



Stool Testing Support Guide

GI *fx* GI Effects
Stool Profiles®

CDSA^{2.0}

GENOVA
DIAGNOSTICS®

Genova Diagnostics Stool Support Guide

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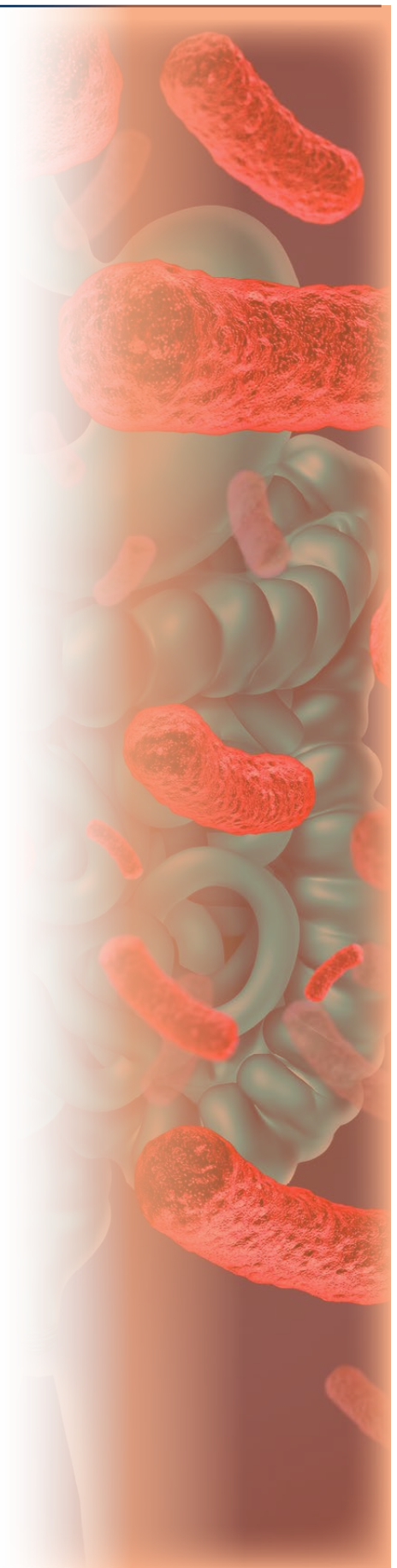
INTRODUCTION

Advances in research, combined with clinical insight, confirm the essential role of the gut in determining overall health and wellness. Genova's stool profiles offer a comprehensive evaluation of GI function paired with the broadest clinical utility available. It is important that clinicians possess these tools since they provide the most accurate and comprehensive assessment of gastrointestinal health.

Genova's line of stool testing provides immediate actionable clinical information for gastrointestinal health management. Utilizing both advanced technologies and premier biomarkers, **the GI Effects Stool Profiles** and **CDSA/CDSA 2.0 Profiles** offer valuable insight into digestive function, intestinal inflammation, as well as the gastrointestinal microbiota. Our tests are designed to identify potential root causes of symptoms. They assist clinicians by providing targeted therapeutics that improve symptoms and overall gut health.

In addition to providing a comprehensive set of GI functional biomarkers, our stool profiles incorporate the most sophisticated tools in evaluating the microbial community of the GI tract, known as the microbiota. Genova uses multiple methodologies to provide the most clinically accurate assessment of bacteria, yeast, and parasites currently available on the market. The GI Effects Profiles include quantitative assessment of commensal bacteria to determine healthy bacterial balance based on research and analysis of hundreds of thousands of patient results. This data-driven, evidence-based analysis establishes a firm foundation from which to base clinical decisions and treatment.

Lastly, the GI Effects utilizes an innovative scoring system that synthesizes the biomarker findings and groups them into 5 key areas relating to GI function: **maldigestion, inflammation, dysbiosis, metabolite imbalance, and infection**. This allows for clearer visualization of patterns among biomarkers. Protocol design and management of abnormal GI function through dietary, lifestyle, nutraceutical, and other relevant interventions are thus enhanced.



GI Effects® Biomarkers Comparison Table	2200*	2205*	2207*
*Not Available in New York			
Digestion and Absorption			
Pancreatic Elastase-1	.		
Products of Protein Breakdown (Total) (Valerate+Isobutyrate+Isovalerate)	.		
Fecal Fat (Total)	.		
Triglycerides	.		
Long Chain Fatty Acids	.		
Cholesterol	.		
Phospholipids	.		
Inflammation and Immunology			
Calprotectin	.		
Eosinophil Protein X (EPX)	.		
Fecal sIgA	.		
Metabolic			
SCFA (Total) (Acetate, n-Butyrate, Propionate)	.		
n-Butyrate Concentration	.		
n-Butyrate %	.		
Acetate %	.		
Propionate %	.		
Beta- glucuronidase	.		
Gastrointestinal Microbiome			
Commensal Bacteria (PCR)			
Bacteroides-Prevotella group			
<i>Bacteroides vulgatus</i>	.	.	
<i>Barnesiella</i> spp.	.	.	
<i>Odoribacter</i> spp.	.	.	
<i>Prevotella</i> spp.	.	.	
Firmicutes Phylum			
<i>Anaerotruncus colihominis</i>	.	.	
<i>Butyrivibrio crossotus</i>	.	.	
<i>Clostridium</i> spp.	.	.	
<i>Coprococcus eutactus</i>	.	.	
<i>Faecalibacterium prausnitzii</i>	.	.	
<i>Lactobacillus</i> spp.	.	.	
<i>Pseudoflavonifractor</i> spp.	.	.	
<i>Roseburia</i> spp.	.	.	
<i>Ruminococcus</i> spp.	.	.	
<i>Veillonella</i> spp.	.	.	
Actinobacteria Phylum			
<i>Bifidobacterium</i> spp.	.	.	
<i>Bifidobacterium longum</i>	.	.	
<i>Collinsella aerofaciens</i>	.	.	
Proteobacteria Phylum			
<i>Desulfovibrio piger</i>	.	.	
<i>Escherichia coli</i>	.	.	
<i>Oxalobacter formigenes</i>	.	.	
Euryarchaeota Phylum			
<i>Methanobrevibacter smithii</i>	.	.	
Fusobacteria Phylum			
<i>Fusobacterium</i> spp.	.	.	
Verrucomicrobia Phylum			
<i>Akkermansia muciniphila</i>	.	.	
Firmicutes/Bacteroidetes (F/B Ratio)	.	.	
Bacteriology	.	.	.
Mycology (Yeast/Fungi)	.	.	.
Parasitology			
Microscopic Exam Results	.	.	.
Parasitology PCR Tests	.	.	.
Other Biomarkers			
Fecal Occult Blood	.	.	
Color	.	.	
Consistency	.	.	
Mic Sensitivities, Yeast or Bacteria	.	.	.
+ Add-ons			
2203 <i>Clostridium difficile</i> EIA	+	+	+
2204 Shiga toxin <i>E. coli</i> EIA	+	+	+
2202 <i>Campylobacter</i> spp. EIA	+	+	+
2206 Fecal Lactoferrin	+	+	
2208 <i>Helicobacter pylori</i> EIA	+	+	+
2331 Macro Exam for Worms	+	+	.
2336 Zonulin Family Peptide	+	+	
2338 KOH Preparation for Yeast	+	+	.

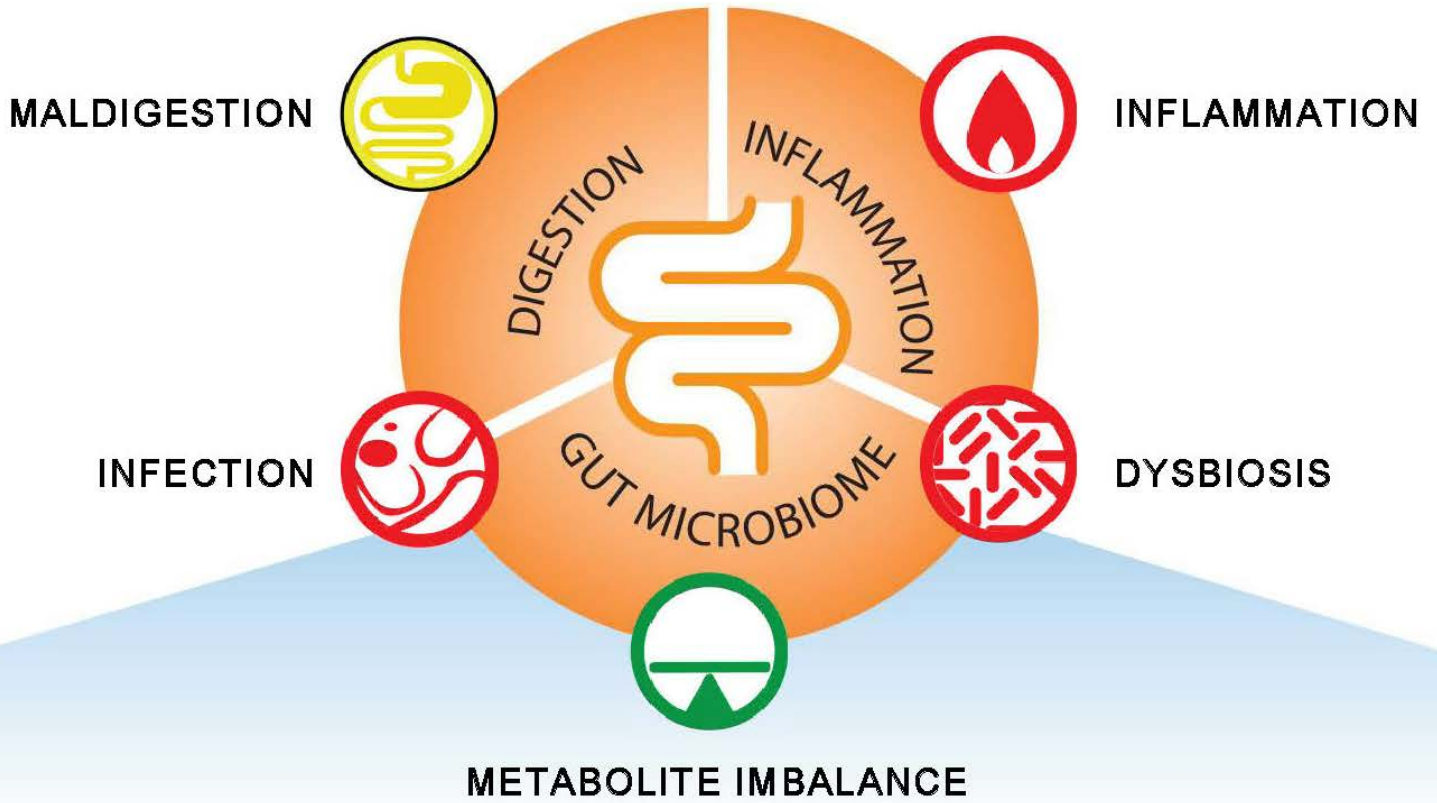
CDSA Biomarkers Comparison Table	CDSA	CDSA/P	CDSA 2.0/P	CDSA 2.0 w/o P
*Not Available in New York				
Digestion and Absorption				
Pancreatic Elastase-1	+	+	•	•
Putrefactive SCFAs	•	•	•	•
Chymotrypsin	•	•	+	+
Fecal Fats	•	•	+	+
Meat Fibers	•	•		
Vegetable Fibers	•	•		
Gut Immunology				
Fecal Lactoferrin	•	•		
Calprotectin	+	+	•	•
Eosinophil Protein X	+	+	•	•
Zonulin Family Peptide			+	
Metabolic Markers				
Beneficial SCFAs	•	•	•	•
SCFA Distribution	•	•	+	+
Beta-glucuronidase	•	•	•	•
pH	•	•	•	•
n-Butyrate	•	•	•	•
Lithocholic acid (LCA)	+	+	•	•
Deoxycholic Acid (DCA)	+	+	•	•
LCA/DCA Ratio	+	+	•	•
Microbiology Markers				
Bacteriology	•	•	•	•
Mycology	•	•	•	•
<i>Campylobacter</i> EIA	+	+	+	+
Shiga-like Toxin <i>E.coli</i> EIA	+	+	+	+
<i>Clostridium difficile</i> EIA	+	+	+	+
HpSA- <i>H. pylori</i> EIA	+	+	+	+
Parasitology				
Microscopic O&P		•	•	
EIA		•	•	
Macroscopic Exam for Worms			+	+

PHOSPHOLIPIDS
 LONG CHAIN
 CHOLESTEROL
 FATTY ACIDS (LCFAs)
 TRIGLYCERIDES

GI EFFECTS RESULTS OVERVIEW

The GI Effects Stool Profile report is organized to provide a quick overview and synthesis of results at the beginning of the test. The results overview graphic reflects the status of the 3 key functions of gut health arranged in the “DIG” format: digestion, inflammation, and the gut microbiome. The gut microbiome section is further broken down into three components: infection, metabolite imbalance, and dysbiosis. These individual gut microbiome sections allow

the practitioner to differentiate between interventions that are antimicrobial versus supportive of the microbiome. The colorimetric circles reflect the need for support in each area and help the practitioner prioritize therapeutic strategies. Green represents low need for support, gray (optional), yellow (moderate), and red (high need).








Functional Imbalance Scores

The functional imbalance scores are generated using weighted algorithms that incorporate biomarkers belonging to each functional category. The biomarkers that are represented in the algorithm are listed below the score in each functional column. A qualitative indicator of whether the biomarker is normal (green circle ●) or abnormal (yellow ▲ or red arrow ▲) is located adjacent

to the biomarker name. The level of need for support in a functional area is reflected both by the color and score in the circle. Green represents a low need for support and corresponds with scores less than 2, grey represents an optional need for support and corresponds with a score of 2 or 3, yellow indicates moderate need with scores of 4-6, and red indicates high need with scores of 7-10.

Functional Imbalance Scores

Key  : Low Need for Support  : Optional Need for Support  : Moderate Need for Support  : High Need for Support

	Need for Digestive Support	Need for Inflammation Modulation	Need for Microbiome Support	Need for Prebiotic Support	Need for Antimicrobial Support
	MALDIGESTION	INFLAMMATION	DYSBIOSIS	METABOLIC IMBALANCE	INFECTION
					
Biomarkers	Pancreatic Elastase ▼ Products of Protein Breakdown ● Fecal Fats ●	Calprotectin ▲ Eosinophil Protein X ▲ Secretory IgA ● Occult Blood ●	IAD/Methane Score ▲ PP Bacteria/Yeast ▲ Reference Variance ▲ Total Abundance ▲	Total SCFA's ● n-Butyrate Conc. ● SCFA (%) ● Beta-glucuronidase ●	Parasitic Infection ▲ PP Bacteria/Yeast ▲ Total Abundance ▲ Pathogenic Bacteria ●

Therapeutic Support Options

Therapeutic support options are listed at the bottom of each column. Therapeutic support options are static on every report to serve as potential treatment ideas. Clinician discretion is advised when selecting appropriate

therapeutics for individual patients. More information on therapeutic support options are discussed throughout this guide as they relate to each biomarker.

Therapeutic Support Options	<ul style="list-style-type: none"> • Digestive Enzymes • Betaine HCl • Bile Salts • Apple Cider Vinegar • Mindful Eating Habits • Digestive Bitters 	<ul style="list-style-type: none"> • Elimination Diet/ Food Sensitivity Testing • Mucosa Support: Slippery Elm, Althea, Aloe, DGL, etc. • Zinc Carnosine • L-Glutamine • Quercetin • Turmeric • Omega-3's • GI Referral (If Calpro is Elevated) 	<ul style="list-style-type: none"> • Pre-/Probiotics • Increase Dietary Fiber Intake • Consider SIBO Testing • Increase Resistant Starches • Increase Fermented Foods • Meal Timing 	<ul style="list-style-type: none"> • Pre-/Probiotics • Increased Dietary Fiber Intake • Increase Resistant Starches • increase Fermented Foods • Calcium D-Glucarate (for high beta-glucuronidase) 	<ul style="list-style-type: none"> • Antibiotics (if warranted) • Antimicrobial Herbal Therapy • Antiparasitic Herbal Therapy (if warranted) • <i>Saccharomyces boulardii</i>
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GI EFFECTS COMMENSAL MICROBIOME ANALYSIS

The GI Effects features a synthesis of the patient's microbiome data. In addition to listing amounts of the 24 commensal bacteria, Genova has developed unique algorithms that account for the levels of bacteria and

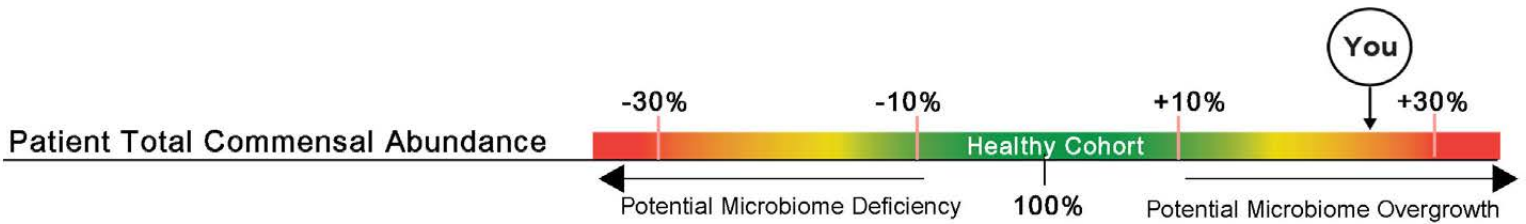
translate the patient's microbiome data into clinically actionable information. The commensal microbiome analysis focuses in the areas of abundance, dysbiosis, and balance.

Commensal Abundance

Total Commensal Abundance

The total commensal abundance is a sum-total of the reported commensal bacteria compared to a healthy cohort. Results are denoted with circle (You) and reported as a percent variance from healthy cohort levels. Low levels of commensal bacteria are often observed after

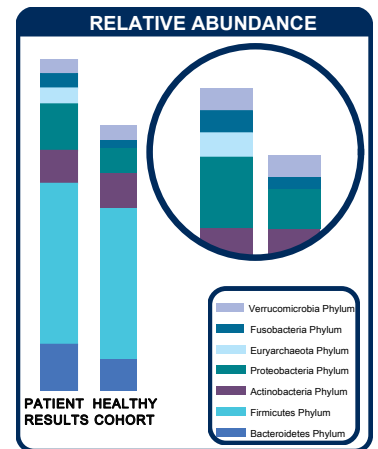
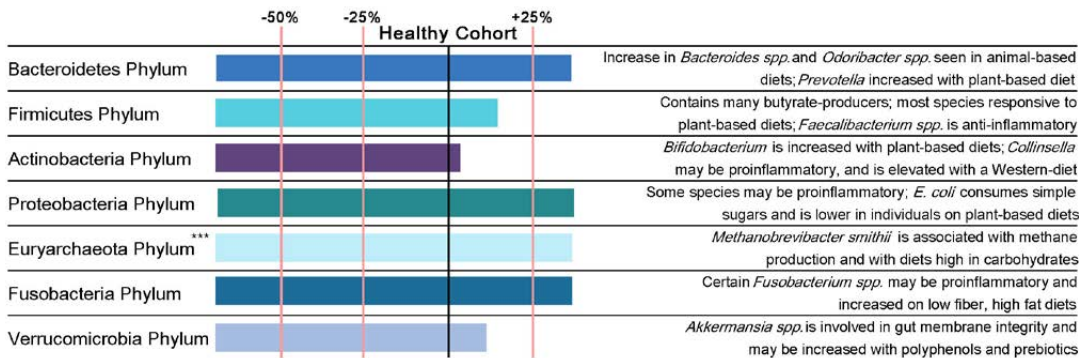
antimicrobial therapy, or in diets lacking fiber and/or prebiotic-rich foods and may indicate the need for microbiome support. Conversely, higher total commensal abundance may indicate potential bacterial overgrowth or probiotic supplementation.



Relative Commensal Abundance

The relative abundance compares the quantity of each of 7 major bacterial phyla to a healthy cohort. Phyla are represented by various colors and each is reported as a percent variance from healthy cohort levels. This can indicate broader variances in the patient's gut microbiome profile. Certain interventions may promote or limit individual phyla when clinically appropriate.

Relative Commensal Abundance



Commensal Dysbiosis Patterns

Genova's data analysis has led to the development of unique dysbiosis patterns, related to key physiologic disruptions, such as immunosuppression and inflammation. These patterns are based on the commensal bacteria and may represent dysbiotic changes that could pose clinical significance.

Inflammation-Associated Dysbiosis (IAD) Score

The Inflammation-Associated Dysbiosis score was developed from a pattern-based algorithm. When grouping patients according to their IAD scores, the group mean IAD score was negatively associated with commensal abundance and positively associated with fecal calprotectin, EPX, and sIgA. The score was validated in clinical studies including Genova's database of IBD patients, and an independent UCLA study with a cohort of IBD patients.¹ More information about the IAD score can be found in the publication: <https://link.springer.com/article/10.1007/s10620-019-05828-8>

It is unknown whether inflammation-associated dysbiosis is a cause and/or an effect of inflammation. A low IAD score with elevated inflammatory markers indicates the gut microbiome may not play a role in the inflammatory condition and other etiologies should be investigated (see zone 1 pattern description below). Longitudinal studies are needed to determine the significance of a high IAD score with normal inflammatory markers. It is possible that an inflammatory microbiome pattern may precede the rise of inflammatory markers.



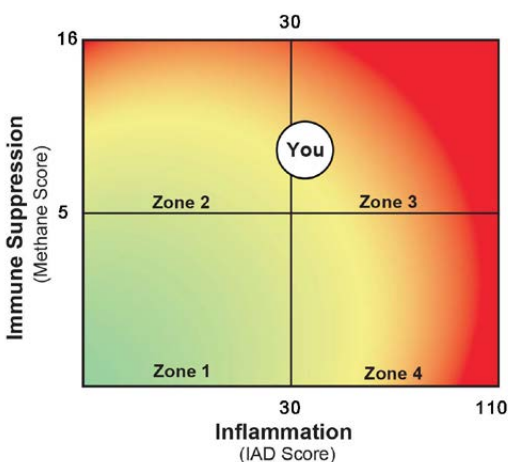
Methane Dysbiosis Score (Immune Suppression)

The Methane Dysbiosis score was derived from an analysis of breath methane test results that correlated with certain markers on the GI Effects stool test. Genova's unpublished data found a unique correlation with markers indicating immune suppression (low fecal sIgA and EPX) and the presence of methanogens, potentially pathogenic bacteria, bacterial overgrowth, and certain parasitic organisms. (See zones 2 and 3 below for more

information.) This dysbiosis pattern is associated with immune suppression and is distinct from the IAD pattern. It is unknown whether methane/methanogenic organisms are a cause and/or an effect of immune suppression. An elevated methane dysbiosis score may warrant treating potentially pathogenic organisms depending on the clinical picture. Additionally, intestinal barrier therapies may be helpful in supporting intestinal immune function.



