GI Effects Stool Profiles

CDSA 2.0™

STOOL TESTING SUPPORT GUIDE

GUT HEALTH

GENOVA DIAGNOSTICS®

Contents

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.

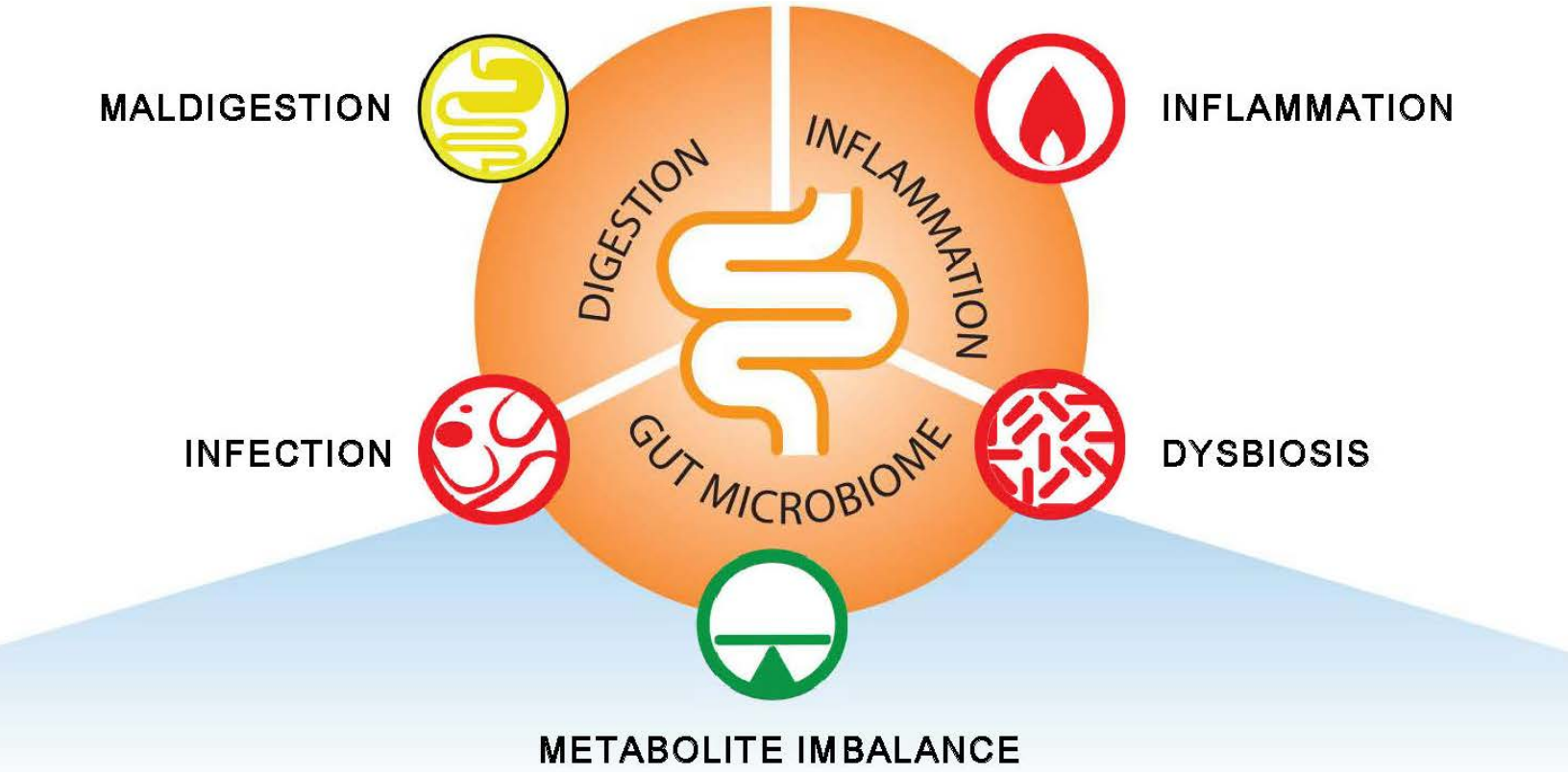
Lastly, the GI Effects utilizes an innovative scoring system that synthesizes the biomarker findings and groups them into **5 key areas relating to GI function: maldigestion, inflammation, dysbiosis, metabolite imbalance, and infection**. This allows for clearer visualization of patterns among biomarkers. 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)	•		
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)			
Bacteroidetes Phylum	•	•	
Bacteroides-Prevotella group	•	•	
Bacteroides vulgatus	•	•	
Barnesiella spp.	•	•	
Odoribacter spp.	•	•	
Prevotella spp.	•	•	
Firmicutes Phylum	•	•	
Anaerotruncus colihominis	•	•	
Butyrivibrio crossotus	•	•	
Clostridium spp.	•	•	
Coprococcus eutactus	•	•	
Faecalibacterium prausnitzii	•	•	
Lactobacillus spp.	•	•	
Pseudoflavonifractor spp.	•	•	
Roseburia spp.	•	•	
Ruminococcus spp.	•	•	
Veillonella spp.	•	•	
Actinobacteria Phylum	•	•	
