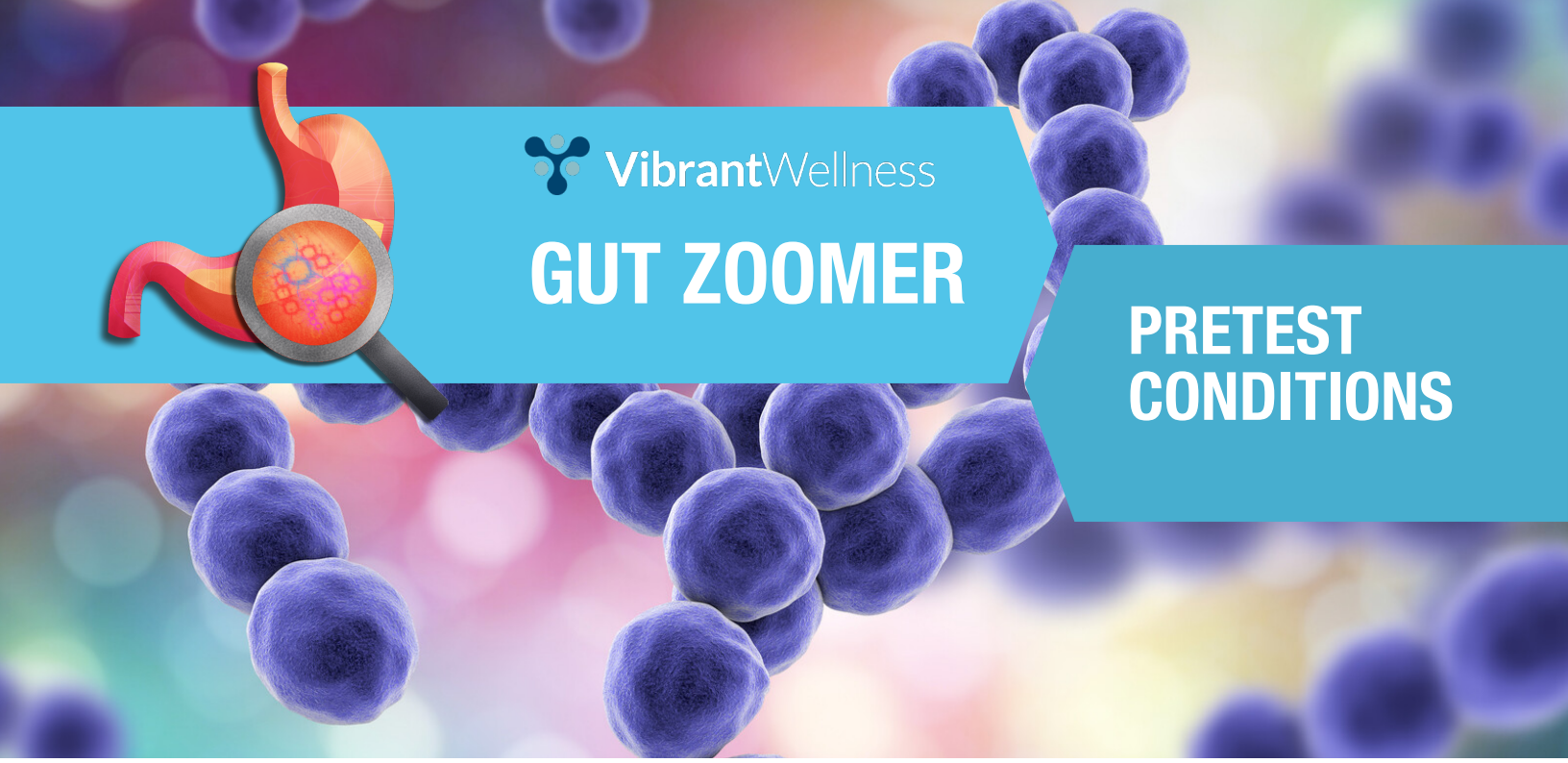
Probiotics can play a vital role in helping to re-populate and maintain balance of one's microbiome ecosystem. There is no one-size-fits-all approach for recommending probiotic supplements. Practitioners should individualize recommendations based on patients' presenting symptoms and degree of dysbiosis.