Dysbiosis Pattern Zones



The IAD and methane scores are placed on the x- and y-axis, respectively, and certain cut points create 4 distinct zones. Each zone is associated with different clinical associations and treatment considerations.

Zone 1: The commensal profile in this zone does not align with profiles associated with intestinal inflammation or immunosuppression. Clinically, if a patient in this zone has elevated inflammatory biomarkers, other causes of intestinal inflammation other than dysbiosis need to be excluded. Other causes include infectious pathogens, celiac disease, food allergies and sensitivities, or more serious pathologies.

Zone 2: Profiles that demonstrate this pattern of bacteria are associated with a suppressed innate immune system (low fecal sIgA and EPX) and potentially impaired intestinal barrier function. Patients in this zone statistically have higher rates of opportunistic infections (e.g. *Blastocystis* spp. & *Dientamoeba fragilis*) as well as fecal fat malabsorption. In general, commensal abundance is high in this group suggesting potential bacterial overgrowth. Treating potentially pathogenic organisms and microbiome modulation is suggested to reduce methanogens and improve gut-barrier function.

Zone 3: A small fraction of patients are found with this pattern of commensal bacteria. Patients in this zone may have more inflammation compared to those in zone 4.

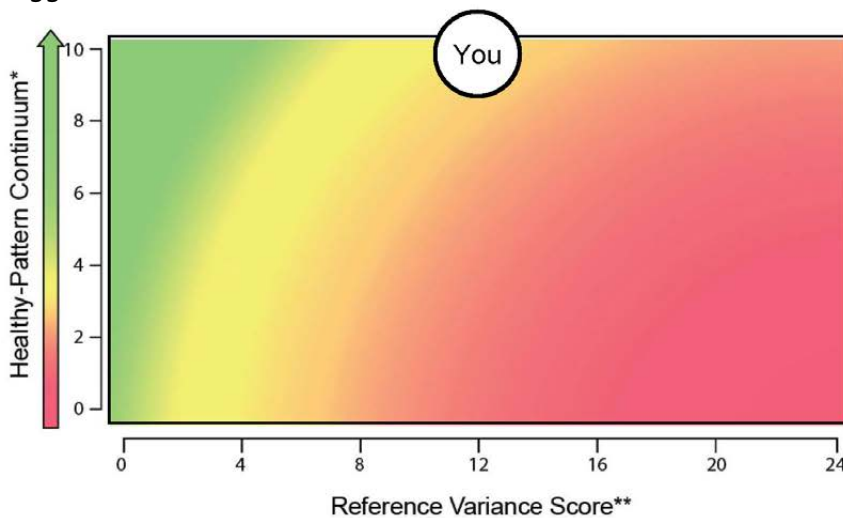
However, commensal abundance is usually higher making use of antimicrobial therapy relatively safer. Patients in this zone may have higher rates of pathogenic infections.

Zone 4: The commensal profile in this zone is associated with increased intestinal inflammation. Patients with IBD are more frequently found with this pattern of bacteria compared to non-IBD patients, and patients are more likely to present with diarrhea. Commensal abundance is lower in this zone and is associated with higher inflammatory biomarkers. Due to the decreased total abundance commonly seen in this group, antibiotic use for GI potential pathogens should be used with caution. In addition to standard treatment for intestinal inflammation, modulation of the commensal gut profile is encouraged.

Commensal Balance

The patient's result on the Commensal Balance infographic is denoted by a circle (You) against a color-coded gradient (green, yellow and red). The position of the patient's result against this background provides an At-a-Glance comparison of the patient's current commensal findings against those seen in healthy and diseased cohorts. Green suggests a balanced commensal health profile, yellow suggests borderline, and red suggests an imbalance.

The Commensal Balance graphic is a combination of the Healthy Pattern Continuum (y-axis) and the Reference Variance Score (x-axis). These scores combine to offer insight into dysbiosis by comparing a patient's commensal bacteria PCR results to that of a healthy cohort.



Balanced	Represents 95% of healthy individuals
Borderline	Represents 5% of healthy individuals
Imbalanced	Represents 60% of unhealthy individuals

*A progressive ranking scale based on a Genova proprietary algorithm that differentiates healthy and unhealthy commensal patterns.

**The total number of Commensal Bacteria (PCR) that are out of reference ranges for this individual.

Healthy-Pattern Continuum

The Healthy-Pattern Continuum is a progressive ranking scale based on a Genova proprietary algorithm which differentiates healthy and unhealthy commensal patterns. This algorithm is applied to an individual patient's GI Effects commensal bacteria (PCR) findings and produces a numeric result ranging from 0 to 10 denoted by the 'y' axis of the Commensal Balance infographic.

Reference Variance Score

The Reference Variance Score reflects the total number of an individual patient's commensal bacteria (PCR) results that are out of reference range. This number ranges from zero to 24 and is denoted by the 'x' axis of the Commensal Balance infographic.

DIGESTION AND ABSORPTION

Digestion and Absorption			
Pancreatic Elastase 1 †	158 L		>200 mcg/g
Products of Protein Breakdown (Total*) (Valerate, Isobutyrate, Isovalerate)	6.0		1.8-9.9 micromol/g
Fecal Fat (Total*)	19.5		3.2-38.6 mg/g
Triglycerides	1.1		0.3-2.8 mg/g
Long-Chain Fatty Acids	12.9		1.2-29.1 mg/g
Cholesterol	0.5		0.4-4.8 mg/g
Phospholipids	5.0		0.2-6.9 mg/g

Pancreatic Elastase 1 (PE-1)

Pancreatic elastase 1 is a digestive enzyme secreted exclusively by the pancreas. PE-1 measurement in the stool provides insight into pancreatic exocrine function.

Biomarker Key Points:

PE-1 is highly stable and is not degraded during passage through the gastrointestinal tract.² Fecal PE-1 levels are a good reflection of the pancreatic output of elastase, as well as other pancreatic enzymes, such as amylase, lipase, and trypsin.

PE-1 is not affected by transit time, though profuse watery stool samples may result in a falsely low PE-1 due to dilution.

PE-1 is not affected by pancreatic enzyme replacement therapy (PERT); therefore, it is a true reflection of pancreatic exocrine function.³ Genova utilizes the Schebo ELISA method using a monoclonal antibody which is highly specific for human PE-1. The monoclonal antibodies used in the test do not cross react with elastases of animal origin, which are contained in enzyme substitution preparations. Therefore, PE-1 should not be used to monitor PERT.

Fecal PE-1 (mcg/g)	Interpretation
>200	Normal exocrine pancreatic function
100 to 199	Mild-to-moderate exocrine pancreatic insufficiency (EPI)
<100	Severe pancreatic insufficiency

PE-1 correlates with the gold-standard test for pancreatic insufficiency, the secretin-cerulean test. Additionally, low PE-1 levels correlate with gold-standard morphological tests for chronic pancreatitis, including endoscopic retrograde pancreatography (ERCP) and magnetic resonance cholangiopancreatography (MRCP).

Reference values are adopted from an FDA-cleared kit and are based on correlation with the gold-standard testing for exocrine pancreatic insufficiency (EPI) as described in the literature. Since the reference range for PE-1 was evaluated using patients with severe EPI, the fecal PE-1 test does not have a high sensitivity for mild and moderate EPI. An optimal range of PE-1 may be higher than 200µg/g. Although the sensitivity and specificity of fecal PE-1 in EPI varied among studies, in several healthy cohorts, most individuals had average values $\geq 500\mu\text{g/g}$.⁴⁻⁷

Symptoms:

Exocrine pancreatic insufficiency (EPI) is a reduction of pancreatic digestive enzymes or enzyme activity leading to maldigestion and malabsorption. Clinical symptoms may not manifest until approximately 90% of pancreatic exocrine function has been lost.⁸ Some patients can have mild to moderate EPI, which may not be associated with maldigestion and/or malabsorption signs and symptoms.⁸

Signs and symptoms of EPI include:

- Diarrhea
- Steatorrhea
- Foul-smelling stools
- Bloating
- Excess flatulence
- Abdominal discomfort
- Weight loss⁹⁻¹¹

Causes of EPI:

Exocrine pancreatic insufficiency can occur secondary to:

- Cystic Fibrosis¹²
- Chronic pancreatitis (CP)¹³
- Pancreatic resection¹⁴
- Autoimmune pancreatitis¹⁵
- Gallstones¹⁶
- Pancreatic tumor/cancer¹⁷
- GI surgery (i.e. gastric bypass, pancreatic resection)¹⁸

Other clinical factors associated with EPI through unknown mechanisms include:

- Celiac disease¹⁹⁻²³
- Inflammatory Bowel Disease (IBD)²⁴
- Zollinger-Ellison syndrome^{25,26}
- Aging^{8,27}
- Excessive alcohol consumption²⁸
- Small Intestinal Bacterial Overgrowth (SIBO)^{8,29 30,31}
- Smoking²⁸
- Obesity³²
- Vegan/vegetarian diets³³
- Diabetes^{34,35}

Therapeutic considerations:

1. Further investigation to determine the underlying cause of dysfunction (see above lists)
2. Support patients with pancreatic enzyme replacement therapy (PERT) with meals at doses appropriate for the size of the meal/snack^{10,11 36,37}
3. Consider small, frequent meals, smoking cessation, and reduced alcohol consumption^{10,11}
4. Consider **SIBO testing** if there is an elevated Relative Abundance of commensal bacteria, high products of protein breakdown, high fecal fats, high short chain fatty acids, or high levels of *Methanobrevibacter smithii* via PCR.

Products of Protein Breakdown

Dietary protein that is not digested or absorbed in the small intestine may be fermented by colonic bacteria to produce products of protein breakdown, also called putrefactive short chain fatty acids. Genova's products of protein breakdown (PPB) biomarker assesses total concentration of three short chain fatty acids (SCFAs)- valerate, isobutyrate, and isovalerate- which are bacterial fermentation protein products.

Biomarker Key Points:

Human studies on the exact physiologic and pathophysiologic roles these SCFAs play are rare. Most of our evidence-based knowledge regarding products of protein breakdown come from Genova's internal data analysis. Products of protein breakdown results should be considered in conjunction with patient lifestyle, other fecal biomarkers, as well as commensal bacteria profiles.

Bacteria ferment protein to produce putrefactive short chain fatty acids. They also ferment fiber to produce other short chain fatty acids (e.g. butyrate, acetate, and propionate). Dysbiosis can result in imbalanced levels of the short chain fatty acids.

In the literature, short chain fatty acid imbalances (from both protein and fiber fermentation) are associated with multiple conditions, including:

- Colorectal cancer^{38,39}
- Depression⁴⁰
- Small Intestinal Bacterial Overgrowth (SIBO)⁴¹
- Antibiotics⁴²
- Increased protein consumption⁴³
- Diverticulosis⁴⁴
- Celiac disease⁴⁵
- Autism
- GI bleeding⁴⁶
- Chronic pancreatitis, steatorrhea⁴⁷

Causes of high fecal products of protein breakdown:

- Exocrine pancreatic insufficiency⁴⁸
- High protein diet
- Small intestinal bacterial overgrowth (SIBO)⁴⁹
- Low gastric HCL (hypochlorhydria, acid-blocking medications)⁵⁰
- Certain types of dysbiosis
- GI bleeding⁴⁶

Causes of low fecal products of protein breakdown:

- Very low protein diet
- Antibiotic use
- Low commensal bacteria abundance
- Intestinal inflammation

Therapeutic considerations for elevated PPB:

- 1. Evaluate dietary protein intake**
- 2. Assess for, and treat, root causes of insufficient protein digestion:**
 - Hypochlorhydria
 - » Assess/reduce use of acid-blocking medications (as clinically indicated)
 - » Consider betaine HCl challenge (as clinically indicated)
 - Exocrine pancreatic insufficiency
 - » Evaluate fecal PE1- If lower than 200 mcg/g, support with PERT as clinically indicated
- 3. Assess for small intestinal bacterial overgrowth and consider SIBO breath testing if any of these apply:**
 - Relative abundance of commensal bacteria is high
 - Fecal fats are elevated
 - SCFAs are elevated
 - *Methanobrevibacter smithii* is high via PCR
- 4. Review, assess, and treat any abnormal inflammatory biomarkers or infection.**

Therapeutic considerations for low PPB:

1. Evaluate dietary protein intake
2. Evaluate relative abundance of commensal bacteria
 - » Consider prebiotics, probiotics, and fermented foods
3. Assess inflammatory biomarkers (Calprotectin, EPX, fecal sIgA) and treat causes of inflammation

Genova's fecal fat analysis evaluates multiple lipid analytes including **triglycerides (TG), long chain fatty acids (LCFAs), phospholipids, cholesterol, and total fecal fat**. Stool fecal fats are used clinically as surrogate markers for fat maldigestion and malabsorption. The total fecal fat is derived from a sum of the lipid analytes. The total fecal fat is usually dominated by the long chain fatty acid component, which has the greatest concentration among the four fats.

Because stool fat concentrations were measured without controls of fat ingestion, all test results need to be considered with a patient's diet.

Biomarker Key Points:

Triglycerides (TGs) and cholesterol make up most, if not all, of our dietary fat intake. TG are broken down to form LCFAs. The fate of dietary fatty acids depends on their size. Smaller fatty acids passively diffuse through the enterocyte wall and are absorbed. LCFA absorption needs to be mediated by a transporter.

- **Triglycerides:** Increased fecal TG signifies maldigestion.^{51,52}
- **LCFAs:** Increased fecal LCFAs are often indicators of malabsorption.^{51,52}
- **Cholesterol:** Fecal cholesterol can come from different sources: diet, bile, and intestinal secretion.⁵³ Our daily fecal cholesterol excretion may exceed cholesterol intake.⁵³ With this, fecal cholesterol should not be used in isolation to determine maldigestion or malabsorption.
- **Phospholipids:** Fecal phospholipids can be derived from the diet, bile, shed epithelial cells, and bacterial cells. The diet is unlikely to contribute a dominant fraction to the fecal phospholipid pool. Dietary phosphatidylcholine (PC) is generally hydrolyzed and absorbed by the small intestine. On the other hand, PC is the major phospholipid in bile, and accounts for 90% of intestinal mucus.⁵⁴ Elevations in fecal phospholipids can be due to mucosal cell turnover, malabsorption, or bile.

Causes of fat maldigestion:

1. **Exocrine pancreatic insufficiency (EPI)**⁵⁵
2. **Bile salt insufficiency**⁵⁵
3. **PPI usage and hypochlorhydria**⁵⁶
 - PPI's increase the secretion of most pancreatic enzymes, but reduce the secretion of colipase.⁵⁷ Pancreatic colipase is secreted as a pro-protein and needs proteolytic enzyme activation. A deficiency in colipase production or activation can cause fat maldigestion, even when pancreatic lipase is normal or increased.
4. **Small intestinal bacterial overgrowth due to:**
 - Acidic small-intestinal pH (impairment of small intestinal digestive enzymes)⁵⁶
 - Bile acid deconjugation^{49,58}
5. **Use of medications** designed to impair intestinal lipase activity (Orlistat, Xenical, Alli), or use of synthetic fat-like products, indigestible by normal lipase (Olestra)⁵⁹

Causes of fat malabsorption

1. Intestinal dysbiosis and SIBO⁶⁰
2. Intestinal parasites⁶¹
3. Gastric bypass, ileal resection, or other surgeries that limit absorptive surface area⁶²
4. Irritable bowel syndrome (often as a symptom of pancreatic exocrine insufficiency or bile acid malabsorption)^{55,63} – more likely with the constipation subtype
5. Inflammatory bowel disease⁶⁴
6. Celiac disease⁶⁵

Therapeutic considerations for elevated fecal fats:

Target evaluation and treatment for common etiologies of fat maldigestion:

- **Pancreatic exocrine insufficiency**
 - If PE-1 is less than 200 mcg/g, consider PERT
- **Small Intestinal Bacterial Overgrowth (SIBO)**
 - Consider SIBO breath testing if any of these apply:
 - » Relative abundance of commensal bacteria is high
 - » Products of Protein Breakdown are elevated
 - » SCFAs are elevated
 - » *Methanobrevibacter smithii* is high via PCR
- **Hypochlorhydria**
 - Assess for acid blocking medication (PPIs) and reduce/remove if clinically indicated
 - Consider betaine HCl challenge then treat as indicated
- **Bile Salt Insufficiency**
 - Assess for causes, including liver damage and/or impaired gall bladder function
 - Consider addition of bile salts and/or cholagogues

Target evaluation and treatment for common etiologies of fat malabsorption:

- **Assess and treat for infection**
- **Celiac Disease**
 - Consider [Celiac and Gluten Sensitivity Panel](#)
- **IBD**
 - Review calprotectin, if greater than 120 µg/g, GI referral

Further Evaluation:

- Fat malabsorption or digestion may be associated with deficiencies in fat or fat-soluble nutrients
- Consider nutritional assessment of essential fatty acids and fat-soluble vitamins

Therapeutic considerations for low fecal fats (<3.2 mg/g):

Low fecal fats can be seen in low-fat diets. Genova's data analysis found specific associations between low fecal fats and inflammation markers. By evaluating inflammatory biomarkers (calprotectin and EPX), one may draw a distinction between lifestyle choice versus low-fat diet selection due to intestinal inflammation-related symptoms.

Chymotrypsin

Chymotrypsin is one of the numerous digestive enzymes secreted by the exocrine portion of the pancreas. Specifically, it is a protein-digesting enzyme which can be useful when monitoring pancreatic exocrine function in patients with normal stool transit time.

Unlike PE-1, chymotrypsin has not been correlated with the secretin-pancreozymin test,^{66,67} although it has been compared to the 72-hour fecal fat test.⁶⁸

Biomarker Key Points:

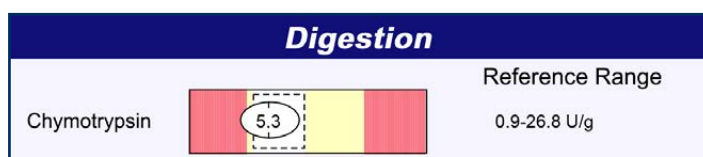
- Chymotrypsin is a noninvasive biomarker of pancreatic exocrine (i.e., digestive) function. It is affected by exogenous supplementation making it an ideal marker for monitoring dosing adequacy.⁶⁸
- Altered gut transit may effect chymotrypsin.⁶⁹ Degradation of proteases can result in higher chymotrypsin levels in response to diarrhea, and lower levels in response to slow transit time.⁶⁹

Causes of Abnormal Chymotrypsin:

- Low levels of chymotrypsin (<0.9U/g) in the presence of normal transit time are indicative of exocrine pancreatic insufficiency. Low chymotrypsin can also result from slowed transit time (constipation).⁷⁰
- High levels of chymotrypsin (>26.8U/g) suggests a rapid transit time (diarrhea) or may be due to excessive pancreatic enzyme supplementation.⁷⁰

Therapeutic Considerations for Abnormal Chymotrypsin:

1. Patients with altered chymotrypsin levels should undergo further investigation to determine the underlying causes of their dysfunction; refer to the Pancreatic Elastase-1 section for information about additional testing.
2. Pancreatic dysfunction typically leads to malabsorption, the severity of which is relative to the degree of exocrine pancreatic impairment. Evaluation of absorptive markers will provide valuable insight into the degree of malabsorption present.



Meat Fibers & Vegetable Fibers

Meat and vegetable fibers in the stool have been used to identify adequate digestion and absorption when used in combination with clinical presentation and other biomarkers, such as Pancreatic Elastase-1. Food fibers can help determine digestive insufficiency and monitor treatment progression.

Biomarker Key Points:

Meat and vegetable fibers are digested in the upper gastrointestinal tract, therefore the presence of these food fibers are suggestive of maldigestion and malabsorption or increased gut transit (diarrhea).⁷¹

The presence of meat fiber or more than a few vegetable fibers in the stool suggests incomplete digestion (e.g. pancreatic insufficiency, hypochlorhydria).^{71,72}

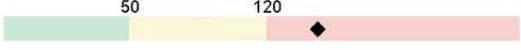


- Elevated levels can also result from inadequate mastication⁷² or hypermotility.^{74,75}

Therapeutic Considerations for the Presence of Meat/Vegetable Fibers in Stool:

Certain lifestyle, medication, and supplement interventions may be appropriate for patients with the presence of stool food fibers (e.g. pancreatic enzyme therapy). Refer to other biomarkers of digestion and absorption in conjunction with the clinical presentation of the patient to best determine which therapeutic option to select for the patient.

	Inside	Outside	Reference Range
Meat Fibers ♦	None		None
Vegetable Fibers	Rare		None - Few

INFLAMMATION AND IMMUNOLOGY

Inflammation and Immunology			
Calprotectin †	145 H		<=50 mcg/g
Eosinophil Protein X (EPX) †	4.9 H		<=4.6 mcg/g
Fecal secretory IgA	206		<=885 mcg/g

Calprotectin

Calprotectin is a calcium-binding protein with antimicrobial properties.⁷⁶ It accounts for 60% of neutrophil cytosolic content and is also found in monocytes and macrophages.⁷⁷ Calprotectin is released from the intestinal mucosa into the stool in intestinal inflammation.