Bifidobacterium spp.	•	•	
Bifidobacterium longum	•	•	
Collinsella aerofaciens	•	•	
Proteobacteria Phylum	•	•	
Desulfovibrio piger	•	•	
Escherichia coli	•	•	
Oxalobacter formigenes	•	•	
Euryarchaeota Phylum	•	•	
Methanobrevibacter smithii	•	•	
Fusobacteria Phylum	•	•	
Fusobacterium spp.	•	•	
Verrucomicrobia Phylum	•	•	
Akkermansia muciniphila	•	•	
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 *Clostridium difficile* EIA	+	+	+
2204 Shiga toxin *E. coli* EIA	+	+	+
2202 *Campylobacter* spp. EIA	+	+	+
2206 Fecal Lactoferrin	+	+	
2208 *Helicobacter pylori* 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	+	+	•	•
Products of Protein Breakdown (Total)	•	•	•	•
Chymotrypsin	•	•	+	+
Fecal Fats	•	•	+	+
Meat Fibers	•	•		
Vegetable FIbers	•	•		
Gut Immunology				
Fecal Lactoferrin	•	•		
Calprotectin	+	+	•	•
Esosinophil 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	•	•	•	•
Campylobacter EIA	+	+	+	+
Shiga-like Toxin *E.coli* EIA	+	+	+	+
Clostridium difficile EIA	+	+	+	+
Helicobacter pylori EIA	+	+	+	+
Parasitology				
Microscopic O&P		•	•	
EIA		•	•	
Macroscopic Exam for Worms		+	+	+