The Vibrant Gut Zoomer 3.0 provides individualized probiotics recommendations for each disease state association section listed in the report, displayed in the section titled ***"your level of probiotic organisms".***

Vibrant presents a list of commercially available probiotic organisms that clinical literature has demonstrated to be beneficial for the specific disease-state association listed above. These organisms are also measured on Vibrant Gut Zoomer 3.0 and an individual's abundance score for each organism is given. These recommendations and results can help a practitioner define an individual probiotic recommendation and protocol.

How are the reference ranges and abundance scores for commensal microorganisms established?

Reference ranges have been established using a sample cohort of 192 relatively healthy stool samples. The cut-off for the healthy reference range is set between 2.5% and 97.5%, and the high-risk range is set to greater than 97.5%. The healthy reference range for each bacteria is individually determined and available in the Gut Zoomer Commensal Validation report



VibrantWellness

GUT ZOOMER

PRETEST CONDITIONS

Potential Side Effects on Stool Sample

Diet	We recommend completing the stool test while following the diet that is most normal for you. Realize that intake of dietary fibers, probiotic foods, and foods treated with antibiotics, as well as your dietary macronutrient ratio, can profoundly influence the microbiota and digestive health.
Colonoscopy Procedure	Can influence functional markers due to tissue contamination; wait to provide sample until 14 days post colonoscopy.
Anti-inflammatory (Aspirin)	Could influence calprotectin; wait to provide sample until 2 days after use.
Steroids (prednisone, etc.)	Could influence calprotectin; not recommended to discontinue use.
Autoimmune medications (biologics, immune-suppressants, etc.)	Could influence calprotectin; not recommended to discontinue use.
Antacids: proton pump inhibitors	Can cause false negatives for H. Pylori; acid blocking medications may influence levels of digestion/absorption markers. Wait 5 -14 days after last use (14 days if checking for H. Pylori).
Antacids: Tums and H2 Blockers	May influence the level of digestion and absorption markers. Wait 2-3 days after last use.

Potential Side Effects on Stool Sample

Probiotics	The Vibrant Gut ZoomerTM measures microorganism 16sRNA from a person's stool sample. The results reflect the relative abundance of these microorganisms (bacteria, candida yeast, parasites) compared to a reference range at the time of the sample. Thus, if a person is using a probiotic supplement in the days/weeks leading up to the sample collection, their sample will reflect the ecosystem as influenced by the probiotics. Our practitioners usually like to use the Gut Zoomer™ in one of two ways: 1. They will either have the patient remove or discontinue the probiotic for ~2 weeks before sample collection. The sample will then represent a person's "baseline" microbiome ecosystem and a practitioner can individually recommend probiotic supplementation from baseline. 2. The second strategy would be to run a Gut Zoomer™ about one month into probiotic supplementation to determine if that particular product is effective for that individual. If you choose to do a probiotic wash out for a baseline assessment; discontinue use for ~30 days.
Bentonite Clay	Should not have an effect on microbial DNA measurement. Wait 2-3 days after last use.
Betaine HCL	May influence markers of digestion and absorption. Wait 2-3 days after last use.
Digestive Enzymes	May influence markers of digestion and absorption. Wait 2-3 days after last use.
Laxatives	If they have a dramatic effect on transit time, may influence markers of digestion and absorption.
Activated Charcoal	Should not have an effect on microorganism DNA measurement.
Antimicrobials (antibiotics; anti-fungals; anti-parasitics; anti-helminthics)	May have significant influences on all microorganisms (bacteria, yeast, parasites, and worms). Wait 14-30 days after last use.
Rectal Suppositories	Can alter stool sample density and result in inaccurate biomarker findings. Wait 2-3 days after last use.

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