Biomarker Key Points:

- Calprotectin is not subject to proteolytic degradation in feces.⁷⁸
- The Genova fecal calprotectin test is measured by an FDA approved ELISA assay.
- The normal range for fecal calprotectin is considered <50 mcg/g of feces.
- Dietary substances have not been found to interfere with the assay.
- Fecal calprotectin is useful in differentiating IBD from IBS and monitoring IBD treatment.⁷⁹

According to the literature, calprotectin levels can vary with age. It is higher in children younger than 5 years old due to increased intestinal mucosal permeability and differences in intestinal flora. Fecal calprotectin for children between 2 to 9 years is considered normal if <166 mcg/g, in individuals between 10 and 59 years if <51 mcg/g, and after 60 years if <112 mcg/g.⁸⁰

Causes of elevated calprotectin:

- Age (children younger than 5 years old, and patients greater than 60)⁸¹
- IBD, not in remission⁸²
- Colorectal cancer and polyps⁸²
- Infection (bacteria and some parasitic organisms)⁸²
- Non-steroidal anti-inflammatory medication use (NSAIDs) and NSAID enteropathy⁸²
- Diverticular disease^{84,85}
- IBS patients may also have increased fecal

calprotectin (at a much lower rate and level compared to IBD), indicating an inflammatory component to IBS (especially the diarrhea subtype). It is important to exclude IBD in patients with IBS-like symptoms when fecal calprotectin is high.^{82,86}

- Proton pump inhibitor (PPI) use is associated with elevated fecal calprotectin levels, although the cause-effect relationship is not clear.⁸⁷

Therapeutic considerations for elevated calprotectin:

- Eicosapentaenoic acid (EPA) from fish oil⁸⁸

Calprotectin 50 to 120 mcg/g

- Address cause of inflammation:
 - Infection
 - Suspected or history of IBD
 - Chronic NSAID use
- Recheck calprotectin in 4-6 weeks

Calprotectin >120 mcg/g

- Refer to GI specialist to rule out IBD, malignancy, or other cause of significant GI inflammation

*****NOTE: All patients over 50 should have independent colorectal cancer screening per USPSTF recommendations. Although a normal fecal calprotectin does have a high negative predictive value for colorectal cancer, no single biomarker on the GI Effects panel is intended to exclusively rule out or to diagnose cancer.**

Eosinophil Protein X (EPX)

EPX, also known as eosinophil-derived neurotoxin (EDN), is one of the four basic eosinophil granule proteins (i.e. major basic protein, eosinophil cationic protein, EPX, and eosinophil peroxidase).

Biomarker Key Points:

- Under steady-state conditions, the digestive tract's mucosa harbors a substantial number of eosinophils, which, if need be, are activated and exert several effector and immunoregulatory functions.⁸⁹
- While small-intestinal eosinophils are anti-inflammatory, large-intestinal eosinophils, when activated, secrete proinflammatory cytokines that can aggravate colitis. Although eosinophils are present throughout the intestine, large-intestinal eosinophils are scarce in a steady state. They can dramatically increase only under intestinal inflammatory conditions.⁸⁹

Causes of EPX elevation:

- Immune-mediated food hypersensitivity, atopic dermatitis, and food allergies^{90,91}
- IBD⁹²
- Certain parasitic infections⁹³
 - According to Genova's data analysis, stool inflammatory biomarker levels were parasite specific. In general, *Giardia* and *Cryptosporidium* were associated with high calprotectin, EPX, and sIgA. Additionally, Genova's analysis showed lower EPX in patient groups positive for *Blastocystis* and *Dientamoeba fragilis*. EPX was higher in the *Cryptosporidium* group compared to a healthy cohort or parasite negative group. Due to the low incidence of intestinal worms in the U.S. population at large, Genova's data set did not allow for a conclusion as to whether EPX would be expected to be elevated with all, or only certain worm infections.

- Microscopic colitis
 - A definitive diagnosis of microscopic colitis is only possible by histological analysis, which further classify these clinical entities as collagenous colitis (CC), lymphocytic colitis (LC), or other conditions.
 - Elevated fecal EPX, without neutrophilic inflammation, may predict CC but not LC.⁹⁴
- Eosinophilic gastrointestinal disorders
 - Eosinophilic gastroenteritis, eosinophilic esophagitis, and eosinophilic colitis make up a group of disorders called eosinophilic gastrointestinal disorders. Currently, there are no specific studies that evaluated EPX stool levels in these diseases. However, these are conditions worthy of consideration, especially when the patient is not responding to an elimination diet.
- Age (children younger than 4 years old)⁹⁵

Therapeutic considerations for elevated EPX:

Target evaluation and treatment for etiologies for EPX abnormalities:

- IgE-mediated allergy (Consider [IgE Food Antibody Profile](#) - If positive consider elimination diet)
- IBD (review Calprotectin level)
- Evaluate for parasitic infection

As the most abundant class of antibody found in the human intestinal lumen, secretory IgA (sIgA) is recognized as a first line of defense in protecting the intestinal epithelium from enteric pathogens and toxins. It is used to assess gastrointestinal barrier function.

Biomarker Key Points:

- As part of the gut epithelial barrier, sIgA is important in the development of immune tolerance for normal, beneficial commensal gut organisms, as well as common molecular epitopes found in foods.⁹⁶⁻¹⁰⁰
- Early studies of sIgA focused on immune exclusion (the prevention of pathological material and organisms from entering the general circulation). Recent studies also show sIgA plays a role in immune inclusion (delivery of commensal bacteria and their products to the gut and systemic immune system) for recognition. This leads to the development of immune tolerance. Immune inclusion spares beneficial organisms from destruction by the immune system which helps to support the immune system in a noninflammatory way to preserve local homeostasis.^{97,100}
- Although secretory IgA is the major antibody in the intestinal mucosa, the prevalence of GI disorders in patients with systemic IgA deficiency is not as high as one might expect. It is thought that the transportation of IgM from the mucosa can compensate for a lack of IgA.¹⁰¹

In people with genetic immunodeficiency of systemic sIgA, GI symptoms such as diarrhea have been reported.¹⁰² Systemically IgA-deficient patients more often have airway infections since compensatory sIgM is lacking in the airways (in contrast to the gut). Adaptive sIgA responses may allow the host to respond to fluctuations in commensal bacteria to favor mucosal homeostasis.¹⁰³

Causes of elevated fecal sIgA:

- Any defective epithelial barrier¹⁰⁴⁻¹⁰⁶
 - A defective epithelial barrier allows bacterial and microbial penetration, which is the strongest stimulator of sIgA production.
- Celiac disease¹⁰⁷
- Colon cancer¹⁰⁸
- Infections
- IBS (especially the diarrhea subtype)

Therapeutic considerations for elevated fecal sIgA:

Assess for and treat root causes of immune upregulation/inflammation:

- Infection (bacterial, parasitic, and/or viral pathogen, potential pathogen)
- Compromised intestinal barrier function (i.e., intestinal permeability)
- Heightened response to noninfectious stimuli (i.e., food sensitivity/allergy, etc.)
 - Consider Food Antibody testing
 - » If positive, consider elimination diet

Considerations for low fecal sIgA:

Because of the lack of clinical evidence, there is no clear cut-off value for low fecal sIgA.

Patients with systemic IgA deficiency can have low levels of fecal secretory IgA. There is a demonstrated link between IgA deficiency and several GI diseases, including celiac disease, giardiasis, nodular lymphoid hyperplasia, ulcerative colitis, Crohn's disease, pernicious anemia, and gastric and colonic adenocarcinoma. Low sIgA may reflect a loss of GI immune response resiliency.

Fecal sIgA may be low in severe/prolonged IBD patients due to a switch from intestinal IgA to IgG production as well as a deficiency in producing IgA dimers and polymers.¹⁰⁰

Secretory IgA demonstrates an array of activities integral to the maintenance of intestinal homeostasis. It influences the composition of intestinal microbiota, down-regulates pro-inflammatory responses normally associated with the uptake of highly pathogenic bacteria and potentially allergenic antigens, and promotes the retro-transport of antigens across the intestinal epithelium to gut-associated lymphoid tissue (GALT). Therefore, a low sIgA is clinically significant.¹⁰³ This test result should be considered together with the patient's medical condition, other biomarkers, and microbiome profiles when interpreting the data.

Probiotics have been shown to support sIgA levels.¹⁰⁹⁻¹¹²

Fecal Lactoferrin

Lactoferrin is an iron-binding glycoprotein secreted by mucosal membranes as a granular component of neutrophils. It is liberated by neutrophils in response to inflammation.

Biomarker Key Points:

Lactoferrin can also be found in most exocrine secretions, including breast milk, tears, nasal secretions, saliva, intestinal mucus, and genital secretions.¹¹³

Lactoferrin has antimicrobial properties by depriving pathogens of iron, or disrupting their plasma membranes through its highly cationic charge. It also exhibits immunomodulatory activities by up- and down-regulating innate and adaptive immune cells.¹¹³

Genova's assessment uses an enzyme immunoassay to assess polyclonal antibodies to lactoferrin. The result is qualitative and expressed as a positive or negative finding. Subsequent calprotectin testing can provide additional useful information and assist in triage for endoscopic referral.

Immunology			
	Inside	Outside	Reference Range
Fecal Lactoferrin ♦	Negative		Negative

GASTROINTESTINAL MICROBIOME

The GI microbiome biomarkers provide information regarding the health, function, and diversity of the trillions of GI tract microbial cells. They indicate how well the microbiome is performing the metabolic functions that are shared with the human host.

There are several different GI microbiome stool biomarker categories on Genova's stool profiles:

Metabolic Indicators: This category includes β -glucuronidase, short chain fatty acids (butyrate, acetate, and propionate), and secondary bile acids. These biomarkers reflect specific and vital metabolic functions performed by the microbiota.

Commensal Bacteria: GI Effects measures 24 commensal bacteria using semi-quantitative polymerase chain reaction (PCR). More than 95% of commensal gut organisms are anaerobic and are difficult to recover by traditional (aerobic) culture techniques. Genova's proprietary algorithms produce scores for composition and relative abundance of stool bacteria.

Bacteriology and Mycology Culture with Sensitivities: Culture demonstrates the presence of specific live beneficial and pathogenic organisms. Sensitivities to prescriptive and natural antimicrobial agents are provided to guide therapeutic interventions when clinically indicated. Culture is the only method that accurately and reproducibly evaluates an organism's response to prescriptive and natural antimicrobial agents.

Potassium Hydroxide (KOH) Preparation: KOH prep is offered as standard on the Gut Pathogen Profile. It is an add-on to other stool profiles. This microscopic evaluation reflects all yeast regardless of viability.

Parasitology: Genova's assessment includes comprehensive testing for all parasites on every parasitology exam ordered. Microscopic ova and parasite (O&P) examination is offered on all parasitology profiles, while GI Effects profiles also offer PCR detection. Six targets are chosen to detect common protozoan parasites. These include *Blastocystis* spp. with reflex subtyping¹⁻⁹, *Cryptosporidium parvum/hominis*, *Cyclospora cayetanensis*, *Dientamoeba fragilis*, *Entamoeba histolytica*, and *Giardia*. PCR for pathogenic organisms has emerged as a preferred, highly sensitive method for infectious organism detection. Additionally, the CDSA profiles offer Enzyme Immunoassay for select parasites. By utilizing multiple detection tools, Genova offers the most comprehensive parasitology examination currently available.

Macroscopic evaluation for worms is offered as standard on the Gut Pathogen Profile, and as an add-on to other stool profiles.

METABOLIC INDICATORS

Metabolic			
Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	81.3		>=23.3 micromol/g
n-Butyrate Concentration	18.1		>=3.6 micromol/g
n-Butyrate %	22.3		11.8-33.3 %
Acetate %	63.1		48.1-69.2 %
Propionate %	14.6		<=29.3 %
Beta-glucuronidase	2,297		368-6,266 U/g

Short Chain Fatty Acids (SCFAs)

Short chain fatty acids (SCFAs) are organic acids that consist of one to six carbons, of which acetate, propionate, and butyrate are the most abundant ($\geq 95\%$). Acetate, propionate, and butyrate are produced by bacterial fermentation of dietary fiber and resistant starch. They can also be produced using endogenous epithelial-derived mucus by specific colonic anaerobic bacteria.^{114,115}

Short Chain Fatty Acids function to:

1. Maintain intestinal barrier function
2. Provide fuel for colonocytes
3. Regulate colonic absorption of water, electrolytes, and nutrients
4. Salvage unabsorbed carbohydrates
5. Support commensal bacteria
6. Modulate anti-inflammatory and antimicrobial activities

Biomarker Key Points:

It is important to note that fecal SCFA results may not completely reflect how much of the SCFA was produced and absorbed in the intestine. A low fecal SCFA test result can be a consequence of low production or high absorption. A high fecal SCFA test result can be a consequence of high production or low absorption.

Results are reported as total SCFA concentration, n-butyrate concentration, and n-butyrate, acetate, and propionate percentages of the total concentration. The total concentration is important to focus on, with causes of elevated or low levels outlined below. Skewed percentages of the individual SCFAs may reflect an imbalanced microbiome or diet.

SCFA production from fiber is dependent on the specific enzymes each gut bacteria possesses.¹¹⁵ The table below lists the commensal bacteria listed on the GI Effects profile, and the type of short chain fatty acid they primarily produce, based on literature. When bacteria are imbalanced, SCFA may also be imbalanced.

Butyrate:

Butyrate is the primary fuel source for colonocytes. Inadequate levels are associated with disordered colonic health.^{116,117}

Based on the literature, the three major butyrate-producers are *Faecalibacterium*, *Eubacterium*, and *Roseburia*.¹¹⁴

Various mixtures of dietary fibers, some types of resistant starch, fructooligosaccharides (FOS), and beta glucan are important for butyrate production.¹¹⁸

Acetate:

Acetate is the most abundant SCFA in the colon and makes up more than half of the total SCFAs.

Acetate has two main routes of production. The primary route is carbohydrate fermentation by enteric bacteria. Acetate is formed directly from acetyl-CoA, gets released into systemic circulation, and is taken up by the liver. It is then used as an energy source, as well as a substrate for the synthesis of cholesterol and long-chain fatty acids.¹¹⁹

Acetate is recognized as a volatile signal for biofilm formation.¹²⁰

Inulin supplementation has been shown to increase acetate levels.¹²¹ Pectin is also an important substrate for acetate production.¹¹⁸

Literature-Based Short Chain Fatty Acid Production		
Butyrate Producer (C4:0)	Acetate Producer (C2:0)	Propionate Producer (C3:0)
<i>F. prausnitzii</i> <i>B. crossotus</i> <i>A. colihominis</i> <i>Clostridium</i> spp. <i>C. eutactus</i> <i>Roseburia</i> spp.	<i>Prevotella</i> spp. <i>Odoribacter</i> spp. <i>A. colihominis</i> <i>Clostridium</i> spp. <i>C. eutactus</i> <i>Lactobacillus</i> spp. <i>Ruminococcus</i> spp. <i>Veillonella</i> spp. <i>Bifidobacterium</i> spp. <i>A. muciniphila</i>	<i>Prevotella</i> spp. <i>Odoribacter</i> spp. <i>Clostridium</i> spp. <i>Veillonella</i> spp. <i>A. muciniphila</i>
Butyrate Utilizer	Acetate Utilizer	Propionate Utilizer
<i>Clostridium</i> spp.	<i>Roseburia</i> spp.	<i>Clostridium</i> spp.

Propionate:

Propionate is a minor energy source for the colonocytes, though it has anti-inflammatory effects.¹²²

Propionate acts as a precursor for gluconeogenesis in the liver.¹¹⁹

Systemic propionate inhibits acetate incorporation into cholesterol.¹²¹

Guar gum can support propionate levels.¹¹⁸

Causes of low SCFAs:

- Diarrhea (rapid transit leading to decreased SCFA production)
- Constipation (increased SCFA absorption)
- Inflammation (high calprotectin and/or high EPX/sIgA)
- Chronic antibiotic use
- Decreased carbohydrate/fiber consumption¹²³⁻¹²⁵
- Chronic illness with restricted diet (e.g. low fermentable fiber)
- Severe dysbiosis (e.g. some commensal bacteria are very high, while others are very low)

Therapeutic considerations for low SCFAs:

- Dietary fiber, resistant starches (e.g. seeds and legumes, whole grains, green bananas, potatoes) and/or butyrate supplementation
- Arabinogalactans and β -glucan, as found in whole grains¹²⁶
- Inulin supplementation¹²¹
- Probiotics and fermented foods to balance the microbiome

Causes of elevated SCFAs:

- Elevated commensal bacteria abundance or bacterial overgrowth¹²⁷
- High dietary intake of fiber and resistant starches

Optimal levels of SCFAs have not been established. However, in general, higher levels are considered beneficial.

Therapeutic considerations for high SCFAs:

- May be optimal
- Consider SIBO testing if any of these apply:
 - Relative abundance of commensal bacteria is high
 - Products of Protein Breakdown are elevated
 - Fecal fats are elevated
 - *Methanobrevibacter smithii* is high via PCR

Beta-glucuronidase

Beta-glucuronidase is an enzyme which is produced by colonocytes and by some intestinal bacteria (particularly *E. coli*, but also *Ruminococcus*, *Bacteroides*, *Eubacterium*, *Peptostreptococcus*, *Staphylococcus*, and *Clostridium*).¹²⁸

Biomarker Key Points:

Beta-glucuronidase breaks down complex carbohydrates and increases the bioavailability and reabsorption of plant polyphenols (lignans, flavonoids, ceramides, and glycyrrhethinic acid).¹²⁹

Beta-glucuronidase deconjugates glucuronide molecules from a variety of toxins, carcinogens, hormones (i.e. estrogens) and drugs. Deconjugation permits reabsorption via enterohepatic circulation, with the potential to elevate systemic levels of potentially harmful compounds and hormones.¹²⁸ The intestinal bacterial microbiome related to estrogen metabolism is collectively called the 'estrobolome' and is illustrated in the figure below.¹³⁰

Limited research suggests an association between elevated fecal beta-glucuronidase and cancer risk, primarily colorectal and breast cancer.¹³¹⁻¹³⁴

Evaluating beta-glucuronidase may be of specific interest to clinicians interested in evaluating levels of important substances such as hormones, vitamin D, and phytonutrients.

Causes of elevated beta-glucuronidase:

- Dysbiosis
- Western diet, high in red meat and protein^{128,135}

Therapeutic consideration for elevated beta-glucuronidase:

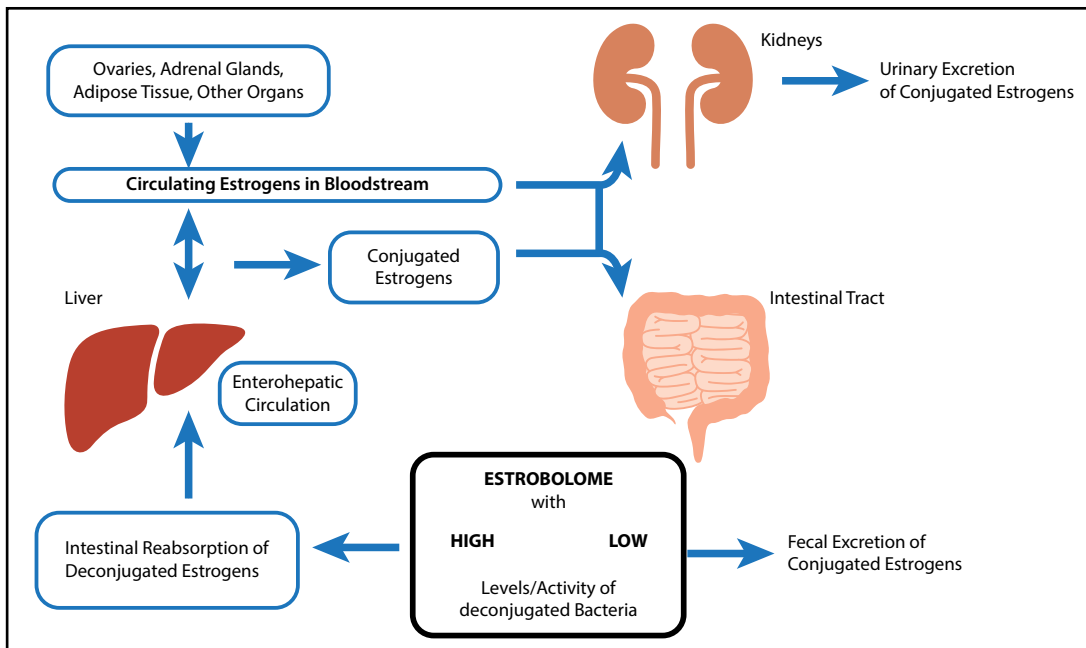
- Probiotics^{136,137}
- Dietary fiber, prebiotics¹³⁶⁻¹³⁹
- Calcium-D-glucarate
 - Calcium-D-glucarate is the calcium salt of D-glucaric acid. It is found in fruits and vegetables (oranges, apples, grapefruit, and cruciferous vegetables).¹⁴⁰
 - Oral supplementation inhibits the enzymatic activity of beta-glucuronidase.¹⁴⁰
- Milk thistle^{141,142}
- Low-calorie and vegetarian diets^{128,143}

Causes of low beta-glucuronidase:

- Dysbiosis
- Antibiotic use^{144,145}

Therapeutic considerations for low beta-glucuronidase:

Abnormally low levels may diminish the bioavailability of many phytonutrients. There is no literature indicating the need to treat low fecal β -glucuronidase. However, because it is produced in the intestinal endothelium and by commensal bacteria, maintaining a healthy commensal balance may be helpful to optimize levels.



Fecal pH indicates the relative acidity or alkalinity of the feces. The pH of the stool should not be confused with stomach pH (GI tract pH fluctuates significantly, depending on location), and therefore is not directly influenced by hydrochloric acid in the stomach.¹⁴⁶ Fecal pH is a standard accepted laboratory procedure, but is a non-specific test.

Biomarker Key Points:

Factors that have an impact on stool pH include fiber and food constituent intake,¹⁴⁷⁻¹⁴⁹ fermentive processes, bacterial populations, antibiotics,¹⁵⁰ and stool transit time.¹⁵¹

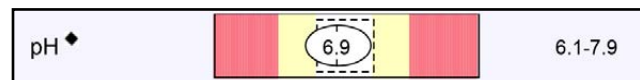
Causes of Abnormal Stool pH:

A normal fecal pH is associated with a mildly acidic stool (often a result of SCFA production), which encourages beneficial bacteria and discourages intestinal pathogens that prefer a more neutral pH.¹⁴⁶

- Abnormally low fecal pH of <6.1 (stool acidity) may be related to malabsorption of carbohydrates (including lactose),^{146,152} or to small bowel bacterial overgrowth.¹⁵³ Osmotic diarrhea is another possible cause of a low fecal pH,¹⁵⁴ such as from using osmotic laxative agents.
- Abnormally high fecal pH of >7.9 (stool alkalinity) may be due to hypochlorhydria,¹⁴⁶ slow transit time/constipation, antibiotics,¹⁴⁶ inadequate dietary fiber,¹⁵⁵ or a high protein/low carbohydrate diet.¹⁵⁶

Therapeutic considerations for abnormal stool pH:

No direct clinical action is necessary; however, follow-up with identifying and addressing a possible cause is recommended.