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 report. 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 color-coded 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 (< 2) : Low Need for Support (2-3) : Optional Need for Support (4-6) : Moderate Need for Support (7-10) : 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
	5	10	10	0	10
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	• Digestive Enzymes • Betaine HCl • Bile Salts • Apple Cider Vinegar • Mindful Eating Habits • Digestive Bitters	• 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)	• Pre-/Probiotics • Increase Dietary Fiber Intake • Consider SIBO Testing • Increase Resistant Starches • Increase Fermented Foods • Meal Timing	• Pre-/Probiotics • Increased Dietary Fiber Intake • Increase Resistant Starches • increase Fermented Foods • Calcium D-Glucarate (for high beta-glucuronidase)	• Antibiotics (if warranted) • Antimicrobial Herbal Therapy • Antiparasitic Herbal Therapy (if warranted) • *Saccharomyces boulardii*

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 is discussed throughout this guide as they relate to each biomarker.

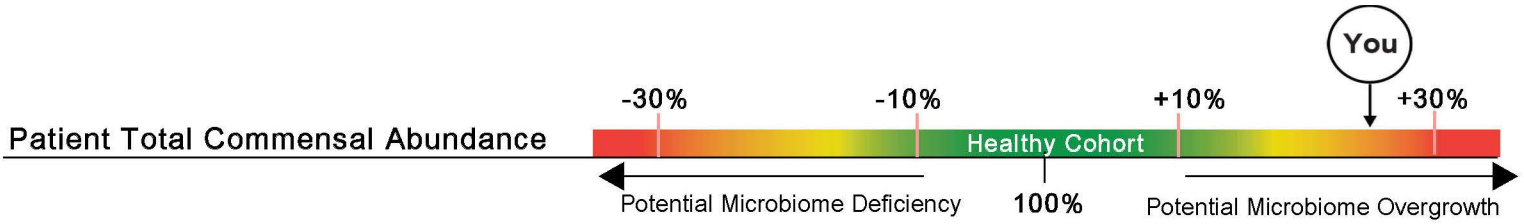
GI Effects Commensal Microbiome Analysis

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

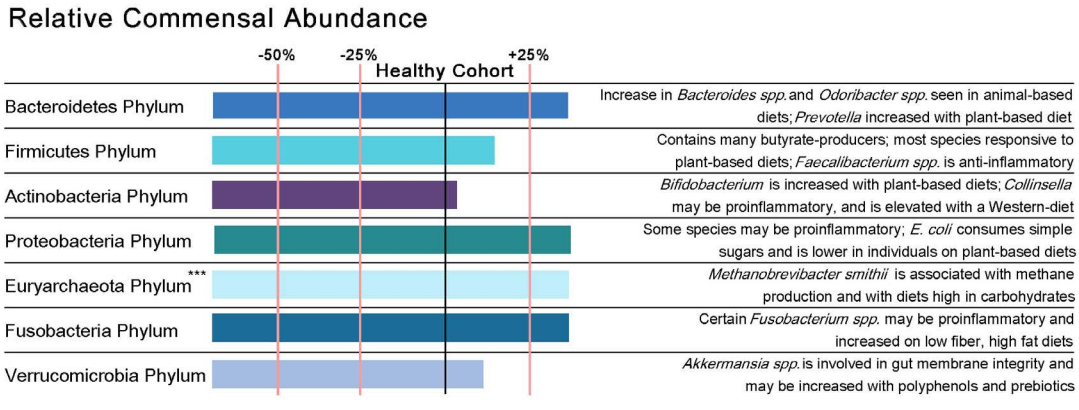
Total Commensal Abundance

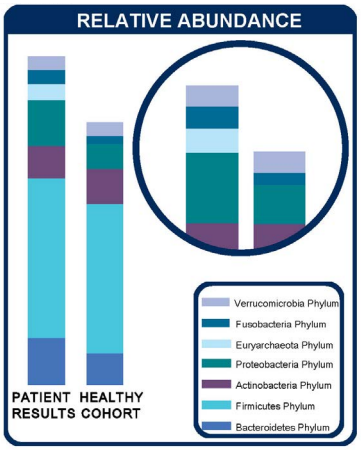
The total commensal abundance is a sum-total of the reported commensal bacteria compared to a healthy cohort. Results are denoted with a circle (You) and reported as a percent variance from healthy cohort levels. Low levels of commensal bacteria are often observed after antimicrobial therapy, or in diets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





Commensal Dysbiosis Patterns

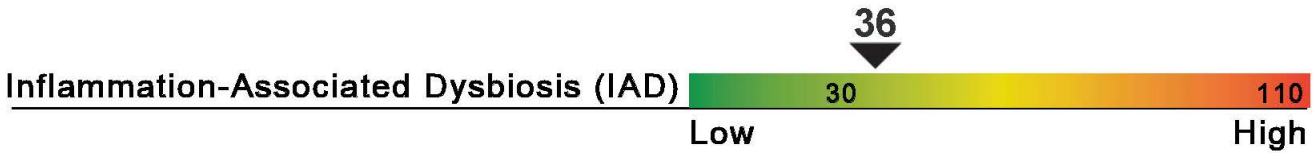
Genova's data analysis has led to the development of unique dysbiosis patterns, related to key physiologic disruptions, such as immunosuppression and inflammation. These patterns are based on the commensal bacteria and may represent dysbiotic changes that could pose clinical significance.

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 profile. 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

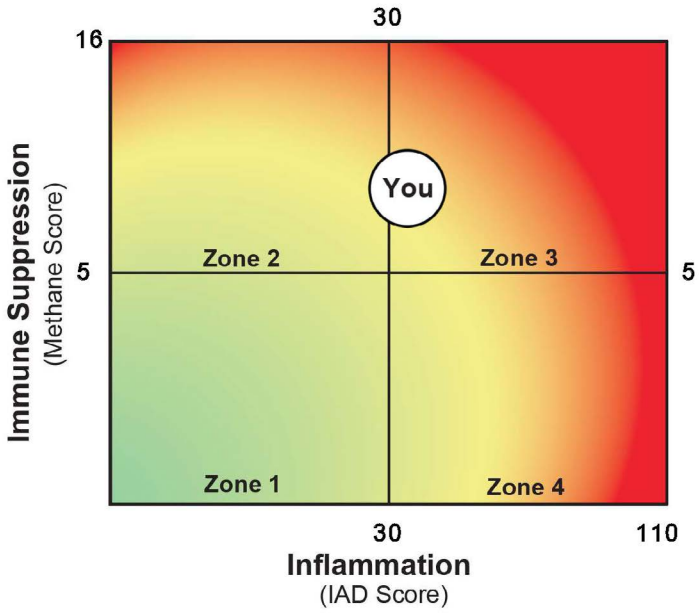
The IAD and methane scores are placed on the x- and y-axis, respectively, and certain cut points create 4 distinct zones. Each zone is associated with different clinical associations and treatment considerations.

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.

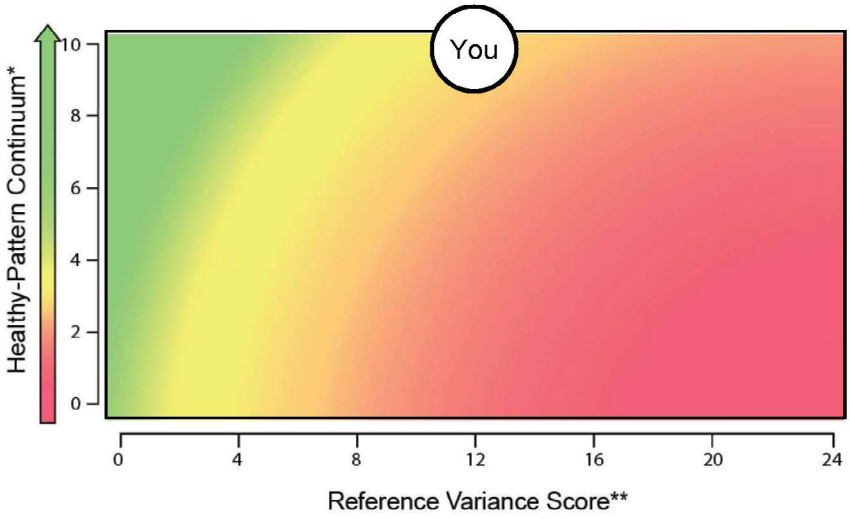
Zone 4: The commensal profile in this zone is associated with increased intestinal inflammation. Patients with IBD are more frequently found with this pattern of bacteria compared to non-IBD patients, and patients are more likely to present with diarrhea. Commensal abundance is lower in this zone and 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.