Secondary Bile Acids

Bile acids are the end products of hepatic cholesterol metabolism and are responsible for fat emulsification, aiding lipid absorption and digestion in the small intestine. Primary bile acids, chenodeoxycholic acid (CDCA) and cholic acid (CA) are derived from cholesterol. Once they enter the colon, they are acted upon by anaerobic bacteria to produce the secondary bile acids lithocholic acid (LA) and deoxycholic acid (DCA). CDCA is modified into LA and CA is modified to DCA.

Biomarker Key Points:

- Secondary bile acids have been shown to have carcinogenic and mutagenic properties.¹⁵⁷⁻¹⁶³
- The specific bacteria involved in primary bile acid deconjugation to secondary bile acids include *Clostridium*, *Enterococcus*, *Bacteroides* and *Lactobacillus*.¹⁶⁴
- Diet can have a significant impact: Increased dietary fiber can reduce secondary bile acids, while high saturated fat and high meat diets are associated with elevated levels.^{163,165}
- Secondary bile acids have been associated with increases of oxidative stress and DNA damage.^{163,166,167}
- LCA is thought to be more toxic than DCA, partly due to its inhibitory effects on glutathione-S-transferase (GST) in colocytes.¹⁶⁰⁻¹⁶⁶

Interpretation:

Elevated LCA/DCA ratio has been associated with:

- Colorectal cancer¹⁶⁸⁻¹⁷¹
- Gallstone formation¹⁷²
- Cholecystectomy^{173,174}

Elevated secondary bile acids have also been associated with:

- Impaired gallbladder function or cholesterol gallstone formation^{175,176}
- Inflammation within colonic mucosa, which is thought to also compromise intestinal permeability^{167,177}

Low secondary bile acids may result from:



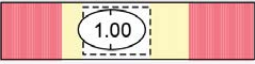
- Broad-spectrum antibiotics¹⁷⁸
- Reduced cholesterol intake or absorption

Therapeutic consideration for abnormal secondary bile acids:

Measurement of secondary bile acids can help guide treatment of patients with GI disorders or give insight into a more serious GI pathology which would require further testing. Certain dietary recommendations and supplement interventions may be appropriate for patients with abnormal secondary bile acids:

Dietary Support:

- Fiber and probiotics can help reduce an elevated bile acid ratio. Fiber reduces the concentration of secondary bile acids in the stool.
- Resistant starch, insoluble fiber contained in wheat bran, legumes and certain vegetables, decreases the level of secondary bile acids. These insoluble fibers enhance short-chain fatty acids production in the proximal colon, thus lowering intestinal pH. A reduction in pH inhibits 7 alpha-hydroxylase activity, which reduces the concentrations of LCA, DCA, and the LCA:DCA ratio.¹⁷⁹⁻¹⁸¹
- Probiotics and prebiotics have been found to reduce the conversion of CDCA to LCA.¹⁸²
- Lowering saturated fat and meat intake may decrease secondary bile acid levels.¹⁶³

Secondary Bile Acids		
Lithocholic Acid (LCA)		0.65-5.21 mg/g
Deoxycholic Acid (DCA)		0.67-6.76 mg/g
LCA/DCA Ratio		0.39-2.07

COMMENSAL BACTERIA

Gastrointestinal Microbiome							
Commensal Bacteria (PCR)	Result CFU/g stool	QUINTILE DISTRIBUTION					Reference Range CFU/g stool
		1st	2nd	3rd	4th	5th	
Bacteroidetes Phylum							
<i>Bacteroides-Prevotella</i> group	2.4E8						3.4E6 -1.5E9
<i>Bacteroides vulgatus</i>	1.2E9						<=2.2E9
<i>Barnesiella</i> spp.	3.6E7						<=1.6E8
<i>Odoribacter</i> spp.	7.1E7						<=8.0E7
<i>Prevotella</i> spp.	1.4E8 H						1.4E5 -1.6E7
Firmicutes Phylum							
<i>Anaerotruncus colihominis</i>	3.4E7 H						<=3.2E7
<i>Butyrivibrio crossotus</i>	5.0E7 H						5.5E3 -5.9E5
<i>Clostridium</i> spp.	2.1E8						1.7E8 -1.5E10
<i>Coprococcus eutactus</i>	1.0E8						<=1.2E8
<i>Faecalibacterium prausnitzii</i>	7.5E8						5.8E7 -4.7E9
<i>Lactobacillus</i> spp.	1.6E8						8.3E6 -5.2E9
<i>Pseudoflavonifractor</i> spp.	3.0E8 H						4.2E5 -1.3E8
<i>Roseburia</i> spp.	7.6E7 L						1.3E8 -1.2E10
<i>Ruminococcus</i> spp.	1.9E9 H						9.5E7 -1.6E9
<i>Veillonella</i> spp.	1.5E8 H						1.2E5 -5.5E7
Actinobacteria Phylum							
<i>Bifidobacterium</i> spp.	1.5E8						<=6.4E9
<i>Bifidobacterium longum</i>	1.4E8						<=7.2E8
<i>Collinsella aerofaciens</i>	5.1E8						1.4E7 -1.9E9
Proteobacteria Phylum							
<i>Desulfovibrio piger</i>	8.7E7 H						<=1.8E7
<i>Escherichia coli</i>	1.3E8 H						9.0E4 -4.6E7
<i>Oxalobacter formigenes</i>	5.0E7 H						<=1.5E7
Euryarchaeota Phylum							
<i>Methanobrevibacter smithii</i>	1.4E8 H						<=8.6E7
Fusobacteria Phylum							
<i>Fusobacterium</i> spp.	2.3E7 H						<=2.4E5
Verrucomicrobia Phylum							
<i>Akkermansia muciniphila</i>	3.1E7						>=1.2E6
Firmicutes/Bacteroidetes Ratio							
<i>Firmicutes/Bacteroidetes</i> (F/B Ratio)	11 L						12-620

The gray-shaded portion of a quintile reporting bar represents the proportion of the reference population with results below detection limit.

Commensal results and reference range values are displayed in a computer version of scientific notation, where the capital letter "E" indicates the exponent value (e.g., 7.3E6 equates to 7.3 x 10⁶ or 7,300,000).

The Firmicutes/Bacteroidetes ratio (F/B Ratio) is estimated by utilizing the lowest and highest values of the reference range for individual organisms when patient results are reported as <DL or >UL.

The vast majority of microorganisms within the body reside in the colon and are called 'microbiota'; their genetic components are collectively termed 'the microbiome.' The microbiome is viewed as an integral part of the body that is essential to proper organ function. The individual species in these communities were long considered "commensal" organisms—literally "at the same table"—with the implication that such microorganisms were neither pathogenic nor particularly harmful when in their natural site and in a proper amount.

After a child reaches 2–3 years old, a relative stability in gut microbiota composition has been demonstrated. Richness and diversity of gut microbiota shaped in early life characterize a healthy gut microbiome.¹⁸³ However, optimal healthy gut microbiota composition is different for each individual. The composition of each person's microbiome is highly variable and can change according to age, ethnicity, location, diet, lifestyle, medications, and environmental factors.¹⁸³

Rather than concentrating on any one commensal bacteria, understanding overall microbiome patterns is essential in connecting dysbiosis to clinical symptomatology. Genova's GI Effects Comprehensive Stool Profile and the Microbial Ecology Profile test 24 commensal gut bacteria (at genus or species levels) using PCR methodology.

**Please refer to the Commensal Bacteria Chart online regarding these 24 measured commensal bacteria.*

The commensal gut microbiota interacts extensively with the host, influencing multiple metabolic and physiological functions, such as:¹⁸⁴⁻¹⁸⁶

- Regulating the gut's development
- Facilitating digestion
- Producing SCFAs
- Shaping the immune system
- Preventing the growth of harmful microflora species
- Synthesizing nutrients (such as B vitamins and vitamin K)
- Neutralizing toxins
- Stimulating the intestinal immune system
- Modulating gastrointestinal hormone production
- Oxidative response
- Barrier function

Metabolomics of the commensal bacteria reveal the interaction between the microbiome and its host. Commensal bacteria and SCFAs are closely related. Commensal bacteria each have differing functions. The balance of products and processes helps to establish partnerships, depending on which bacteria are in the gut. There are many literature-based associations between commensal bacteria and important bacterial fermentation end products.

<p>Butyrate Producer (C4:0) Increases with fermentation of starch and inulin-type fructans.¹⁸⁷</p>	<p>Acetate Producer (C2:0) The majority of acetate is produced by most enteric bacteria from carbohydrate fermentation. One-third of acetate comes from acetogenic bacteria, which synthesize acetate from hydrogen and carbon dioxide or formic acid.¹¹⁴</p>	<p>Propionate Producer (C3:0) Increases with fermentation of oat bran and β-glucan, pectin, pulses, wheat dextrin, and pyrodextrins.¹⁸⁷</p>
<ul style="list-style-type: none"> • <i>F. prausnitzii</i> • <i>B. crossotus</i> • <i>A. Colihominis</i> • <i>Clostridium</i> spp. • <i>C. eutactus</i> • <i>Roseburia</i> spp. 	<ul style="list-style-type: none"> • <i>Prevotella</i> spp. • <i>Odoribacter</i> spp. • <i>A. Colihominis</i> • <i>Clostridium</i> spp. • <i>C. eutactus</i> • <i>Lactobacillus</i> spp. • <i>Ruminococcus</i> spp. • <i>Veillonella</i> spp. • <i>Bifidobacterium</i> spp. • <i>A. muciniphila</i> 	<ul style="list-style-type: none"> • <i>Prevotella</i> spp. • <i>Odoribacter</i> spp. • <i>Clostridium</i> spp. • <i>Veillonella</i> spp. • <i>A. muciniphila</i>
<p>Lactate Producer Higher concentrations of lactate have been noted in IBD. Lactate is converted to acetate, butyrate, and propionate, generally at a higher pH, and there may be reduced conversion and lactate accumulation at a lower pH.¹⁸⁸</p>	<p>H₂-producing (hydrogenogenic) H₂ is a primary by-product of human microbiota biology. Endogenous H₂ is either passed in flatus or absorbed into the circulation and released by respiration. New research is evaluating it as an anti-inflammatory biomolecule that safeguards against tissue injury.¹⁸⁹ H₂ is used by intestinal bacterial methanogens, acetogens, and SRB.</p>	<p>Sulfate reducing bacteria (SRB) H₂S Producer: AA metabolism utilizes sulfate (SO₄²⁻) and reduces it to hydrogen sulfide (H₂S). SRB are part of a normal gut microbiota, though increased levels may contribute to disease. Excess is not absorbed and is available for rapid exogenous H₂S production by the SRB.^{190,191}</p>
<ul style="list-style-type: none"> • <i>B. crossotus</i> • <i>Lactobacillus</i> spp. • <i>Bifidobacterium</i> spp. • <i>B. longum</i> 	<ul style="list-style-type: none"> • <i>Odoribacter</i> spp. • <i>Clostridium</i> spp. • <i>E. coli</i> 	<ul style="list-style-type: none"> • <i>Odoribacter</i> spp. • <i>D. piger</i>
<p>Degrades Lactate Lactate-Utilizing Bacteria (LUB) metabolize lactate to form different end-products. The balance between H₂-producing and H₂-utilizing LUB might contribute to colic symptoms.¹⁹²</p>	<p>H₂-using (hydrogenotrophic) H₂ consumers include reductive acetogens, methanogenic archaea, and sulfate-reducing bacteria (SRB).</p>	<p>Methane Producer – Methanogens Methane producers produce methane by utilizing hydrogen and carbon dioxide. Approximately 30% to 62% of individuals harbor methane-producing bacteria.¹⁹³</p>
<ul style="list-style-type: none"> • <i>Roseburia</i> spp. • <i>Ruminococcus</i> spp. • <i>Veillonella</i> spp. 	<ul style="list-style-type: none"> • <i>Ruminococcus</i> spp. • <i>D. piger</i> • <i>M. smithii</i> 	<ul style="list-style-type: none"> • <i>M. smithii</i>

Firmicutes/Bacteroidetes (F/B) Ratio

Literature suggests that a high **Firmicutes/Bacteroidetes (F/B)** ratio may be associated with a greater risk of metabolic syndrome, diabetes, and obesity.¹⁹⁴⁻¹⁹⁶ However, the literature is mixed on this subject. Additionally, not all sources calculate the ratio using the same methodology.

At Genova, the Firmicutes/Bacteroidetes ratio calculation is made by adding the abundance of *Anaerotruncus colihominis*, *Butyrivibrio crossotus*, *Clostridium* spp., *Faecalibacterium prausnitzii*, *Lactobacillus* spp., *Pseudoflavonifractor* spp., *Roseburia* spp., *Ruminococcus* spp., and *Veillonella* spp. This total is then divided by the

sum of the *Bacteroidetes-Prevotella* group, *Barnesiella*, and *Odoribacter* species. Results are placed within a reference range based on a questionnaire-qualified healthy cohort.

Genova's F/B ratio should be used to evaluate commensal microbial balance. Since there is no standardized F/B calculation, disease associations may not always apply. Treatment strategies, including pre- and probiotics, fermented foods, lifestyle modification, and a varied diet, should be used to achieve a balance between the two phyla.

Dysbiosis

The term 'dysbiosis' is often used to describe altered microbiome patterns as compared to a healthy cohort.¹⁹⁷ Others define dysbiosis as the changes in gut microbiota composition associated with disease.¹⁹⁸ Genova's data analysis reveals that dysbiosis and commensal microbial patterns may contribute to, and be a root cause of, many clinical conditions. In Genova's data analysis, statistically significant correlations were found between commensal bacteria and self-reported clinical conditions such as inflammatory bowel disease, metabolic syndrome, chronic fatigue, autoimmune dysfunction, type 2 diabetes mellitus, high blood pressure, mood disorder, and ROME III criteria irritable bowel syndrome.

Dysbiosis can result from medication use (antibiotics, PPIs, etc.), stress, alcohol, disruption in circadian rhythms, and poor diet.¹⁹⁹⁻²⁰³

On the GI Effects Comprehensive profile, the Commensal Microbiome Analysis section assesses dysbiosis. These graphics were outlined previously on pages 8-10.

Therapeutic considerations:

Therapeutic interventions, such as dietary macronutrient content, fiber supplementation, prebiotics, probiotics, symbiotics, lifestyle modification, and the environment have been shown to modulate the individual microbiome.^{204,205}

Commensal and Biomarker Clinical Associations

As part of Genova's ongoing data analysis, statistically significant clinical associations were noted between commensal bacteria, stool biomarkers, and various conditions. To create its Clinical Associations charts, Genova utilized the extensive GI Effects test-results database which allowed comparison of commensal and biomarker results in patients with self-reported clinical conditions (IBD, Metabolic Syndrome, Chronic Fatigue, Autoimmune dysfunction, Type 2 Diabetes, High Blood Pressure, Mood Disorders and IBS (ROME III criteria) to those found in the healthy cohort.

Differences between the healthy cohort and individuals with clinical conditions are denoted by the arrows in the Clinical Associations charts.

Interpretation At-a-Glance									
Commensal Bacteria	Patient Results Out of Reference Range	Genova Diagnostics Commensal Bacteria Clinical Associations*							
		IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-Immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
Bacteroidetes Phylum									
<i>Bacteroides-Prevotella</i> group		↑	↑	↑	↑	↑	↑	↑	↑
<i>Bacteroides vulgatus</i>		↑			↑	↑		↑	↑
<i>Barnesiella</i> spp.									
<i>Odoribacter</i> spp.									
<i>Prevotella</i> spp.	H	↑		↑	↑	↑		↑	↑
Firmicutes Phylum									
<i>Anaerotruncus colihominis</i>	H	↑	↑	↑	↑	↑	↑	↑	↑
<i>Butyrivibrio crossotus</i>	H								
<i>Clostridium</i> spp.									
<i>Coprococcus eutactus</i>		↑			↑	↑		↑	↑
<i>Faecalibacterium prausnitzii</i>		↑				↑			↑
<i>Lactobacillus</i> spp.									
<i>Pseudoflavonifractor</i> spp.	H	↑	↑	↑	↑	↑	↑	↑	↑
<i>Roseburia</i> spp.	L		↓						
<i>Ruminococcus</i> spp.	H	↕↑	↓	↓	↓	↕↑	↕↑	↕↑	↕↑
<i>Veillonella</i> spp.	H	↑	↑	↑	↑	↑	↑		↑
Actinobacteria Phylum									
<i>Bifidobacterium</i> spp.									
<i>Bifidobacterium longum</i>									
<i>Collinsella aerofaciens</i>		↕↑	↕↑	↓	↕↑	↕↑	↕↑	↕↑	↕↑
Proteobacteria Phylum									
<i>Desulfovibrio piger</i>	H								↑
<i>Escherichia coli</i>	H	↑	↑	↑	↑	↑	↑	↑	↑
<i>Oxalobacter formigenes</i>	H	↑		↑	↑				↑
Euryarchaeota Phylum									
<i>Methanobrevibacter smithii</i>	H	↑				↑			↑
Fusobacteria Phylum									
<i>Fusobacterium</i> spp.	H	↑	↑	↑	↑	↑	↑	↑	↑
Verrucomicrobia Phylum									
<i>Akkermansia muciniphila</i>		↓	↓	↓	↓	↓	↓	↓	↓

*Information derived from GDx results data comparing a healthy cohort to various clinical condition cohorts. The chart above showing a comparison of patient results to clinical conditions is meant for informational purposes only; it is not diagnostic, nor does it imply that the patient has a specific clinical diagnosis or condition.

Interpretation At-a-Glance

Biomarker	Patient Results Out of Reference Range	Genova Diagnostics Biomarker Clinical Associations*							
		IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
Pancreatic Elastase	L	↓	↓	↓	↓	↓	↓	↓	↓
Products of Protein Breakdown (Total)							↑↓		
Fecal Fat (Total*)		↑		↑	↑	↑	↓↑	↑	↑
Triglycerides		↑			↑	↑	↑	↑	↑
Long-Chain Fatty Acids		↑			↑	↑	↓↑	↑	↑
Cholesterol							↓↑	↑	
Phospholipids		↑	↑	↑	↑	↑	↑	↑	↑
Calprotectin	H		↑					↑	
Eosinophil Protein X (EPX)	H		↑						
Fecal secretory IgA		↑	↑	↑	↑	↑	↑	↑	↑
Short-Chain Fatty Acids (SCFA) (Total)					↓	↓			
n-Butyrate Concentration				↓					
n-Butyrate %									
Acetate %					↑↓		↓↑		
Propionate %				↑			↑	↑	
Beta-glucuronidase						↑↓			↑↓

*Information derived from GDX results data comparing a healthy cohort to various clinical condition cohorts. The chart above showing a comparison of patient results to clinical conditions is meant for informational purposes only; it is not diagnostic, nor does it imply that the patient has a specific clinical diagnosis or condition.

The arrows indicate Genova's clinical condition cohort test results falling below ↓ or above ↑ the reference range that is greater than that of Genova's healthy cohort.

↑↓ Indicates Genova's clinical condition cohort test results falling below and above the reference range that are greater than that of Genova's healthy cohort.

Cells with bolded arrows indicate Genova's clinical condition cohort had more test results falling above versus below ↓↑ or more below versus above ↑↓ the reference range compared to that of Genova's healthy cohort.

BACTERIOLOGY AND MYCOLOGY

Bacteriology and Mycology Culture

Traditional culture complements DNA-based testing by providing a more complete survey of a patient's gut microbiota beyond the specific organisms targeted by PCR. Culture methods have established clinical utility and are recognized as the 'gold standard' in traditional clinical diagnostics. Culture is necessary to determine therapeutic interventions, such as sensitivities to pharmaceutical or botanical antimicrobial agents.

Bacteriology and mycology culture results are reported as 'No Growth' (NG) or growth using quantification (1+, 2+, 3+ 4+) and a color coding system: Non-pathogen (NP) in green, potential pathogen (PP) in yellow, or known Pathogen (P) in red.





Non-pathogens are normal, commensal flora which have not been recognized as disease-causing. Potential pathogens are considered opportunistic organisms capable of causing symptoms. Pathogens are organisms which are well-recognized in literature to cause disease regardless of the quantity. Since the human microflora is influenced by many factors, pathogenic significance should be based on the patient's clinical presentation.

Beneficial Bacteria Culture:

- *Lactobacillus*, *Escherichia coli*, and *Bifidobacterium* are cultured to offer a more complete microbiome assessment. They are also measured via PCR for quantification.
- *Lactobacillus*, *Escherichia coli*, and *Bifidobacterium* are known to exert positive local and systemic effects in the microbiome.²⁰⁶⁻²⁰⁹
- Lower levels of these beneficial bacteria have been associated with disease.^{210,211}

Additional Bacteria and Mycology Culture:

Any aerobic bacteria or yeast that is grown in culture will be identified using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF) and a Vitek-MS library. Vitek-MS using MALDI-TOF relies on the most extensive FDA-cleared library of microbial targets available on the market, which can accurately identify approximately 200 different additional bacteria and yeast. It should be noted that the technology is capable of identifying a limitless number of organisms. Any organism identified will be reported.

Microbiology Legend			
NG	NP	PP	P
			
No Growth	Non-Pathogen	Potential Pathogen	Pathogen

		1+	2+	3+	4+
Bacteriology (Culture)					
<i>Lactobacillus spp.</i>	2+ NP				
<i>Escherichia coli</i>	4+ NP				
<i>Bifidobacterium</i>	2+ NP				
Additional Bacteria					
<i>alpha haemolytic Streptococcus</i>	4+ NP				
<i>Yersinia enterocolitica</i>	3+ P				
<i>Proteus mirabilis</i>	4+ PP				
Mycology (Culture)					
<i>Candida species</i>	4+ PP				
<i>Candida albicans</i>	3+ PP				

***Please refer to the Pathogenic Bacteria and Yeast Chart online regarding specific pathogenic or potentially pathogenic bacteria and yeast.**

Bacteriology and Mycology Sensitivities

Antimicrobial sensitivities to both pharmaceutical and botanical agents are automatically offered for any pathogenic or potentially pathogenic organism to help guide therapy. The decision to treat should be based on the patient's clinical presentation and symptoms.