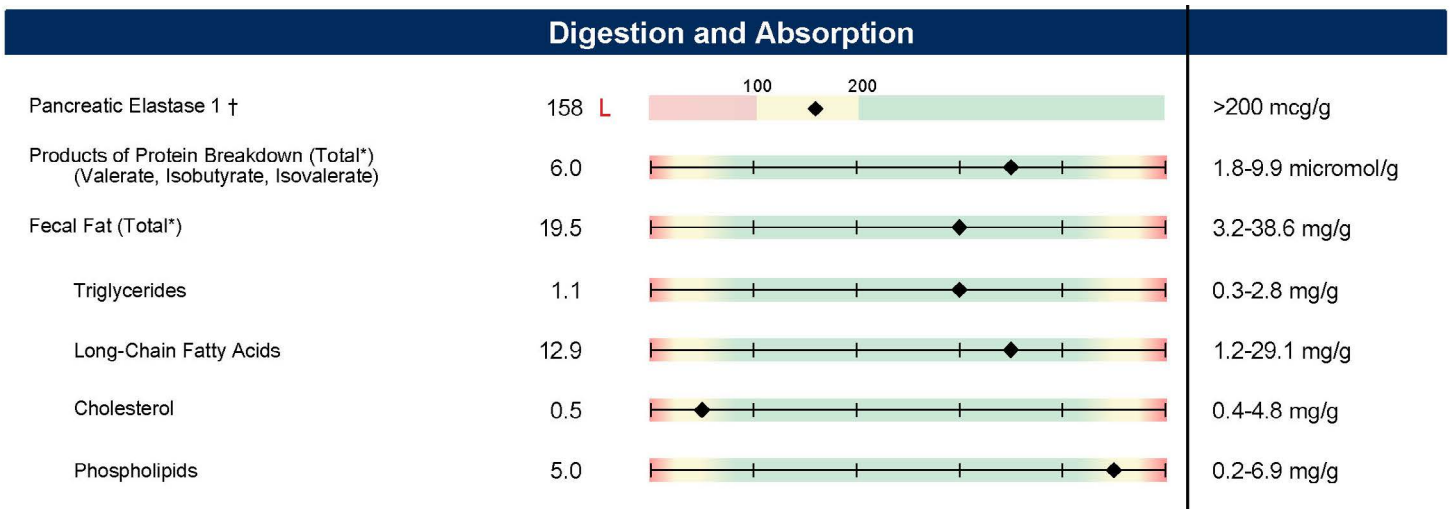
Healthy-Pattern Continuum

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



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.

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 ≥ 500µg/g.[4-7]

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

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 loss[9-11]

Causes of EPI:

Exocrine pancreatic insufficiency may be due to:

- Cystic Fibrosis[12]
- Chronic pancreatitis (CP)[13]
- Pancreatic resection[14]
- Autoimmune pancreatitis[15]
- Gallstones[16]
- Pancreatic tumor/cancer[17]
- GI surgery (i.e., gastric bypass, pancreatic resection)[18]

Other clinical factors associated with EPI through unknown mechanisms include:

- Celiac disease[19-23]
- Inflammatory Bowel Disease (IBD)[24]
- Zollinger-Ellison syndrome[25,26]
- Aging[8,27]
- Excessive alcohol consumption[28]
- Small Intestinal Bacterial Overgrowth (SIBO)[8,29 30-32]
- Smoking[28]
- Obesity[33]
- Vegan/vegetarian diets[34]
- Diabetes[35,36]
- Infectious enteritis[37]

Although exact mechanisms are unknown, it is thought that any condition that damages small intestine mucosa or causes villous atrophy may be associated with EPI. A damaged mucosa results in reduced enteric cholecystokinin (CCK) secretion.[19,22,37] CCK is responsible for stimulating the exocrine pancreas as well as postprandial gallbladder emptying.[38] Low pancreatic elastase levels may reflect damaged small intestine mucosa with levels normalizing when the mucosa is repaired. For example, in celiac disease, avoidance of gluten results in restoration of the mucosa and reverses pancreatic impairment.[19,37]

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 39,40]
3. Consider small, frequent meals, smoking cessation, and reduced alcohol consumption[10,11]
4. Consider SIBO testing if there is an elevated Total 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, or branched-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. Increased protein fermentation is associated with malodorous flatus, IBS, ulcerative colitis, and colorectal cancer, as shown in studies on other protein fermentation products including ammonia, phenols, and hydrogen sulfide.[41] 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[42-44]
- Depression[45]
- Small Intestinal Bacterial Overgrowth (SIBO)[46]
- Antibiotics[47]
- Increased protein consumption[48]
- Diverticulosis[49]
- Celiac disease[50]
- Autism[51]
- GI bleeding[52]
- Chronic pancreatitis, steatorrhea[53]
- Bariatric surgery[54]

Causes of high fecal products of protein breakdown:

- Exocrine pancreatic insufficiency[55]
- High protein diet
- Small intestinal bacterial overgrowth (SIBO)[56]
- Low gastric HCL (hypochlorhydria, acid-blocking medications)[57]
- Certain types of dysbiosis
- GI bleeding and/or endogenous protein exudates associated with inflammatory and/or ulcerative conditions of the bowel[41,52]
- Rapid transit time[41]

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**
   - Studies indicate that high-protein, low complex carbohydrate diets result in higher concentrations of PPB; dietary supplementation with complex carbohydrates results in a decrease in levels of PPB[58]
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:**
   - Total 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 total abundance of commensal bacteria
   » Consider prebiotics, probiotics, and fermented foods
3. Assess inflammatory biomarkers (calprotectin, EPX, fecal sIgA) and treat causes of inflammation

Fecal Fats

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.[59,60]
- **LCFAs**: Increased fecal LCFAs are often indicators of malabsorption.[59,60]
- **Cholesterol**: Fecal cholesterol can come from different sources: diet, bile, and intestinal secretion.[61] Our daily fecal cholesterol excretion may exceed cholesterol intake.[61] 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.[62] Elevations in fecal phospholipids can be due to mucosal cell turnover, malabsorption, or bile.

Causes of fat maldigestion:

1. Exocrine pancreatic insufficiency (EPI)[63]
2. Bile salt insufficiency[63]
3. PPI usage and hypochlorhydria[64]
   - PPI's increase the secretion of most pancreatic enzymes, but reduce the secretion of colipase.[65] 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)[64]
   - Bile acid deconjugation[56,66]
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)[67]

Causes of fat malabsorption

1. Intestinal dysbiosis and SIBO[68]
2. Intestinal parasites[69]
3. Gastric bypass, ileal resection, or other surgeries that limit absorptive surface area[70]
4. Irritable bowel syndrome (often as a symptom of pancreatic exocrine insufficiency or bile acid malabsorption)[63,71] – more likely with the constipation subtype
5. Inflammatory bowel disease[72]
6. Celiac disease[73]

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:
    - » Total 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 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

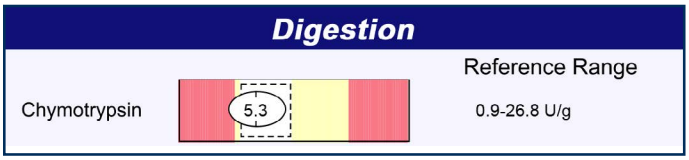
Chymotrypsin is one of the numerous digestive enzymes secreted by the exocrine portion of the pancreas. Specifically, it is a protein-digesting enzyme which can be useful when monitoring pancreatic exocrine function in patients with normal stool transit time.

Unlike PE-1, chymotrypsin has not been correlated with the secretin-pancreozymin test,[74,75] although it has been compared to the 72-hour fecal fat test.[76]

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.[76]

Altered gut transit may effect chymotrypsin.[77] Degradation of proteases can result in higher chymotrypsin levels in response to diarrhea, and lower levels in response to slow transit time.[77]



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).[78]
- High levels of chymotrypsin (>26.8U/g) suggests a rapid transit time (diarrhea) or may be due to excessive pancreatic enzyme supplementation.[78]

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).[79]

The presence of meat fiber or more than a few vegetable fibers in the stool suggests incomplete digestion (e.g. pancreatic insufficiency, hypochlorhydria).[79,80]