Bacteria Sensitivity					
Prescriptive Agents					
<i>Citrobacter amalonaticus</i>	R	I	S-DD	S	NI
Ampicillin	R				
Amox./Clavulanic Acid	R				
Cephalothin	R				
Ciprofloxacin				S	
Tetracycline				S	
Trimethoprim/Sulfa				S	
Natural Agents					
<i>Citrobacter amalonaticus</i>	LOW INHIBITION				HIGH INHIBITION
Berberine	[Shaded bar from 0 to ~60%]				
Oregano	[Shaded bar from 0 to ~50%]				
Plant Tannins	[Shaded bar from 0 to ~75%]				
Uva-Ursi	[Shaded bar from 0 to ~70%]				

For prescriptive agents, an 'R' for resistant or 'S' for sensitive will be placed in the appropriate column:

- **R – (Resistant)** category implies the isolated organism is not inhibited by that prescriptive agent.
- **I – (Intermediate)** category includes isolates which have minimum inhibitory concentration (MIC) values that are obtainable but may be lower than for susceptible isolates.
- **S-DD (Susceptible- Dose Dependent)** category implies better clinical efficacy when a higher than normal drug dosage is used to achieve maximal concentration.
- **S – (Susceptible)** column implies that the isolated organism is inhibited by the prescriptive agent.
- **NI – (No Interpretive Guidelines Established)** category is used for organisms that currently do not have established guidelines for MIC interpretation. Any numerical value placed in this column signifies some inhibition.

For natural agents, inhibition levels indicate how effective the substance was at limiting the organism's growth in vitro. Higher inhibition reflects a greater ability by the substance to limit growth.

The decision to treat any pathogen or potential pathogen should be based on the patient's clinical presentation.

Mycology Sensitivity

Azole Antifungals

<i>Candida species</i>	R	I	S-DD	S	NI
Fluconazole				0.5	
Voriconazole				<=0.008	
Nystatin	=50				

Natural Agents

<i>Candida species</i>	LOW INHIBITION	HIGH INHIBITION
Berberine		
Caprylic Acid		
Garlic		
Undecylenic Acid		
Plant tannins		
Uva-Ursi		

Potassium Hydroxide (KOH) Prep for Yeast

Potassium hydroxide (KOH) is a strong alkali used to clear cellular material and better visualize fungal elements. Results are reported as the amount of yeast detected microscopically:

- Rare: 1-2 per slide
- Few: 2-5 per high power field (HPF)
- Moderate: 5-10 per HPF
- Many: >10 per HPF

These yeast usually represent the organisms isolated by culture. In the presence of a negative yeast culture, microscopic yeast reflects organisms not viable enough to grow in culture. The presence of yeast on the KOH prep should be correlated with the patient's symptoms. However, moderate yeast suggests overgrowth.

Pathogenic Bacteria EIA Testing

The utility of pathogenic bacteria EIA testing is best placed in the context of appropriate differential diagnosis. Clinicians should consider a patient's symptoms and establish a high index of suspicion for a clinically known syndrome or symptom complex. Testing of non-symptomatic patients is not recommended.

***Please refer to the Pathogenic Bacteria and Yeast Chart online regarding specific pathogenic or potentially pathogenic bacteria and yeast.**

- ***Clostridium difficile* (Toxin A/B):**

- *C. difficile* is an opportunistic anaerobic bacterium which causes symptoms ranging from mild diarrhea to pseudomembranous colitis when the normal flora has been altered (as in antibiotic use).
- *C. difficile* produces two toxins. Toxin A is a tissue-damaging enterotoxin, while toxin B is referred to as a cytotoxin.
- A prerequisite for *C. difficile* EIA toxin testing is a stool consistency of 7 on the Bristol stool scale, whereby the sample takes the shape of the container.
- Genova's EIA kit measures antibodies to both toxin A and B. Clinical relevance is determined by the presence of toxin A/B. When these toxins are present, correlation with patient symptoms is recommended.

- **Shiga toxin *E. coli*:**

- Most *E. coli* harmlessly colonize the GI tract as normal flora. However, some have acquired virulence factors such as Shiga toxin.
- Shiga toxin *E. coli* symptoms include bloody diarrhea, vomiting, and can progress to hemolytic uremic syndrome (HUS).
- All enterohemorrhagic *E. coli* (EHEC) can produce Shiga toxin (ST). ST-1 and ST-2 are the most common and EHEC can produce both or either. Therefore, ST detection is a better diagnostic strategy than serotype in the determination of EHEC associated disease.
- Genova's enzyme immunoassay measures monoclonal anti-Shiga toxin antibodies.

- ***Campylobacter* spp.:**

- *Campylobacter* is bacterial pathogen associated with a wide range of symptoms and gastrointestinal conditions. It can cause watery or bloody diarrhea, fever, nausea, and abdominal pain. It is also associated with IBD, Barrett's esophagus, colorectal cancer, and reactive arthritis.²¹²
- Genova's enzyme immunoassay measures a *Campylobacter*-specific antigen.

- ***Helicobacter pylori*:**

- *H. pylori* is an important cause of peptic ulcer disease (PUD) and gastric cancer. It may also have a role in functional dyspepsia, ulcer risk in patients taking low-dose aspirin or starting NSAID therapy, unexplained iron deficiency anemia, and idiopathic thrombocytopenic purpura (ITP).
- According to the American College of Gastroenterology, the indications to test for *H. pylori* infection include active PUD, a history of PUD, low-grade mucosa-associated lymphoid tissue (MALT) lymphoma, or endoscopic early gastric cancer. Patients initiating chronic aspirin or NSAID treatment, those with unexplained iron deficiency anemia, and patients with ITP should be tested.²¹³
- Patients with typical GERD symptoms without a history of PUD, need not be tested for *H. pylori*; however, those who are tested and found to be infected should be treated.²¹³
- Genova uses an enzyme-immunoassay platform that utilizes antibodies to detect *H. pylori* antigen present in the stool sample.

PARASITOLOGY

Currently, there is not one methodology that provides a complete examination for all parasites. The most effective approach is to provide a combination of methodologies to account for varying sensitivities and specificities for all parasitic organisms. Utilizing a single technology cannot fully capture the complex dynamics of the microbiome. Genova's stool profiles offer the most comprehensive parasitology assessment available including:

- Microscopic ova and parasites (O&P)
- PCR for 6 protozoan targets, including reflex *Blastocystis* subtyping 1-9
- Macroscopic examination for worms
- Enzyme Immunoassay (EIA)

When clinical suspicion for a parasitic infection is high, a three-day sample collection is recommended. This traditional recommendation in textbooks and lab manuals to collect at least three samples has been challenged, in an attempt to reduce cost and improve patient ease of use.²¹⁴⁻²¹⁶ Many intestinal protozoa

irregularly shed. Data suggests that a single stool specimen submitted for microscopic examination will detect 58 to 72% of protozoa present. The three specimen evaluation increases the yield by 22.7% for *E. histolytica*, 11.3% for *Giardia*, and 31.1% for *D. fragilis*.¹⁹⁹ However, older studies demonstrated that in at least 90% of cases, examination of only one stool sample was sufficient to detect an enteric parasite.²¹⁴

Purge testing refers to the administration of a laxative prior to sample collection, with the assumption that parasite recovery will be enhanced. Genova has not noted any significant difference in parasite recovery when comparing purged with non-purged specimens. Therefore, it is not necessary to purge prior to specimen collection.

****Please refer to the Parasitic Organisms Chart online regarding specific pathogenic or potentially pathogenic parasites.***

Microscopic Ova & Parasites (O&P)

Microscopic examination of stool specimens for ova and parasites (O&P) is considered the gold-standard stool parasite testing methodology for traditional laboratories. Factors that influence the sensitivity of microscopic parasite examinations include the specimen collection interval, patient medications, and stool preservation prior to testing.²¹⁷

The organism's correct identification is subjective, and highly dependent on the technician's training and experience. Genova's microbiology staff is highly trained and employs technicians with decades of experience. Based on Genova's proficiency test scores, our sensitivity (detecting a parasite present) is >97%, and our accuracy (correctly identifying it) is >98%.

While the O&P exam can detect any and all parasites, some parasites are more difficult to detect due to their small size, irregular shedding schedules, etc. Additional testing methods are recommended to enhance sensitivity, such as PCR or EIA.

A negative O&P microscopy result is reported as "Not Detected." A positive finding is reported as the amount of that organism (rare, few, moderate, many), followed by the organism's morphology characteristics (trophozoites, cysts, ova.)

- Rare: 1-2 per slide
- Few: 1-2 per high powered field (HPF)
- Moderate: 2-5 per HPF
- Many: >5 per HPF

Parasitology**	
Microscopic O&P Results	
Microscopic O&P is capable of detecting all described gastrointestinal parasites. The organisms listed in the box represent those commonly found in microscopic stool analysis. Should an organism be detected that is not included in the list below, it will be reported in the Additional Results section. For an extensive reference of all potentially detectable organisms, please visit www.gdx.net/product/gi-effects-comprehensive-stool-test	
Genus/species	Result
Nematodes - roundworms	
<i>Ancylostoma/Necator</i> (Hookworm)	Not Detected
<i>Ascaris lumbricoides</i>	Not Detected
<i>Capillaria philippinensis</i>	Not Detected
<i>Enterobius vermicularis</i>	Not Detected
<i>Strongyloides stercoralis</i>	Not Detected
<i>Trichuris trichiura</i>	Not Detected
Cestodes - tapeworms	
<i>Diphyllobothrium latum</i>	Not Detected
<i>Dipylidium caninum</i>	Not Detected
<i>Hymenolepis diminuta</i>	Not Detected
<i>Hymenolepis nana</i>	Not Detected
<i>Taenia</i> spp.	Not Detected
Trematodes - flukes	
<i>Clonorchis/Opisthorchis</i> spp.	Not Detected
<i>Fasciola</i> spp./ <i>Fasciolopsis buski</i>	Not Detected
<i>Heterophyes/Metagonimus</i>	Not Detected
<i>Paragonimus</i> spp.	Not Detected
<i>Schistosoma</i> spp.	Not Detected
Protozoa	
<i>Balantidium coli</i>	Not Detected
<i>Blastocystis</i> spp.	Rare Detected
<i>Chilomastix mesnili</i>	Not Detected
<i>Cryptosporidium</i> spp.	Not Detected
<i>Cyclospora cayetanensis</i>	Not Detected
<i>Dientamoeba fragilis</i>	Moderate Detected
<i>Entamoeba coli</i>	Not Detected
<i>Entamoeba histolytica/dispar</i>	Not Detected
<i>Entamoeba hartmanii</i>	Not Detected
<i>Entamoeba polecki</i>	Not Detected
<i>Endolimax nana</i>	Not Detected
<i>Giardia</i>	Not Detected
<i>Iodamoeba buetschlii</i>	Not Detected
<i>Cystoisospora</i> spp.	Not Detected
<i>Trichomonads</i> (e.g. <i>Pentatrichomonas</i>)	Not Detected
Additional Findings	
White Blood Cells	Not Detected
Charcot-Leyden Crystals	Not Detected
Other Infectious Findings	

Other Microscopic Findings:

Charcot-Leyden crystals may be seen under the microscope. This is an eosinophil breakdown product and is present in patients with tissue-invading parasites and allergic conditions.^{218,219} They are observed more commonly in the sputum of asthmatics, but are rarely found in the stool.²²⁰ Studies show that Charcot-Leyden crystals can be present with *E. histolytica* and *Blastocystis* infections.²²¹ Allergy assessment may be warranted in symptomatic patients that do not have a parasite and may include ordering a serum IgE allergy panel. While rare, Charcot-Leyden crystals may indicate eosinophilic gastroenteritis which requires evaluation with endoscopy.^{220,222}

White blood cells (WBC) indicate an immune response that can be seen in infectious conditions or inflammatory bowel disease (IBD).

Red blood cells (RBC) indicate blood in the stool. RBCs can be seen with bleeding hemorrhoids or menstrual blood, as well as serious conditions such as malignancy

or IBD. If a serious condition is suspected, a follow-up fecal occult blood test or colonoscopy is recommended. *Entamoeba histolytica* can engulf RBCs which can distinguish the pathogenic *E. histolytica* from the non-pathogenic *E. dispar*.²²³

Vegetable and meat fibers are undigested food particles that are sometimes seen microscopically or macroscopically. They may indicate maldigestion and/or malabsorption. Correlation with symptoms and other biomarkers of maldigestion/malabsorption is recommended. Biomarkers of maldigestion and malabsorption include pancreatic elastase 1, products of protein breakdown, and fecal fats.

PCR Parasitology- Protozoa

Polymerase Chain Reaction (PCR) is a method that utilizes probes targeting specific DNA segments, which allow identification of specific organisms. It is sometimes called “molecular photocopying” since small DNA segments are amplified, or copied.²²⁴

Genova’s 6 parasite targets include *Cryptosporidium parvum/hominis*, *Entamoeba histolytica*, *Giardia*, *Blastocystis* spp., *Cyclospora cayetanensis*, and *Dientamoeba fragilis*. They are assessed via real-time PCR (also known as quantitative PCR, or qPCR.)

Certain organisms are difficult to recover or visualize

microscopically. PCR offers enhanced sensitivity. This is especially important for those organisms that present a public health concern, such as *Entamoeba histolytica*, *Cyclospora cayetanensis* or *Cryptosporidium parvum/hominis*.

Until all potential human parasitic pathogens are included in molecular panels, PCR will remain highly sensitive but will fail to detect the scope of possible pathogens that can be found via an O&P microscopic exam.²²⁵

Parasitology					
PCR Parasitology - Protozoa**			Methodologies: DNA by PCR, Next Generation Sequencing		
Organism	Result	Units		Expected Result	
<i>Blastocystis</i> spp.	6.00e2	femtograms/microliter C&S stool	Detected	Not Detected	
<i>Cryptosporidium</i> spp.	<4.87e2	genome copies/microliter C&S stool	Not Detected	Not Detected	
<i>Cyclospora cayetanensis</i>	<2.65e2	genome copies/microliter C&S stool	Not Detected	Not Detected	
<i>Dientamoeba fragilis</i>	6.40e2	genome copies/microliter C&S stool	Detected	Not Detected	
<i>Entamoeba histolytica</i>	<1.14e3	genome copies/microliter C&S stool	Not Detected	Not Detected	
<i>Giardia</i>	<1.57e2	genome copies/microliter C&S stool	Not Detected	Not Detected	
<i>Blastocystis</i> spp. Reflex Subtyping					
Type 1:	Not Detected	Type 4:	Not Detected	Type 7:	Not Detected
Type 2:	Detected	Type 5:	Not Detected	Type 8:	Not Detected
Type 3:	Not Detected	Type 6:	Not Detected	Type 9:	Not Detected

The PCR results for the 6 organisms are reported as detected or not detected.

The *Blastocystis* subtyping is a reflex exam that will only populate if the organism is detected. Subtypes 1-9 have been developed, and only those subtypes found will be reported.

Positive PCR, negative microscopy

A positive PCR means the organism’s DNA was detected, but the organism itself could not be found or visualized under the microscope. In the case of irregular organism shedding, it can be difficult to detect an organism microscopically. Additionally, parasite DNA may be detected regardless of organism viability; it is possible the organism was dead upon transmission. Correlation with symptoms is always recommended regardless of any test findings. The clinical effect of nonviable parasite DNA passing through the host organism is not known.

Negative PCR, positive microscopy

Possible reasons for this finding include sample mishandling, interfering substances, PCR assay step failure, or misidentification on microscopic exam. Additionally, the PCR testing is performed on the third-day vial, while microscopy is performed using a homogenized sample mixing all three days of stool. If a parasite intermittently sheds, it may be possible to miss in PCR since only one stool sample is tested.

Additionally, approximately 15% of samples submitted for parasite detection via PCR will demonstrate inhibition of the PCR reaction. This inhibition rate can be due to many factors, such as medications, excessive unrelated DNA, and other constituent stool factors. With dilution of the extracted DNA, the rate of reaction inhibition can be cut in half. This has been documented in peer reviewed literature as well as studies supporting FDA approval of these commercial assays. Genova’s internal data review

and external validation studies have confirmed a similar inhibition rate for our laboratory developed assay. Genova performs sample dilution to lower the percentage of inhibition, however, for those samples continuing to exhibit inhibition we will not report results. This is due to the fact that further dilution will adversely impact the limit of detection and may result in false negatives. Additionally Genova will not increase the number of amplification cycles in order to compensate for the reduced sensitivity due to dilution. This approach may result in amplification of artifact and thus generate false positives.

With any laboratory-developed test, it is critical that there be agreement with a proven, clinically valid FDA method. PCR parasitology should always be validated by comparison to proven standards, such as enzyme-linked

immunoassay or microscopic ova and parasite methods. Genova combines microscopic parasite detection with PCR for relevant parasites. This multi-pronged approach results in a comprehensive, highly sensitive, and highly specific assessment of parasite infection. It also helps mitigate the impact of sporadic shedding, rare parasite presence and PCR inhibition that can adversely impact the results given when using a single technology.

This PCR assay inhibition is rarely seen when reporting results for commensal bacterial DNA. This is due to the much higher concentration of these bacteria relative to the low levels of parasites in stool specimens. Thus dilution of these samples can overcome inhibition of the PCR reaction but not at the expense of the detection limit of the assay.

EIA Parasitology

Enzyme-immunoassay (EIA) is a methodology that detects specific parasite antigens. Genova offers FDA-cleared EIA for *Giardia*, *Entamoeba histolytica*, and *Cryptosporidium*. Genova combines microscopic parasite detection with EIA for relevant parasites. This multi-pronged approach results in a comprehensive, highly sensitive, and highly specific assessment of parasite infection.

PARASITOLOGY EIA TESTS:		
	In Range	Out of Range
Cryptosporidium ♦	Negative	
Giardia lamblia ♦	Negative	
Entamoeba histolytica ♦	Negative	

Macroscopic Examination for Worms

Most nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms) to a lesser degree, are primarily diagnosed by ova in the stool during the microscopic O&P exam.

The technician performs a gross examination of the entire specimen to look for macroscopic evidence of proglottids (tapeworm segments) or whole worms prior to doing the microscopic examination.

If a patient sees worms in the stool, they should remove the worm from the stool and place it in the vial clean of any stool, or in a separate container for transport to the lab.

While pinworm eggs can be seen in a stool sample submitted for O&P exam, there is often a low yield. The best way to diagnose pinworms is the "tape test," or "Scotch tape test."

****Please refer to the Parasitic Organisms Chart online regarding specific pathogenic or potentially pathogenic parasites.***

Therapeutic considerations for Parasitology:

Correct identification of the organism allows the clinician to choose appropriate treatment protocols aimed toward infection resolution. Treatment should be patient specific.

Genova is unable to provide sensitivities for parasitic organisms. The collection vial contains a fixative/preservative such that the organism arrives dead. Only live organisms can be cultured for sensitivities.

Intestinal parasites are spread via soil, food, water, and surfaces that are contaminated with feces from infected humans or animals.²²⁶ Optimizing personal and community hygiene, in addition to sanitary measures to prevent contamination with fecal material, are essential (i.e. hand washing, washing and peeling raw vegetables and fruit, avoiding unboiled tap water when traveling).^{225,227}

The following resources provide valuable insight into the practical, clinical management of parasitic infections:

- The Sanford Guide to Antimicrobial Therapy
- Centers for Disease Control – monographs on individual parasites <https://www.cdc.gov/dpdx/>
- World Health Organization – maps showing geographic prevalence <http://www.who.int/>
- American Journal of Gastroenterology 2018 article “Beyond O&P Times Three.” This article outlines multiple organisms, their symptomatology, and differential diagnoses, and discusses testing and management.²¹⁶

- Garcia, et al. 2018 article “Laboratory Diagnosis of Parasites from the Gastrointestinal Tract.” This is an 81-page guide on lab diagnosis, versus clinical features.²¹⁸
- CDC hotline for healthcare providers with questions regarding parasites:
 - Parasitic Diseases Hotline (M-F; 8am-4pm EST) 404-718-4745
 - Emergency, after-hours hotline 770-488-7100

Generally, symptom resolution does not warrant follow-up testing.²²⁸ Retesting PCR should not be used to document cure.^{229,230}

Reflex *Blastocystis* Subtyping 1-9

If *Blastocystis* is found via PCR, reflex subtyping (ST) for ST1-ST9 will be performed. *Blastocystis* subtyping is performed using Next Generation DNA Sequencing.

Biomarker Key Points:

The pathogenicity of individual *Blastocystis* subtypes has been studied, with some subtypes presumed to be more pathogenic than others, although literature is still evolving.

Although most individuals host only a single ST, it is possible to have more than one subtype, or a mixed-ST infection.^{231,232}

If *Blastocystis* is found either on microscopic O&P or via PCR, but subtypes are not detected, then the patient has a subtype other than ST1-ST9. Finding a subtype other than ST1-ST9 in humans is rare. There are 17 known STs, and ST1-ST9 have been reported in humans.