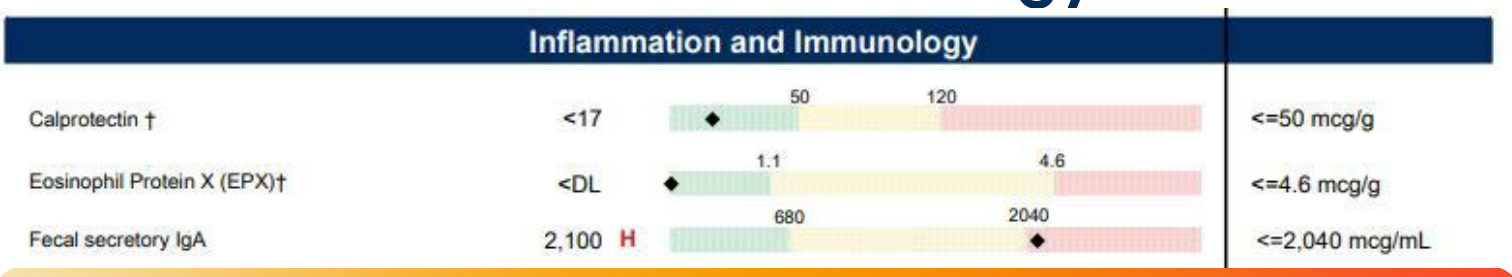
- Elevated levels can also result from inadequate mastication[81] or hypermotility.[82,83]

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



Calprotectin

Calprotectin is a calcium-binding protein with antimicrobial properties.[84] It accounts for 60% of neutrophil cytosolic content and is also found in monocytes and macrophages.[85] 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.[86]
- 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.[87]

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.[88]

Causes of elevated calprotectin:

- Age (children younger than 5 years old, and patients greater than 60)[89]
- IBD, not in remission[90]
- Colorectal cancer and polyps[90]
- Infection (bacteria and some parasitic organisms)[90]
- Non-steroidal anti-inflammatory medication use (NSAIDs) and NSAID enteropathy[91]
- Diverticular disease[92,93]
- 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.[90,94]
- Proton pump inhibitor (PPI) use is associated with elevated fecal calprotectin levels, although the cause-effect relationship is not clear.[95]
- Bariatric surgery such as Roux-en-Y gastric bypass[96-98]

Therapeutic considerations for elevated calprotectin:

- Eicosapentaenoic acid (EPA) from fish oil[99,100]

Calprotectin 50 to 120 mcg/g

Address cause of inflammation:

- Infection
- Suspected or history of IBD
- Chronic NSAID, PPI 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.[101]

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.[101]

Causes of EPX elevation:

- Immune-mediated food hypersensitivity, atopic dermatitis, and food allergies[102,103]
- IBD[104,105]
- Certain parasitic infections[106]
  - » 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.[107]
- 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)[108]

Therapeutic considerations for elevated EPX:

Target evaluation and treatment for etiologies for EPX abnormalities:

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

Fecal secretory IgA

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.[109-113]

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.[110,113]

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.[114]

In people with genetic immunodeficiency of systemic sIgA, GI symptoms such as diarrhea have been reported.[115] 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.[116]

Causes of elevated fecal sIgA:

- Any defective epithelial barrier[117-119]
  - » A defective epithelial barrier allows bacterial and microbial penetration, which is the strongest stimulator of sIgA production.
- Celiac disease[120]
- Colon cancer[121]
- 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
    - o 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.[113]

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.[116] 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.[122-125]

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.[126]

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.[126]

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.

Gastrointestinal (GI) 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 dysbiosis scores as well as 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., *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 (≥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.[127,128]

SCFAs 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.

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

SCFA production from fiber is dependent on the specific enzymes each gut bacteria possesses.[128] The table 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.[129,130]

Based on the literature, the three major butyrate-producers are *Faecalibacterium, Eubacterium*, and *Roseburia*.[127]

Various mixtures of dietary fibers, some types of resistant starch, fructooligosaccharides (FOS), and beta glucan are important for butyrate production.[131]

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.[132]

Acetate is recognized as a volatile signal for biofilm formation.[133]

Inulin supplementation has been shown to increase acetate levels.[134] Pectin is also an important substrate for acetate production.[131]

Propionate

Propionate is a minor energy source for the colonocytes, though it has anti-inflammatory effects.[135]

Propionate acts as a precursor for gluconeogenesis in the liver.[132]

Systemic propionate inhibits acetate incorporation into cholesterol.[134]

Guar gum can support propionate levels.[131]