ST1-ST4 are the most prevalent, representing approximately 90% of all isolates. ST3 is the most common, with a predominance of around 60% of all isolates.^{227,231,233}

There is regional distribution of specific *Blastocystis* subtypes - each with differing zoonotic transmissions.^{221,227,231,234-242}

Subtype	Geographic Distribution	Animal Transmission	Associated Symptoms/ Conditions
Subtype 1	Worldwide	Pigs, monkeys, cattle, birds, rodents, dogs	Seen in GI symptomatic patients; possibly most virulent subtype; IBS-D, traveler’s diarrhea, asymptomatic
Subtype 2	China (Eryuan, Yunnan), Denmark, Germany, Greece, Japan, Turkey, Ireland, USA, S America	Pigs, monkeys, cattle, birds, rodents, dogs	Less pathogenic; possible bloating; diarrhea
Subtype 3 most common in humans	Worldwide	Primates, pigs, dogs, cattle, rodents	Most prevalent in symptomatic patients; Seen in GI symptomatic patients; urticaria; diarrhea; IBD; asymptomatic
Subtype 4	Denmark, Germany, Greece, Japan, Nepal, Spain, Australia, Europe in general, China (Yunnan)	Rodents, primates	Seen in GI symptomatic patients; diarrhea; uses proteases to degrade luminal IgA; dysbiosis
Subtype 5	Thailand	Livestock, apes, monkeys, pigs, birds	N/A (N/A information not available)
Subtype 6	Japan, Pakistan, Egypt, Greece	Birds, livestock	N/A
Subtype 7	Japan, Pakistan, Egypt, Thailand, Turkey, Greece, China (Guangxi)	Birds	Multiple intestinal symptoms; IBS; uses proteases to destroy tissues and degrade luminal IgA; dysbiosis
Subtype 8	N/A	Marsupials, primates, birds	N/A
Subtype 9	Japan	N/A	Multiple intestinal symptoms

Blastocystis has known treatment resistance, with multiple causes:

- Morphologic states (vacuolar, amoeboid, granular, cyst) vary in sensitivity to treatment (i.e., the cyst stage is known to be resistant to metronidazole).²⁴³
- Different subtypes vary in sensitivity to treatment due to different genetic makeup and thus mechanism of pathogenicity.
 - Studies show that the pathogenic potential of ST3 is enhanced when isolates were treated with metronidazole, suggesting a mechanism to produce higher numbers of viable cells to ensure survival during stressed conditions.²⁴⁴⁻²⁴⁶
 - *Blastocystis*-derived proteases found in ST4 and ST7 have been shown to degrade luminal secretory IgA (sIgA), leading to an ineffective immune response, favoring a chronic, symptomatic *Blastocystis* infection.^{235,247} In-vitro studies using cysteine protease inhibitors show efficacy against *Blastocystis* isolates, however there are no commercially available cysteine protease inhibiting drugs.^{243,248}
 - In vitro studies show that certain ST may be more sensitive to drugs that affect nitric oxide (NO), and that ST7 actually has the ability to modulate the antiparasitic host NO defense for its own survival.^{249,250}
- The microbiome may influence treatment outcomes.²⁴⁹
- Reinfection may be mistaken for treatment failure or resistance.
- *Blastocystis* isolates from differing geographical regions have different degrees of metronidazole resistance.²⁴⁹

***Blastocystis* Subtype Treatment**

Research on individual treatments for *Blastocystis* subtypes is in its early stages, and evidence is inconclusive. There is no single drug that is effective across all isolates of different *Blastocystis* subtypes.²⁴⁹ Pre-clinical, in-vitro studies have mixed results.^{251,234}

In-vitro studies are difficult to translate clinically because the isolates are outside of the human microbiome environment, and *Blastocystis* is known to feed on bacteria.²⁴³

Human studies on treating *Blastocystis* subtypes are limited mainly to case studies. Large-scale clinical outcome studies regarding effective treatments for *Blastocystis* subtypes are lacking; however, this presents

an opportunity for Genova and clinicians to correlate patient data as the literature evolves.

The most current, literature-based information on possible therapeutics for individual subtypes is summarized in the tables below.

Note that the findings in the literature may not be consistent with Genova's findings due to different methodologies, thus treatment efficacy may vary. Furthermore, Table 1 shows in-vitro sensitivities for conventional and experimental agents which may or may not translate clinically, Table 2 shows animal studies, and Table 3 shows human case studies representing a very small number of patients. Basing treatment from these findings may or may not be effective.

Certain in-vitro prescriptive agents are experimental and have not been tested clinically for Blastocystis infection. In the meantime, clinicians may decide to treat a symptomatic patient using their current treatment for Blastocystis in general until more definitive information is available on individual subtype treatment.

Treatment selection is at the discretion of the clinician.

Table 1: In-vitro sensitivities (including conventional and experimental agents) for treating *Blastocystis* spp. subtypes.^{243,244,246,248,249,251-256}

TMP/SMX = Trimethoprim/Sulfamethoxazole; MTZ = Metronidazole; NTZ = Nitazoxanide; NAC = N-Acetyl Cysteine; N/A = information not available

	Sensitive	Resistant
ST1	<ul style="list-style-type: none"> • TMP/SMX (most effective) • MTZ (effective ↑ concentrations, but not total clearance) • Albendazole (effective ↓ concentrations) • Garlic • <i>Achillea millefolium</i> (Yarrow) • <i>Artemisia judaica</i> • Egyptian propolis • Note: pathogenic potential may be enhanced by NAC, producing higher numbers of viable cells for survival 	<ul style="list-style-type: none"> • Antifungals (fluconazole, nystatin, itraconazole ketoconazole) • TMP/SMX (lower doses) • Ginger, black pepper, cumin
ST2	<i>Achillea millefolium</i> (Yarrow)	N/A
ST3	<ul style="list-style-type: none"> • MTZ (effective ↑ concentrations, but not total clearance; pathogenic potential may be enhanced, producing higher numbers of viable cells for survival) • TMP/SMX (effective ↓ concentrations) • Garlic • <i>Artemisia judaica</i> • <i>Ferula asafoetida</i> • <i>Achillea millefolium</i> (Yarrow) • <i>Eurycoma longifolia</i> (Tongkat Ali) • Egyptian propolis • Lactic acid bacteria including <i>Lactobacillus rhamnosus</i>, <i>Lactococcus lactis</i> 	<ul style="list-style-type: none"> • Antifungals (fluconazole, nystatin, itraconazole, ketoconazole) • NTZ (lower doses) • Ginger, black pepper, cumin
ST4	<ul style="list-style-type: none"> • TMP/SMX (most effective; 1:2 combination more effective versus 1:5) • MTZ (effective ↑ concentrations, but not total clearance) • Albendazole (effective ↑ concentrations) • Ronidazole • Ornidazole • Nitazoxanide • Furazolidone • Mefloquine • Quinidine • Quinine • Iodoacetamide • Note: pathogenic potential may be enhanced by NAC, producing higher numbers of viable cells for survival 	<ul style="list-style-type: none"> • Antifungals (fluconazole, nystatin, itraconazole) • MTZ • Emetine (literature mixed) • Paromomycin • Chloroquine • Doxycycline • Ampicillin • Pyrimethamine
ST5	MTZ	Ketoconazole
ST6	N/A	N/A
ST7	<ul style="list-style-type: none"> • TMP/SMX (1:2 combination more effective versus 1:5) • Emetine (literature mixed; limited use clinically due to severe side effects) • Ronidazole • Ornidazole, Nitazoxanide • Furazolidone • Mefloquine • Quinidine • Quinine • Iodoacetamide • Note: pathogenic potential may be enhanced by NAC, producing higher numbers of viable cells for survival 	<ul style="list-style-type: none"> • MTZ • Paromomycin • Chloroquine • Doxycycline • Ampicillin • Pyrimethamine
ST8	<ul style="list-style-type: none"> • TMP/SMX (most effective) • MTZ (effective ↑ concentrations, but not total clearance) 	Antifungals (fluconazole, nystatin, itraconazole)
ST9	N/A	N/A

Table 2: Animal studies for treating *Blastocystis* spp. subtypes.²⁵⁷

Subtype(s)	Animal	Treatment
ST3	Rat	Five groups of ST3-infected rats were treated with either <i>Saccharomyces boulardii</i> (Sb), metronidazole (MTZ), Sb extract, Sb+MTZ, or placebo. Combination of Sb+MTZ was most effective with 100% eradication.

Table 3: Human studies for treating *Blastocystis* spp. subtypes.²⁵⁸⁻²⁶⁵

TMP/SMX = Trimethoprim/Sulfamethoxazole; MTZ = Metronidazole; NTZ = Nitazoxanide; TAB = triple antibiotic therapy

Author, year	Study Type	Subtype(s)	Outcomes
Angelici, 2018	Case study; 41 y.o. male with chronic GI symptoms after acute GI symptoms drinking contaminated well water; infection became chronic and patient refused conventional therapies	ST1	<i>Saccharomyces boulardii</i> was effective whereas a probiotic w/ <i>Lactobacillus</i> & <i>Bifidobacterium</i> was not effective
Nagel, 2014	Prospective, longitudinal case study; n=10 IBS-D patients treated with 14 days of TAB (diloxanide furoate, TMP/SMX, secnidazole); follow up testing day 15 and 4 weeks following TAB	ST1, ST3, ST4, ST7, ST8	Successful eradication occurred in 60% of patients, however no correlations could be made between subtype and eradication or subtype and clearance of symptoms
Nagel, 2012	Prospective, longitudinal case study; n=11 symptomatic patients treated with 14 days of either MTZ or TMP/SMX; follow up testing day 15, 28 and 56	ST1, ST3, ST4, ST6	No patient cleared the organism following either monotherapy
Stensvold, 2013	Case study; 40 y.o. female hospitalized with severe traveler's diarrhea and fever, and later developed chronic post-infectious GI symptoms of bloating, flatulence and abdominal pain	ST8	Patient treated with 2 rounds of 10 days of MTZ with little symptom relief, and later 10 days of TMP/SMX with marked symptom improvement and negative stool testing
Roberts, 2013	Prospective, longitudinal case study; n=18 patients with diarrhea, abdominal cramps and bloating treated with multiple regimens including MTZ, iodoquinol, doxycycline, NTZ, furazolidone, secnidazole, ciprofloxacin, tinidazole, norfloxacin, and paromomycin	ST1, ST3, ST4, ST5	Paromomycin was the only therapy that resulted in eradication and symptom resolution in 3 patients with either ST3 or ST5
Katsarou-Katsari, 2008	Case study; 19 y.o. male with 3 week hx of urticaria, soft stools and 2.5 month hx abdominal pain	ST3	Patient treated with MTZ for 10 days and experienced resolution of urticaria and GI symptoms
Vogel-berg, 2010	Case study; 20 y.o. male with chronic urticaria and flatulence	ST2	Patient treated with MTZ with no symptom relief, and later TMP/SMX with no symptom relief; finally treated with combo of MTZ and paromomycin with symptom resolution and negative stool testing
Jones, 2008	Cross-sectional observational study; n=21 symptomatic patients with fatigue, depression, skin rash, joint pain, constipation, abdominal pain, diarrhea; 9/21 patients positive for <i>Blastocystis</i> with six having ST3 and one having ST1	ST1, ST3	Most patients reported failure with MTZ

ADDITIONAL TESTS

Several additional tests have long been used in the analysis of stool. These include stool color and consistency, as well as the presence or absence of occult blood.

Color: Stool color is primarily associated with diet and medication use, though it may indicate various GI health conditions.

Occult Blood

The term 'occult blood' simply means blood that is not evident to the naked eye and present in microscopic quantities only. Genova uses the Hemosure diagnostic kit to measure occult blood.

- The Hemosure diagnostic kit uses fecal immunochemical testing (FIT). It has higher specificity than common guaiac testing because of

Consistency: Stool consistency may vary from hard to watery. This is self-reported by the patients upon submission of the stool sample. The technical ability to measure diagnostic biomarkers from stool may be influenced by consistency extremes.

its use of mono- and polyclonal antibodies specific to human hemoglobin.

- FIT-based diagnostics have been recommended by the American College of Gastroenterology as the preferred test for colorectal cancer screening/detection.

Zonulin Family Peptide

Intestinal barrier transport is mainly regulated by structures of the paracellular pathway called tight junctions, which form barriers between epithelial cells and regulate the transport of ions and small molecules across the intestinal lumen. Zonulin has been identified as a tight junction regulating protein.

Biomarker Key Points:

In this assessment, Genova uses a kit from the manufacturer Immundiagnostik (IDK). A research paper published in *Frontiers in Endocrinology* by Scheffler et al. suggested that the zonulin kits from IDK do not detect zonulin (a precursor of haptoglobin 2). This issue was further confirmed by the kit manufacturer in a statement released to clinical laboratories.²⁶⁶

To the best of Genova's knowledge, the Scheffler paper has impacted the zonulin assay across the United States, including Genova's serum and stool zonulin tests. Because some researchers are conducting studies and have received data from the current zonulin kits, Genova has decided to provide the test **for research use only** with the manufacturer's suggested name: "zonulin family peptide."

The Scheffler paper suggests that the kits may detect properdin, a protein involved in the alternative complement pathway and inflammation. Preliminary study results from an external investigator suggest that properdin may be structurally and functionally similar to zonulin. Another study confirmed that zonulin was not detected, but possibly complement C3, which plays a role in the modulation of intestinal epithelial barrier integrity. The structural similarity of these proteins makes identification challenging.²⁶⁷

Over 60 papers have been published using the IDK kit and clinical associations have been observed ranging from metabolic and liver diseases to mood disorders. The majority of studies have focused on serum concentrations.

Genova's unpublished data analysis (of 13,613 tests) demonstrated that the test results of the current stool zonulin kit (now called zonulin family peptide) were strongly and positively associated with stool EPX and sIgA (but not calprotectin). Levels of zonulin family peptide detected by this kit were also associated with a commensal bacterial profile related to intestinal inflammation. In addition, they were also positively associated with stool biomarkers such as fecal PE-1 and cholesterol. Some biomarkers, such as stool fat and short-chain fatty acids, showed "bell-shaped" distributions. High or low levels of the zonulin family peptide were associated with low levels of stool fat and short-chain fatty acids.

Therapeutic considerations for zonulin family peptide:

- The clinical significance of an **elevated** zonulin family peptide is unknown. It may relate to increased intestinal permeability and results should be confirmed with a follow up lactulose/mannitol **Intestinal Permeability Assessment**. Studies on athletes show a reduction in stool zonulin family peptide levels with colostrum and probiotics.^{268,269}
- **A normal or low zonulin** family peptide finding does not necessarily rule out intestinal permeability. A follow up lactulose/mannitol **Intestinal Permeability Assessment** should be considered if intestinal permeability is suspected.

REFERENCES

- Chen L, Reynolds C, David R, Peace Brewer A. Development of an Index Score for Intestinal Inflammation-Associated Dysbiosis Using Real-World Stool Test Results. *Dig Dis Sci*. 2019.
- Löser C, Möllgaard A, Fölsch U. Faecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test. *Gut*. 1996;39(4):580-586.
- Dominguez-Muñoz JE, Hardt PD, Lerch MM, Löhr MJ. Potential for screening for pancreatic exocrine insufficiency using the fecal elastase-1 test. *Dig Dis Sci*. 2017;62(5):1119-1130.
- Carroccio A, Fontana M, Spagnuolo MI, et al. Pancreatic dysfunction and its association with fat malabsorption in HIV infected children. *Gut*. 1998;43(4):558-563.
- Carroccio A, Iacono G, Ippolito S, et al. Usefulness of faecal elastase-1 assay in monitoring pancreatic function in childhood coeliac disease. *Ital J Gastroenterol Hepatol*. 1998;30(5):500-504.
- Icks A, Haastert B, Giani G, Rathmann W. Low fecal elastase-1 in type I diabetes mellitus. *Z Gastroenterol*. 2001;39(10):823-830.
- Sziegoleit A, Linder D. Studies on the sterol-binding capacity of human pancreatic elastase 1. *Gastroenterology*. 1991;100(3):768-774.
- Singh VK, Haupt ME, Geller DE, Hall JA, Quintana Diez PM. Less common etiologies of exocrine pancreatic insufficiency. *World J Gastroenterol* : *WJG*. 2017;23(39):7059-7076.
- Othman MO, Harb D, Barkin JA. Introduction and practical approach to exocrine pancreatic insufficiency for the practicing clinician. *Int J Clin Pract*. 2018.
- Struyvenberg MR, Martin CR, Freedman SD. Practical guide to exocrine pancreatic insufficiency - Breaking the myths. *BMC Med*. 2017;15(1):29.
- Lindkvist B. Diagnosis and treatment of pancreatic exocrine insufficiency. *World J Gastroenterol*. 2013;19(42):7258-7266.
- Elborn JS. Cystic fibrosis. *Lancet*. 2016;388(10059):2519-2531.
- Majumder S, Chari ST. Chronic pancreatitis. *Lancet*. 2016;387(10031):1957-1966.
- Ghaneh P, Neoptolemos JP. Exocrine pancreatic function following pancreatectomy. *Ann N Y Acad Sci*. 1999;880:308-318.
- Dominguez-Munoz JE, P DH, Lerch MM, Lohr MJ. Potential for Screening for Pancreatic Exocrine Insufficiency Using the Fecal Elastase-1 Test. *Dig Dis Sci*. 2017;62(5):1119-1130.
- Hardt PD, Bretz L, Krauss A, et al. Pathological pancreatic exocrine function and duct morphology in patients with cholelithiasis. *DigDisSci*. 2001;46(3):536-539.
- Vujasinovic M, Valente R, Del Chiaro C, Permert J, Lohr JM. Pancreatic Exocrine Insufficiency in Pancreatic Cancer. *Nutrients*. 2017;9(3).
- Vujasinovic M, Valente R, Thorell A, et al. Pancreatic Exocrine Insufficiency after Bariatric Surgery. *Nutrients*. 2017;9(11).
- Nousia-Arvanitakis S, Fotoulaki M, Tendzidou K, Vassilaki C, Agguridaki C, Karamouzis M. Subclinical exocrine pancreatic dysfunction resulting from decreased cholecystokinin secretion in the presence of intestinal villous atrophy. *J Pediatr Gastroenterol Nutr*. 2006;43(3):307-312.
- Vujasinovic M, Tepes B, Volfand J, Rudolf S. Exocrine pancreatic insufficiency, MRI of the pancreas and serum nutritional markers in patients with coeliac disease. *Postgrad Med J*. 2015;91(1079):497-500.
- Leeds JS, Hopper AD, Hurlstone DP, et al. Is exocrine pancreatic insufficiency in adult coeliac disease a cause of persisting symptoms? *Aliment Pharmacol Ther*. 2007;25(3):265-271.
- Walkowiak J, Herzig KH. Fecal elastase-1 is decreased in villous atrophy regardless of the underlying disease. *Eur J Clin Invest*. 2001;31(5):425-430.
- Gomez JC, Moran CE, Maurino EC, Bai JC. Exocrine pancreatic insufficiency in celiac disease. *Gastroenterology*. 1998;114(3):621-623.
- Pitchumoni CS, Rubin A, Das K. Pancreatitis in inflammatory bowel diseases. *J Clin Gastroenterol*. 2010;44(4):246-253.
- Othman MO, Harb D, Barkin JA. Introduction and practical approach to exocrine pancreatic insufficiency for the practicing clinician. *Int J Clin Pract*. 2018;72(2).
- Baffy G, Boyle JM. Association of Zollinger-Ellison syndrome with pancreatitis: report of five cases. *Dig Dis Sci*. 2000;45(8):1531-1534.
- Herzig KH, Purhonen AK, Rasanen KM, et al. Fecal pancreatic elastase-1 levels in older individuals without known gastrointestinal diseases or diabetes mellitus. *BMC geriatrics*. 2011;11:4.
- Conwell DL, Lee LS, Yadav D, et al. American Pancreatic Association Practice Guidelines in Chronic Pancreatitis: evidence-based report on diagnostic guidelines. *Pancreas*. 2014;43(8):1143-1162.
- Capurso G, Signoretti M, Archibugi L, Stigliano S, Delle Fave G. Systematic review and meta-analysis: Small intestinal bacterial overgrowth in chronic pancreatitis. *United Eur Gastroenterol J*. 2016;4(5):697-705.
- Bures J, Cyraný J, Kohoutova D, et al. Small intestinal bacterial overgrowth syndrome. *World J Gastroenterol*. 2010;16(24):2978-2990.
- Lappinga PJ, Abraham SC, Murray JA, Vetter EA, Patel R, Wu TT. Small intestinal bacterial overgrowth: histopathologic features and clinical correlates in an underrecognized entity. *Arch Pathol Lab Med*. 2010;134(2):264-270.
- Teichmann J, Riemann JF, Lange U. Prevalence of exocrine pancreatic insufficiency in women with obesity syndrome: assessment by pancreatic fecal elastase 1. *Gastroenterology*. 2011;2011:951686.
- Walkowiak J, Madry E, Lisowska A, et al. Adaptive changes of pancreatic protease secretion to a short-term vegan diet: influence of reduced intake and modification of protein. *British J Nutr*. 2012;107(2):272-276.
- Piciocchi M, Capurso G, Archibugi L, Delle Fave MM, Capasso M, Delle Fave G. Exocrine pancreatic insufficiency in diabetic patients: prevalence, mechanisms, and treatment. *Int J Endocrinol*. 2015;2015:595649.
- Rathmann W, Haastert B, Oscarsson J, Berglind N, Lindkvist B, Wareham NJ. Association of faecal elastase 1 with non-fasting triglycerides in type 2 diabetes. *Pancreatology*. 2016;16(4):563-569.
- Lohr JM, Oliver MR, Frulloni L. Synopsis of recent guidelines on pancreatic exocrine insufficiency. *United Eur Gastroenterol J*. 2013;1(2):79-83.
- Durie P, Baillaergeon JD, Bouchard S, Donnellan F, Zepeda-Gomez S, Teshima C. Diagnosis and management of pancreatic exocrine insufficiency (PEI) in primary care: consensus guidance of a Canadian expert panel. *Curr Med Res Opin*. 2018;34(1):25-33.
- Wang X, Wang J, Rao B, Deng L. Gut flora profiling and fecal metabolite composition of colorectal cancer patients and healthy individuals. *Exp Therap Med*. 2017;13(6):2848-2854.
- Amiot A, Dona AC, Wijeyesekera A, et al. (1)H NMR Spectroscopy of Fecal Extracts Enables Detection of Advanced Colorectal Neoplasia. *J Proteome Res*. 2015;14(9):3871-3881.
- Szczesniak O, Hestad KA, Hanssen JF, Rudi K. Isovaleric acid in stool correlates with human depression. *Nutr Neurosci*. 2016;19(7):279-283.
- Hoverstad T, Bjorneklett A, Fausa O, Midtvedt T. Short-chain fatty acids in the small-bowel bacterial overgrowth syndrome. *Scand J Gastroenterol*. 1985;20(4):492-499.
- Kotani A, Miyaguchi Y, Kohama M, Ohtsuka T, Shiratori T, Kusu F. Determination of short-chain fatty acids in rat and human feces by high-performance liquid chromatography with electrochemical detection. *Analytical Sci* . 2009;25(8):1007-1011.
- Geypens B, Claus D, Evenepoel P, et al. Influence of dietary protein supplements on the formation of bacterial metabolites in the colon. *Gut*. 1997;41(1):70-76.
- Tursi A, Mastromarino P, Capobianco D, et al. Assessment of Fecal Microbiota and Fecal Metabolome in Symptomatic Uncomplicated Diverticular Disease of the Colon. *J Clin Gastroenterol*. 2016;50 Suppl 1:S9-S12.
- Camirero A, Nistal E, Herran AR, et al. Differences in gluten metabolism among healthy volunteers, coeliac disease patients and first-degree relatives. *Br J Nutr*. 2015;114(8):1157-1167.
- Holtug K, Rasmussen HS, Mortensen PB. Short chain fatty acids in inflammatory bowel disease. The effect of bacterial fermentation of blood. *Scand J Clin Lab Invest*. 1988;48(7):667-671.
- Nakamura T, Tabeke K, Terada A, et al. Short-chain carboxylic acid in the feces in patients with pancreatic insufficiency. *Acta gastro-enterologica Belgica*. 1993;56(5-6):326-331.
- Pezzilli R, Andriulli A, Bassi C, et al. Exocrine pancreatic insufficiency in adults: a shared position statement of the Italian Association for the Study of the Pancreas. *World J Gastroenterol*. 2013;19(44):7930-7946.
- Bures J, Cyraný J, Kohoutova D, et al. Small intestinal bacterial overgrowth syndrome. *World J Gastroenterol*. 2010;16(24):2978-2990.

50. Revaiah PC, Kochhar R, Rana SV, et al. Risk of small intestinal bacterial overgrowth in patients receiving proton pump inhibitors versus proton pump inhibitors plus prokinetics. *JGH Open*. 2018;2(2):47-53.
51. Thorsgaard PN. Estimation of assimilation of simultaneously ingested 14C-triolein and 3H-oleic acid as a test of pancreatic digestive function. *Scand J Gastroenterol*. 1984;19(2):6.
52. Thorsgaard PN, Halgreen H. Simultaneous assessment of fat maldigestion and fat malabsorption by a double-isotope method using fecal radioactivity. *Gastroenterology*. 1985;88(1 Pt 1):8.
53. van der Velde AE, Vriens CL, van den Oever K, et al. Direct intestinal cholesterol secretion contributes significantly to total fecal neutral sterol excretion in mice. *Gastroenterology*. 2007;133(3):967-975.
54. Turrone S, Fiori J, Rampelli S, et al. Fecal metabolome of the Hadza hunter-gatherers: a host-microbiome integrative view. *Sci Reps*. 2016;6:32826.
55. Alkaade S, Vareedayah AA. A primer on exocrine pancreatic insufficiency, fat malabsorption, and fatty acid abnormalities. *Am J Manag Care*. 2017;23(12 Suppl):s203-s209.
56. Milovic V. Gastrointestinal disease and dosage form performance. In: *Oral Drug Absorption*. CRC Press; 2016:141-151.
57. Foltz E, Azad S, Everett ML, et al. An assessment of human gastric fluid composition as a function of PPI usage. *Physiological reports*. 2015;3(1).
58. Grace E, Shaw C, Whelan K, Andreyev H. Small intestinal bacterial overgrowth—prevalence, clinical features, current and developing diagnostic tests, and treatment. *Aliment Pharmacol Therap*. 2013;38(7):674-688.
59. Berceanu D, Bucur D, Diaconu C. Type 2 diabetes and liver disease: a frequent and harmful connection. *Arch Balkan Med Union vol*. 2016;51(4):506-511.
60. Rana SV, Malik A, Bhadada SK, Sachdeva N, Morya RK, Sharma G. Malabsorption, Orocecal Transit Time and Small Intestinal Bacterial Overgrowth in Type 2 Diabetic Patients: A Connection. *Indian J Clin Biochem*. 2017;32(1):84-89.
61. Mohapatra S, Singh DP, Alcid D, Pitchumoni CS. Beyond O&P Times Three. *Am J Gastroenterol*. 2018;1.
62. O'Keefe SJD, Rakitt T, Ou J, et al. Pancreatic and Intestinal Function Post Roux-en-Y Gastric Bypass Surgery for Obesity. *Clin Transl Gastroenterol*. 2017;8(8):e112.
63. Watson L, Lalji A, Bodla S, Muls A, Andreyev HJ, Shaw C. Management of bile acid malabsorption using low-fat dietary interventions: a useful strategy applicable to some patients with diarrhoea-predominant irritable bowel syndrome? *Clin Med*. 2015;15(6):536-540.
64. Mourad FH, Barada KA, Saade NE. Impairment of Small Intestinal Function in Ulcerative Colitis: Role of Enteric Innervation. *J Crohns Colitis*. 2017;11(3):369-377.
65. Gujral N, Freeman HJ, Thomson ABR. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. *World J Gastroenterol*. 2012;18(42):6036-6059.
66. Dominguez Munoz JE. Diagnosis of chronic pancreatitis: Functional testing. *Best Pract Res Clin Gastroenterol*. 2010;24(3):233-241.
67. Loser C, Mollgaard A, Folsch UR. Faecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test. *Gut*. 1996;39(4):580-586.
68. Lieb JG, Draganov PV. Pancreatic function testing: here to stay for the 21st century. *World J Gastroenterol*. 2008;14(20):3149-3158.
69. Riedel L, Walker AR, Segal I, et al. Limitations of faecal chymotrypsin as a screening test for chronic pancreatitis. *Gut*. 1991;32(3):321-324.
70. Brydon WG, Kingstone K, Ghosh S. Limitations of faecal elastase-1 and chymotrypsin as tests of exocrine pancreatic disease in adults. *Ann Clin Biochem*. 2004;41(Pt 1):78-81.
71. Krishna Das KV. *Clin Med*. 4th ed. Panama City: Jaypee Brothers Medical Publishing; 2013.
72. Amin O. The significance of biomarkers in the interpretation of comprehensive stool analysis for parasite diagnosis. *Parasitologists United J*. 2011;4.
73. Orient JM. *Sapira's Art and Science of Bedside Diagnosis*. 4th ed. Philadelphia, PA: Lippincott, Williams & Wilkins; 2012.
74. Mundt LS, M. S. Graff's *Textbook of Urinalysis and Body Fluids*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2010.
75. Ruiz AR, Jr. Overview of Malabsorption. *Merck Manual; Consumer Version*. 2015. <http://www.merckmanuals.com/home/digestive-disorders/malabsorption/overview-of-malabsorption>. Accessed July 10, 2015.
76. Nakashige TG, Zhang B, Krebs C, Nolan EM. Human calprotectin is an iron-sequestering host-defense protein. *Nat Chem Biol*. 2015;11(10):765-771.
77. Steinbakk M, Naess-Andresen CF, Lingaas E, Dale I, Brandtzaeg P, Fagerhol MK. Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin. *Lancet*. 1990;336(8718):763-765.
78. Lasson A, Stotzer PO, Ohman L, Isaksson S, Sapnara M, Strid H. The intra-individual variability of faecal calprotectin: a prospective study in patients with active ulcerative colitis. *J Crohns Colitis*. 2015;9(1):26-32.
79. Manceau H, Chicha-Cattoir V, Puy H, Peoc'h K. Fecal calprotectin in inflammatory bowel diseases: update and perspectives. *Clin Chem Lab Med*. 2017;55(4):474-483.
80. Joshi S, Lewis SJ, Creanor S, Ayling RM. Age-related faecal calprotectin, lactoferrin and tumour M2-PK concentrations in healthy volunteers. *Ann Clin Biochem*. 2010;47(Pt 3):259-263.
81. Joshi S, Lewis SJ, Creanor S, Ayling RM. Age-related faecal calprotectin, lactoferrin and tumour M2-PK concentrations in healthy volunteers. *Ann Clin Biochem*. 2010;47(3):259-263.
82. D'Angelo F, Felley C, Frossard JL. Calprotectin in Daily Practice: Where Do We Stand in 2017? *Digestion*. 2017;95(4):293-301.
83. Meling TR, Aabakken L, Roseth A, Osnes M. Faecal calprotectin shedding after short-term treatment with non-steroidal anti-inflammatory drugs. *Scand J Gastroenterol*. 1996;31(4):339-344.
84. Gallo A, Ianri G, Montalto M, Cammarota G. The Role of Biomarkers in Diverticular Disease. *J Clin Gastroenterol*. 2016;50 Suppl 1:S26-28.
85. Stimac D, Nardone G, Mazzari A, et al. What's New in Diagnosing Diverticular Disease. *J Gastrointest Liver Dis*. 2020;28 suppl.1:17-22.
86. Hoekman DR, Zeevenhooven J, D'Haens GR, Benninga MA. The prevalence of irritable bowel syndrome-type symptoms in inflammatory bowel disease patients in remission. *Eur J Gastroenterol Hepatol*. 2017;29(9):1086-1090.
87. Lundgren D, Eklof V, Palmqvist R, Hultdin J, Karling P. Proton pump inhibitor use is associated with elevated faecal calprotectin levels. A cross-sectional study on subjects referred for colonoscopy. *Scand J Gastroenterol*. 2019;54(2):152-157.
88. Scaioili E, Sartini A, Bellanova M, et al. Eicosapentaenoic Acid Reduces Fecal Levels of Calprotectin and Prevents Relapse in Patients With Ulcerative Colitis. *Clin Gastroenterol Hepatol*. 2018;16(8):1268-1275. e1262.
89. Yang BG, Seoh JY, Jang MH. Regulatory Eosinophils in Inflammation and Metabolic Disorders. *Immune Netw*. 2017;17(1):41-47.
90. Majamaa H, Laine S, Miettinen A. Eosinophil protein X and eosinophil cationic protein as indicators of intestinal inflammation in infants with atopic eczema and food allergy. *Clin Exp Allergy*. 1999;29(11):1502-1506.
91. van Odijk J, Peterson CG, Ahlstedt S, et al. Measurements of eosinophil activation before and after food challenges in adults with food hypersensitivity. *Int Arch Allergy Immunol*. 2006;140(4):334-341.
92. Bischoff SC, Grabowsky J, Manns MP. Quantification of inflammatory mediators in stool samples of patients with inflammatory bowel disorders and controls. *Dig Dis Sciences*. 1997;42(2):394-403.
93. Jung Y, Rothenberg ME. Roles and regulation of gastrointestinal eosinophils in immunity and disease. *J Immunol*. 2014;193(3):999-1005.
94. Wagner M, Sjoberg K, Vigren L, et al. Elevated fecal levels of eosinophil granule proteins predict collagenous colitis in patients referred to colonoscopy due to chronic non-bloody diarrhea. *Scand J Gastroenterol*. 2016;51(7):835-841.
95. Roca M, Rodriguez Varela A, Donat E, et al. Fecal Calprotectin and Eosinophil-derived Neurotoxin in Healthy Children Between 0 and 12 Years. *J Ped Gastroenterol Nutr*. 2017;65(4):394-398.
96. Mantis NJ, Rol N, Corthesy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunol*. 2011;4(6):603-611.
97. Corthesy B. Secretory immunoglobulin A: well beyond immune exclusion at mucosal surfaces. *Immunopharmacol Immunotoxicol*. 2009;31(2):174-179.
98. Mantis NJ, Forbes SJ. Secretory IgA: arresting microbial pathogens at epithelial borders. *Immunol Invest*. 2010;39(4-5):383-406.
99. Bemark M, Boysen P, Lycke NY. Induction of gut IgA production through T cell-dependent and T cell-independent pathways. *Ann N Y Acad Sci*. 2012;1247:97-116.

100. Brandtzaeg P. Update on mucosal immunoglobulin A in gastrointestinal disease. *Curr Opin Gastroenterol.* 2010;26(6):554-563.
101. Agarwal S, Mayer L. Pathogenesis and treatment of gastrointestinal disease in antibody deficiency syndromes. *J Allergy Clin Immunol.* 2009;124(4):658-664.
102. Ray A, Dittel BN. Interrelatedness between dysbiosis in the gut microbiota due to immunodeficiency and disease penetrance of colitis. *Immunology.* 2015;146(3):359-368.
103. Mantis NJ, Rol N, Corthésy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunol.* 2011;4(6):603-611.
104. Brandtzaeg P, Carlsen HS, Halstensen TS. The B-cell system in inflammatory bowel disease. *Adv Exp Med Biol.* 2006;579:149-167.
105. Chalkias A, Nikotian G, Koutsovasilis A, et al. Patients with colorectal cancer are characterized by increased concentration of fecal hb-hp complex, myeloperoxidase, and secretory IgA. *Am J Clin Oncol.* 2011;34(6):561-566.
106. Dzunkova M, Moya A, Vazquez-Castellanos JF, et al. Active and Secretory IgA-Coated Bacterial Fractions Elucidate Dysbiosis in Clostridium difficile Infection. *mSphere.* 2016;1(3).
107. Matysiak-Budnik T, Moura IC, Arcos-Fajardo M, et al. Secretory IgA mediates retrotranscytosis of intact gliadin peptides via the transferrin receptor in celiac disease. *J Exp Med.* 2008;205(1):143-154.
108. Chalkias A, Nikotian G, Koutsovasilis A, et al. Patients With Colorectal Cancer Are Characterized by Increased Concentration of Fecal Hb-Hp Complex, Myeloperoxidase, and Secretory IgA. *Am J Clin Oncol.* 2011;34(6):561-566.
109. Soldi S, Tagliacarne SC, Valsecchi C, et al. Effect of a multistrain probiotic (Lactoflorene(R) Plus) on inflammatory parameters and microbiota composition in subjects with stress-related symptoms. *Neurobiol Stress.* 2019;10:100138.
110. Kusumo PD, Bela B, Wibowo H, Munasir Z, Suroño IS. Lactobacillus plantarum IS-10506 supplementation increases faecal sIgA and immune response in children younger than two years. *Beneficial microbes.* 2019;10(3):245-252.
111. Wang L, Zhang J, Guo Z, et al. Effect of oral consumption of probiotic Lactobacillus planatarum P-8 on fecal microbiota, SlgA, SCFAs, and TBAs of adults of different ages. *Nutrition.* 2014;30(7-8):776-783. e771.
112. Baldassarre ME, Di Mauro A, Mastromarino P, et al. Administration of a Multi-Strain Probiotic Product to Women in the Perinatal Period Differentially Affects the Breast Milk Cytokine Profile and May Have Beneficial Effects on Neonatal Gastrointestinal Functional Symptoms. A Randomized Clinical Trial. *Nutrients.* 2016;8(11).
113. Drago-Serrano ME, Campos-Rodríguez R, Carrero JC, de la Garza M. Lactoferrin: Balancing Ups and Downs of Inflammation Due to Microbial Infections. *Int J Mol Sci.* 2017;18(3):501.
114. Rios-Covian D, Ruas-Madiedo P, Margolles A, Gueimonde M, de Los Reyes-Gavilan CG, Salazar N. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. *Front Microbiol.* 2016;7:185.
115. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol.* 2014;121:91-119.
116. Mortensen PB, Clausen MR. Short-chain fatty acids in the human colon: relation to gastrointestinal health and disease. *Scand J Gastroenterology Suppl.* 1996;216:132-148.
117. Velazquez OC, Lederer HM, Rombeau JL. Butyrate and the colonocyte. Production, absorption, metabolism, and therapeutic implications. *Adv Experimental Med Biol.* 1997;427:123-134.
118. Basson A, Trotter A, Rodriguez-Palacios A, Cominelli F. Mucosal Interactions between Genetics, Diet, and Microbiome in Inflammatory Bowel Disease. *Front Immunol.* 2016;7:290.
119. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res.* 2013;54(9):2325-2340.
120. Chen Y, Gozzi K, Yan F, Chai Y. Acetic Acid Acts as a Volatile Signal To Stimulate Bacterial Biofilm Formation. *mBio.* 2015;6(3):e00392.
121. Boets E, Deroover L, Houben E, et al. Quantification of in Vivo Colonic Short Chain Fatty Acid Production from Inulin. *Nutrients.* 2015;7(11):8916-8929.
122. Riviere A, Selak M, Lantin D, Leroy F, De Vuyst L. Bifidobacteria and Butyrate-Producing Colon Bacteria: Importance and Strategies for Their Stimulation in the Human Gut. *Front Microbiol.* 2016;7:979.
123. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol.* 2006;40(3):235-243.
124. Valeur J, Roseth AG, Knudsen T, et al. Fecal Fermentation in Irritable Bowel Syndrome: Influence of Dietary Restriction of Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols. *Digestion.* 2016;94(1):50-56.
125. Varju P, Farkas N, Hegyi P, et al. Low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet improves symptoms in adults suffering from irritable bowel syndrome (IBS) compared to standard IBS diet: A meta-analysis of clinical studies. *PLoS One.* 2017;12(8):e0182942.
126. Bach Knudsen KE. Microbial degradation of whole-grain complex carbohydrates and impact on short-chain fatty acids and health. *Adv Nutr.* 2015;6(2):206-213.
127. Ghoshal UC, Shukla R, Ghoshal U. Small Intestinal Bacterial Overgrowth and Irritable Bowel Syndrome: A Bridge between Functional Organic Dichotomy. *Gut Liver.* 2017;11(2):196-208.
128. Mroczynska M, Galecka M, Szachta P, Kamoda D, Libudzisz Z, Roszak D. Beta-glucuronidase and Beta-glucosidase activity in stool specimens of children with inflammatory bowel disease. *Polish J Microbiol.* 2013;62(3):319-325.
129. Flores R, Shi J, Gail MH, Gajer P, Ravel J, Goedert JJ. Association of fecal microbial diversity and taxonomy with selected enzymatic functions. *PLoS One.* 2012;7(6):e39745.
130. Kwa M, Plottel CS, Blaser MJ, Adams S. The Intestinal Microbiome and Estrogen Receptor-Positive Female Breast Cancer. *J Nat Cancer Inst.* 2016;108(8).
131. Kim DH, Jin YH. Intestinal bacterial beta-glucuronidase activity of patients with colon cancer. *Arch Pharmacol Res.* 2001;24(6):564-567.
132. Flores R, Shi J, Gail MH, Gajer P, Ravel J, Goedert JJ. Association of fecal microbial diversity and taxonomy with selected enzymatic functions. *PLoS one.* 2012;7(6):e39745-e39745.
133. Thompson KJ, Ingle JN, Tang X, et al. A comprehensive analysis of breast cancer microbiota and host gene expression. *PLoS one.* 2017;12(11):e0188873-e0188873.
134. Sivieri K, Bedani R, Cavallini DCU, Rossi EA. Probiotics and intestinal microbiota: implications in colon cancer prevention. In: *Lactic Acid Bacteria-R & D for Food, Health and Livestock Purposes.* IntechOpen; 2013.
135. Goldin BR, Swenson L, Dwyer J, Sexton M, Gorbach SL. Effect of diet and Lactobacillus acidophilus supplements on human fecal bacterial enzymes. *J Natl Cancer Inst.* 1980;64(2):255-261.
136. De Preter V, Raemen H, Cloetens L, Houben E, Rutgeerts P, Verbeke K. Effect of dietary intervention with different pre- and probiotics on intestinal bacterial enzyme activities. *Eur J Clin Nutr.* 2008;62(2):225-231.
137. Valerio F, Russo F, de Candia S, et al. Effects of probiotic Lactobacillus paracasei-enriched artichokes on constipated patients: a pilot study. *J Clin Gastroenterol.* 2010;44 Suppl 1:549-53.
138. Liu Z, Lin X, Huang G, Zhang W, Rao P, Ni L. Prebiotic effects of almonds and almond skins on intestinal microbiota in healthy adult humans. *Anaerobe.* 2014;26:1-6.
139. Molan AL, Liu Z, Plimmer G. Evaluation of the effect of blackcurrant products on gut microbiota and on markers of risk for colon cancer in humans. *Phytother Res.* 2014;28(3):416-422.
140. Calcium-D-glucarate. *Altern Med Rev.* 2002;7(4):336-339.
141. Křen V, Walterova D. Silybin and silymarin—new effects and applications. *Biomed papers.* 2005;149(1):29-41.
142. Kim DH, Jin YH, Park JB, Kobashi K. Silymarin and its components are inhibitors of beta-glucuronidase. *Biol Pharm Bull.* 1994;17(3):443-445.
143. Azcarate-Peril MA, Sikes M, Bruno-Barcena JM. The intestinal microbiota, gastrointestinal environment and colorectal cancer: a putative role for probiotics in prevention of colorectal cancer? *Am J Physiol Gastrointest Liver Physiol.* 2011;301(3):G401-424.

144. Koning CJ, Jonkers DM, Stobberingh EE, Mulder L, Rombouts FM, Stockbrugger RW. The effect of a multispecies probiotic on the intestinal microbiota and bowel movements in healthy volunteers taking the antibiotic amoxicillin. *Am J Gastroenterol.* 2008;103(1):178-189.
145. Zhanel GG, Siemens S, Slayter K, Mandell L. Antibiotic and oral contraceptive drug interactions: Is there a need for concern? *Can J Infect Dis.* 1999;10(6):429-433.
146. Osuka A, Shimizu K, Ogura H, et al. Prognostic impact of fecal pH in critically ill patients. *Critical Care.* 2012;16(4):R119.
147. Ahmed R, Segal I, Hassan H. Fermentation of dietary starch in humans. *Am J Gastroenterol.* 2000;95(4):1017-1020.
148. Kashtan H, Stern HS, Jenkins DJ, et al. Manipulation of fecal pH by dietary means. *Prev Med.* 1990;19(6):607-613.
149. Malhotra SL. Faecal urobilinogen levels and pH of stools in population groups with different incidence of cancer of the colon, and their possible role in its aetiology. *J R Soc Med.* 1982;75(9):709-714.
150. Hoverstad T, Carlstedt-Duke B, Lingaas E, et al. Influence of oral intake of seven different antibiotics on faecal short-chain fatty acid excretion in healthy subjects. *Scand J Gastroenterol.* 1986;21(8):997-1003.
151. Oufir LE, Barry JL, Flourie B, et al. Relationships between transit time in man and in vitro fermentation of dietary fiber by fecal bacteria. *Eur J Clin Nutr.* 2000;54(8):603-609.
152. Eherer AJ, Fordtran JS. Fecal osmotic gap and pH in experimental diarrhea of various causes. *Gastroenterology.* 1992;103(2):545-551.
153. Syed SZ. Bacterial Overgrowth Syndrome Workup: Laboratory Studies. . Medscape. 2014. <http://emedicine.medscape.com/article/212861-workup>. Accessed July 10, 2015.
154. Juckett G, Trivedi R. Evaluation of chronic diarrhea. *Am Fam Phys.* 2011;84(10):1119-1126.
155. Vahouny GVK, D. Dietary Fiber in Health and Disease. New York, NY: Springer Science & Business Media; 2013.
156. Russell WR, Gratz SW, Duncan SH, et al. High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr.* 2011;93(5):1062-1072.
157. Makishima M, Lu TT, Xie W, et al. Vitamin D receptor as an intestinal bile acid sensor. *Science.* 2002;296(5571):1313-1316.
158. Imray CH, Radley S, Davis A, et al. Faecal unconjugated bile acids in patients with colorectal cancer or polyps. *Gut.* 1992;33(9):1239-1245.
159. Baek MK, Park JS, Park JH, et al. Lithocholic acid upregulates uPAR and cell invasiveness via MAPK and AP-1 signaling in colon cancer cells. *Cancer letters.* 2010;290(1):123-128.
160. Tadano T, Kanoh M, Matsumoto M, Sakamoto K, Kamano T. Studies of serum and feces bile acids determination by gas chromatography-mass spectrometry. *Japanese J Clin Pathol.* 2006;54(2):103-110.
161. Debruyne PR, Bruyneel EA, Karaguni IM, et al. Bile acids stimulate invasion and haptotaxis in human colorectal cancer cells through activation of multiple oncogenic signaling pathways. *Oncogene.* 2002;21(44):6740-6750.
162. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol.* 2014;12(10):661-672.
163. Ajouz H, Mukherji D, Shamseddine A. Secondary bile acids: an underrecognized cause of colon cancer. *World J Surg Oncol.* 2014;12:164.
164. Gaull GE, Wright CE. Taurine conjugation of bile acids protects human cells in culture. *AdvExpMedBiol.* 1987;217:61-67.
165. Jenkins DJ, Wolever TM, Rao AV, et al. Effect on blood lipids of very high intakes of fiber in diets low in saturated fat and cholesterol. *New Eng J Med.* 1993;329(1):21-26.
166. Degirolamo C, Modica S, Palasciano G, Moschetta A. Bile acids and colon cancer: Solving the puzzle with nuclear receptors. *Trends Mol Med.* 2011;17(10):564-572.
167. Kakiyama G, Hylemon PB, Zhou H, et al. Colonic inflammation and secondary bile acids in alcoholic cirrhosis. *Am J Phys Gastroint Liver Physiol.* 2014;306(11):G929-937.
168. Owen RW, Day DW, Thompson MH. Faecal steroids and colorectal cancer: steroid profiles in subjects with adenomatous polyps of the large bowel. *Eur J Cancer Prev.* 1992;1(2):105-112.
169. Hill MJ. The ratio of lithocholic to deoxycholic acid in faeces: a risk factor in colorectal carcinogenesis. *Eur J Cancer Prev.* 1991;1 Suppl 2:75-78.
170. Owen RW, Thompson MH, Hill MJ, Wilpart M, Mainguet P, Roberfroid M. The importance of the ratio of lithocholic to deoxycholic acid in large bowel carcinogenesis. *Nutr Cancer.* 1987;9(2-3):67-71.
171. Owen RW, Henly PJ, Thompson MH, Hill MJ. Steroids and cancer: faecal bile acid screening for early detection of cancer risk. *J Steroid Biochem.* 1986;24(1):391-394.
172. Van Erpecum KJ, Van Berge-Henegouwen GP. Gallstones: an intestinal disease? *Gut.* 1999;44(3):435-438.
173. Zuccato E, Venturi M, Di Leo G, et al. Role of bile acids and metabolic activity of colonic bacteria in increased risk of colon cancer after cholecystectomy. *Dig Diseases Sci.* 1993;38(3):514-519.
174. Fischer S, Berr F, Paumgartner G. Unchanged levels of keto bile acids in bile after cholecystectomy. *Digestion.* 1991;48(4):202-209.
175. Wang DQ, Cohen DE, Carey MC. Biliary lipids and cholesterol gallstone disease. *JLipid Res.* 2009;50 Suppl:S406-411.
176. Wang HH, Portincasa P, Wang DQ. Molecular pathophysiology and physical chemistry of cholesterol gallstones. *Front Biosci.* 2008;13:401-423.
177. Ridlon JM, Alves JM, Hylemon PB, Bajaj JS. Cirrhosis, bile acids and gut microbiota: unraveling a complex relationship. *Gut Microbes.* 2013;4(5):382-387.
178. Vrieze A, Out C, Fuentes S, et al. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. *J Hepatol.* 2014;60(4):824-831.
179. Van Munster IP, Nagengast FM. The role of carbohydrate fermentation in colon cancer prevention. *Scand J Gastroenterol Suppl.* 1993;200:80-86.
180. Hylla S, Gostner A, Dusel G, et al. Effects of resistant starch on the colon in healthy volunteers: possible implications for cancer prevention. *Am J Clin Nutr.* 1998;67(1):136-142.
181. Thornton JR. High colonic pH promotes colorectal cancer. *Lance.* 1981;1(8229):1081-1083.
182. De Preter V, Hamer HM, Windey K, Verbeke K. The impact of pre- and/or probiotics on human colonic metabolism: does it affect human health? *Mol Nutr Food Res.* 2011;55(1):46-57.
183. Rinninella E, Raoul P, Cintoni M, et al. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms.* 2019;7(1):14.
184. Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease. *BMJ.* 2018;360:j5145.
185. Fava F, Danese S. Intestinal microbiota in inflammatory bowel disease: friend of foe? *World J Gastroenterol.* 2011;17(5):557-566.
186. Rowland I, Gibson G, Heinken A, et al. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr.* 2018;57(1):1-24.
187. Verbeke KA, Boobis AR, Chiodini A, et al. Towards microbial fermentation metabolites as markers for health benefits of probiotics. *Nutr Res Rev.* 2015;28(1):42-66.
188. Belenguer A, Duncan SH, Holtrop G, Anderson SE, Lobley GE, Flint HJ. Impact of pH on lactate formation and utilization by human fecal microbial communities. *Appl Environ Microbiol.* 2007;73(20):6526-6533.
189. Ostojic SM. Non-gut microbiota as a source of bioactive hydrogen. *Postgrad Med J.* 2017;93(1097):170.
190. Figliuolo VR, Dos Santos LM, Abalo A, et al. Sulfate-reducing bacteria stimulate gut immune responses and contribute to inflammation in experimental colitis. *Life Sci.* 2017;189:29-38.
191. Stroot PG. The primary cause of oxidative stress is ultra-exogenous sulfide formation (USF). *Med Hypotheses.* 2014;83(6):766-768.
192. Pham VT, Lacroix C, Braegger CP, Chassard C. Lactate-utilizing community is associated with gut microbiota dysbiosis in colicky infants. *Sci Reports.* 2017;7(1):11176.
193. Ghoshal U, Shukla R, Srivastava D, Ghoshal UC. Irritable Bowel Syndrome, Particularly the Constipation-Predominant Form, Involves an Increase in Methanobrevibacter smithii, Which Is Associated with Higher Methane Production. *Gut and liver.* 2016;10(6):932-938.
194. Koliada A, Syzenko G, Moseiko V, et al. Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiol.* 2017;17(1):120.

195. Indiani C, Rizzardi KF, Castelo PM, Ferraz LFC, Darrieux M, Parisotto TM. Childhood Obesity and Firmicutes/Bacteroidetes Ratio in the Gut Microbiota: A Systematic Review. *Childhood obesity (Print)*. 2018;14(8):501-509.
196. Lyu M, Wang YF, Fan GW, Wang XY, Xu SY, Zhu Y. Balancing Herbal Medicine and Functional Food for Prevention and Treatment of Cardiometabolic Diseases through Modulating Gut Microbiota. *Front Microbiol*. 2017;8:2146.
197. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol*. 2014;16(7):1024-1033.
198. Tang WHW, Kitai T, Hazen SL. Gut Microbiota in Cardiovascular Health and Disease. *Circulation Res*. 2017;120(7):1183-1196.
199. Imhann F, Bonder MJ, Vich Vila A, et al. Proton pump inhibitors affect the gut microbiome. *Gut*. 2016;65(5):740-748. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. *BMJ*. 2018;361:k2179.
200. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. *BMJ*. 2018;361:k2179.
201. Myers SP. The causes of intestinal dysbiosis: a review. *Alt Med Rev*. 2004;9(2):180-197.
202. Engen PA, Green SJ, Voigt RM, Forsyth CB, Keshavarzian A. The Gastrointestinal Microbiome: Alcohol Effects on the Composition of Intestinal Microbiota. *Alcohol Res*. 2015;37(2):223-236.
203. Rea K, Dinan TG, Cryan JF. The microbiome: a key regulator of stress and neuroinflammation. *Neurobiol Stress*. 2016;4:23-33.
204. Houghton D, Stewart CJ, Day CP, Trenell M. Gut Microbiota and Lifestyle Interventions in NAFLD. *Int J Mol Sci*. 2016;17(4):447.
205. Allen AP, Dinan TG, Clarke G, Cryan JF. A psychology of the human brain-gut-microbiome axis. *Soc Personal Psychol Compass*. 2017;11(4):e12309.
206. Frei R, Akdis M, O'Mahony L. Prebiotics, probiotics, synbiotics, and the immune system: experimental data and clinical evidence. *Current Op Gastroenterol*. 2015;31(2):153-158.
207. Sassone-Corsi M, Raffatellu M. No vacancy: how beneficial microbes cooperate with immunity to provide colonization resistance to pathogens. *J Immunol*. 2015;194(9):4081-4087.
208. Tojo R, Suárez A, Clemente MG, et al. Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. *World J Gastroenterol*. 2014;20(41):15163.
209. Kechagia M, Basoulis D, Konstantopoulou S, et al. Health benefits of probiotics: a review. *Nutrition*. 2013;2013.
210. Heeney DD, Gareau MG, Marco ML. Intestinal Lactobacillus in health and disease, a driver or just along for the ride? *Current Op Biotechnol*. 2018;49:140-147.
211. Matsuoka K, Kanai T. The gut microbiota and inflammatory bowel disease. Paper presented at: Seminars in immunopathology 2015.
212. Kaakoush NO, Castano-Rodriguez N, Mitchell HM, Man SM. Global Epidemiology of Campylobacter Infection. *Clin Microbiol Rev*. 2015;28(3):687-720.
213. Chey WD, Leontiadis GI, Howden CW, Moss SF. ACG clinical guideline: treatment of Helicobacter pylori infection. *Am J Gastroenterol*. 2017;112(2):212.
214. Senay H, MacPherson D. Parasitology: diagnostic yield of stool examination. *Can Med Assoc J*. 1989;140(11):1329-1331.
215. Cartwright CP. Utility of multiple-stool-specimen ova and parasite examinations in a high-prevalence setting. *J Clin Microbiol*. 1999;37(8):2408-2411.
216. Mohapatra S, Singh DP, Alcid D, Pitchumoni CS. Beyond O&P Times Three. *Am J Gastroenterol*. 2018;113(6):805-818.
217. McHardy IH, Wu M, Shimizu-Cohen R, Couturier MR, Humphries RM. Detection of intestinal protozoa in the clinical laboratory. *J Clin Microbiol*. 2014;52(3):712-720.
218. Garcia LS, Arrowood M, Kokoskin E, et al. Laboratory Diagnosis of Parasites from the Gastrointestinal Tract. *Clin Microbiol Rev*. 2018;31(1).
219. CDC. Artifacts. DPDx - Laboratory Identification of Parasites of Public Health Concern 2016; <https://www.cdc.gov/dpdx/artifacts/index.html>. Accessed November 15, 2018.
220. Siddique SM, Gilotra NA. Crystal clear: a unique clue to diagnosis in a patient with recurrent nausea and vomiting. *Gastroenterology*. 2014;147(2):e1-2.
221. Tan KS. New insights on classification, identification, and clinical relevance of Blastocystis spp. *Clinical microbiology reviews*. 2008;21(4):639-665.
222. Zuo L, Rothenberg ME. Gastrointestinal eosinophilia. *Immunol Allergy Clin North Am*. 2007;27(3):443-455.
223. CDC. Amebiasis. DPDx - Laboratory Identification of Parasites of Public Health Concern 2017; <https://www.cdc.gov/dpdx/amebiasis/index.html>. Accessed November 14, 2018.
224. NIH. Polymerase Chain Reaction (PCR). Fact Sheets 2015; <https://www.genome.gov/10000207/polymerase-chain-reaction-pcr-fact-sheet/>. Accessed October 30, 2018.
225. Garcia LS. Dientamoeba fragilis, One of the Neglected Intestinal Protozoa. *J Clin Microbiol*. 2016;54(9):2243-2250.
226. CDC. Parasites - Cryptosporidium (also known as "Crypto"). 2017; <https://www.cdc.gov/parasites/crypto/>. Accessed October 15, 2018.
227. Popruk S, Pintong A-r, Radomyos P. Diversity of Blastocystis subtypes in humans. *J Trop Med Parasitol*. 2013;36:88-97.
228. Fullerton KY. Chapter 3 - Giardiasis Travelers' Health 2017; <https://wwwnc.cdc.gov/travel/yellowbook/2018/infectious-diseases-related-to-travel/giardiasis>. Accessed November 2, 2018.
229. Park S, Hitchcock MM, Gomez CA, Banaei N. Is Follow-up Testing with FilmArray Gastrointestinal Multiplex PCR Panel Necessary? *J Clin Microbiol*. 2017;JCM. 02354-02316.
230. Rijsman LH, Monkelbaan JF, Kusters JG. Clinical consequences of polymerase chain reaction-based diagnosis of intestinal parasitic infections. *J Gastroenterol Hepatol*. 2016;31(11):1808-1815.
231. Skotarczak B. Genetic diversity and pathogenicity of Blastocystis. *Ann Agricult Environ Medicine*. 2018;25(3):411-416.
232. Scanlan PD, Stensvold CR, Cotter PD. Development and Application of a Blastocystis Subtype-Specific PCR Assay Reveals that Mixed-Subtype Infections Are Common in a Healthy Human Population. *Appl Environ Microbiol*. 2015;81(12):4071-4076.
233. Tito RY, Chaffron S, Caenepeel C, et al. Population-level analysis of Blastocystis subtype prevalence and variation in the human gut microbiota. *Gut*. 2018;gutjnl-2018-316106.
234. Nagel R. Are Blastocystis species clinically relevant to humans? Australia: School of Veterinary Science, The University of Queensland; 2016.
235. Cifre S, Gozalbo M, Ortiz V, Soriano JM, Merino JF, Trellis M. Blastocystis subtypes and their association with Irritable Bowel Syndrome. *Medical Hypoth*. 2018;116:4-9.
236. Pintong AR, Sunyanusin S, Prasertbun R, et al. Blastocystis subtype 5: Predominant subtype on pig farms, Thailand. *Parasitology Intl*. 2018;67(6):824-828.
237. Dogruman-AI F, Dagci H, Yoshikawa H, Kurt O, Demirel M. A possible link between subtype 2 and asymptomatic infections of Blastocystis hominis. *Parasitology research*. 2008;103(3):685-689.
238. Mirjalali H, Abbasi MR, Naderi N, et al. Distribution and phylogenetic analysis of Blastocystis sp. subtypes isolated from IBD patients and healthy individuals in Iran. *Eur J Clin Microbiol Infect Dis*. 2017;36(12):2335-2342.
239. Jalalou N, Iravani S, Rezaeian M, Alinaghizade A, Mirjalali H. Subtypes Distribution and Frequency of Blastocystis sp. Isolated from Diarrheic and Non-diarrheic Patients. *Iranian J Parasitol*. 2017;12(1):63-68.
240. Terveer EM, van Gool T, Ooijevaar RE, et al. Human transmission of Blastocystis by Fecal Microbiota Transplantation without development of gastrointestinal symptoms in recipients. *Clini infect Dis*. 2019.
241. Kesuma Y, Firmansyah A, Bardosono S, Sari IP, Kurniawan A. Blastocystis ST-1 is associated with Irritable Bowel Syndrome-diarrhoea (IBS-D) in Indonesian adolescences. *Parasite Epidemiol Control*. 2019;6:e00112.
242. Yason JA, Liang YR, Png CW, Zhang Y, Tan KSW. Interactions between a pathogenic Blastocystis subtype and gut microbiota: in vitro and in vivo studies. *Microbiome*. 2019;7(1):30.
243. Mirza H, Teo JD, Upcroft J, Tan KS. A rapid, high-throughput viability assay for Blastocystis spp. reveals metronidazole resistance and extensive subtype-dependent variations in drug susceptibilities. *Antimicrobial Agents Chemother*. 2011;55(2):637-648.
244. Dhurga DB, Suresh KG, Tan TC, Chandramathi S. Apoptosis in Blastocystis spp. is related to subtype. *Trans Royal Soc Trop Med Hyg*. 2012;106(12):725-730.

245. Balakrishnan DD, Kumar SG. Higher Caspase-like activity in symptomatic isolates of *Blastocystis* spp. *Parasites Vectors*. 2014;7:219.
246. Dhurga DB, Suresh K, Tan TC. Granular Formation during Apoptosis in *Blastocystis* sp. Exposed to Metronidazole (MTZ). *PloS one*. 2016;11(7):e0155390.
247. Nagel R, Traub RJ, Kwan MM, Bielefeldt-Ohmann H. *Blastocystis* specific serum immunoglobulin in patients with irritable bowel syndrome (IBS) versus healthy controls. *Parasites Vectors*. 2015;8:453.
248. Mokhtar AB, El-Gayar EK, Habib ES. In Vitro Anti-Protozoal Activity Of Propolis Extract And Cysteine Proteases Inhibitor (Phenyl Vinyl Sulfone) On *Blastocystis* Species. *J Egypt Soc Parasitol*. 2016;46(2):261-272.
249. Yason JA, Koh K, Tan KSW. Viability Screen of LOPAC(1280) Reveals Phosphorylation Inhibitor Auranofin as a Potent Inhibitor of *Blastocystis* Subtype 1, 4, and 7 Isolates. *Antimicrobial Agents Chemother*. 2018;62(8).
250. Mirza H, Wu Z, Kidwai F, Tan KS. A metronidazole-resistant isolate of *Blastocystis* spp. is susceptible to nitric oxide and downregulates intestinal epithelial inducible nitric oxide synthase by a novel parasite survival mechanism. *Infection Immunity*. 2011;79(12):5019-5026.
251. Roberts T, Bush S, Ellis J, Harkness J, Stark D. In Vitro Antimicrobial Susceptibility Patterns of *Blastocystis*. *Antimicrobial agents and chemotherapy*. 2015;59(8):4417-4423.
252. Lepczynska M, Bialkowska J, Dzika E, Piskorz-Ogorek K, Korycinska J. *Blastocystis*: how do specific diets and human gut microbiota affect its development and pathogenicity? *Eur J Clin Microbiol Infect Dis*. 2017;36(9):1531-1540.
253. El Deeb HK, Al Khadrawy FM, Abd El-Hameid AK. Inhibitory effect of *Ferula asafoetida* L. (Umbelliferae) on *Blastocystis* sp. subtype 3 growth in vitro. *Parasitol Res*. 2012;111(3):1213-1221.
254. Yakoob J, Abbas Z, Beg MA, et al. In vitro sensitivity of *Blastocystis hominis* to garlic, ginger, white cumin, and black pepper used in diet. *Parasitol Res*. 2011;109(2):379-385.
255. Lepczynska M, Dzika E. The influence of probiotic bacteria and human gut microorganisms causing opportunistic infections on *Blastocystis* ST3. *Gut pathogens*. 2019;11:6.
256. Mokhtar AB, Ahmed SA, Eltamany EE, Karanis P. Anti-*Blastocystis* Activity In Vitro of Egyptian Herbal Extracts (Family: Asteraceae) with Emphasis on *Artemisia judaica*. *Int J Environ Res Public Health*. 2019;16(9).
257. Meabed EMH, Abdelhafez DN, Abdelaliem YF. *Saccharomyces boulardii* inhibits the expression of pro-inflammatory cytokines and inducible nitric oxide synthase genes in the colonic mucosa of rats experimentally-infected with *Blastocystis* subtype-3 cysts. *Parasitology*. 2019;146(12):1532-1540.
258. Jones MS, Whipps CM, Ganac RD, Hudson NR, Boorom K. Association of *Blastocystis* subtype 3 and 1 with patients from an Oregon community presenting with chronic gastrointestinal illness. *Parasitol Res*. 2009;104(2):341-345.
259. Angelici MC, Nardis C, Scarpelli R, Ade P. *Blastocystis hominis* transmission by non-potable water: a case report in Italy. *New Microbiologica*. 2018;41(2):173-177.
260. Nagel R, Bielefeldt-Ohmann H, Traub R. Clinical pilot study: efficacy of triple antibiotic therapy in *Blastocystis* positive irritable bowel syndrome patients. *Gut Pathogens*. 2014;6:34.
261. Nagel R, Cuttall L, Stensvold CR, Mills PC, Bielefeldt-Ohmann H, Traub RJ. *Blastocystis* subtypes in symptomatic and asymptomatic family members and pets and response to therapy. *Int Med J*. 2012;42(11):1187-1195.
262. Stensvold CR, Arendrup MC, Nielsen HV, Bada A, Thorsen S. Symptomatic infection with *Blastocystis* sp. subtype 8 successfully treated with trimethoprim-sulfamethoxazole. *Ann Trop Med Parasitol*. 2008;102(3):271-274.
263. Roberts T, Ellis J, Harkness J, Marriott D, Stark D. Treatment failure in patients with chronic *Blastocystis* infection. *J Med Microbiol*. 2014;63(Pt 2):252-257.
264. Katsarou-Katsari A, Vassalos CM, Tzanetou K, Spanakos G, Papadopoulou C, Vakalis N. Acute urticaria associated with amoeboid forms of *Blastocystis* sp. subtype 3. *Acta dermato-venereologica*. 2008;88(1):80-81.
265. Vogelberg C, Stensvold CR, Monecke S, et al. *Blastocystis* sp. subtype 2 detection during recurrence of gastrointestinal and urticarial symptoms. *Parasitol Int*. 2010;59(3):469-471.
266. Scheffler L, Crane A, Heyne H, et al. Widely Used Commercial ELISA Does Not Detect Precursor of Haptoglobin2, but Recognizes Properdin as a Potential Second Member of the Zonulin Family. *Front Endocrinol*. 2018;9(22).
267. Ajamian M, Steer D, Rosella G, Gibson PR. Serum zonulin as a marker of intestinal mucosal barrier function: May not be what it seems. *PloS one*. 2019;14(1):e0210728.
268. Lamprecht M, Bogner S, Schippinger G, et al. Probiotic supplementation affects markers of intestinal barrier, oxidation, and inflammation in trained men; a randomized, double-blinded, placebo-controlled trial. *J Int Soc Sports Nutr*. 2012;9(1):45.
269. Halasa M, Maciejewska D, Baskiewicz-Halasa M, Machalinski B, Safranow K, Stachowska E. Oral Supplementation with Bovine Colostrum Decreases Intestinal Permeability and Stool Concentrations of Zonulin in Athletes. *Nutrients*. 2017;9(4).



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