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 consumption[136-138]
- 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-grains[139]
- Inulin supplementation[134]
- Probiotics and fermented foods to balance the microbiome

Causes of elevated SCFAs:

- Elevated commensal bacteria abundance or bacterial overgrowth[140]
- 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:
  - » Total 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*).[141]

Biomarker Key Points:

Beta-glucuronidase breaks down complex carbohydrates and increases the bioavailability and reabsorption of plant polyphenols (lignans, flavonoids, ceramides, and glycyrrhetinic acid).[142]

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.[141] The intestinal bacterial microbiome related to estrogen metabolism is collectively called the 'estrobolome' and is illustrated in the figure below.[143]

Limited research suggests an association between elevated fecal beta-glucuronidase and cancer risk, primarily colorectal and breast cancer.[144-147]

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[141,148]

Therapeutic considerations for elevated beta-glucuronidase:

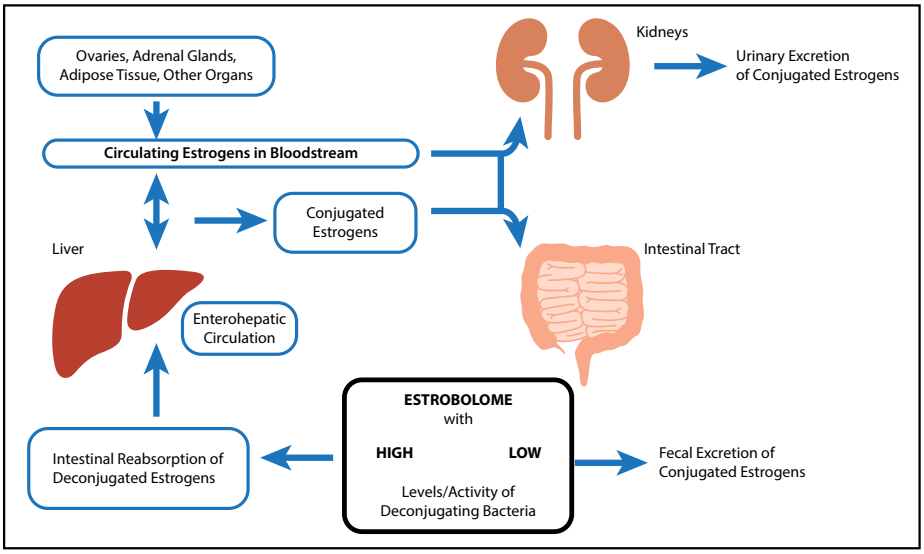
- Probiotics[149,150]
- Dietary fiber, prebiotics[149-152]
- Calcium-D-glucarate
  - » Calcium-D-glucarate is the calcium salt of D-glucaric acid. It is found in fruits and vegetables (oranges, apples, grapefruit, and cruciferous vegetables). [153]
  - » Oral supplementation inhibits the enzymatic activity of beta-glucuronidase[153]
- Milk thistle[154,155]
- Low-calorie and vegetarian diets[141,156]

Causes of low beta-glucuronidase:

- Dysbiosis
- Antibiotic use[157,158]

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.



pH

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.[159] 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,[160-162] fermentive processes, bacterial populations, antibiotics,[163] and stool transit time.[164]

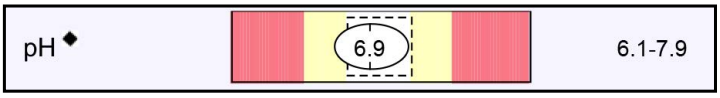
Causes Of Abnormal Stool Ph

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.[159]

- Abnormally low fecal pH of <6.1 (stool acidity) may be related to malabsorption of carbohydrates (including lactose),[159,165] or to small bowel bacterial overgrowth.[166] Osmotic diarrhea is another possible cause of a low fecal pH,[167] such as from using osmotic laxative agents.
- Abnormally high fecal pH of >7.9 (stool alkalinity) may be due to hypochlorhydria,[159] slow transit time/constipation, antibiotics,[159] inadequate dietary fiber,[168] or a high protein/low carbohydrate diet.[169]

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.

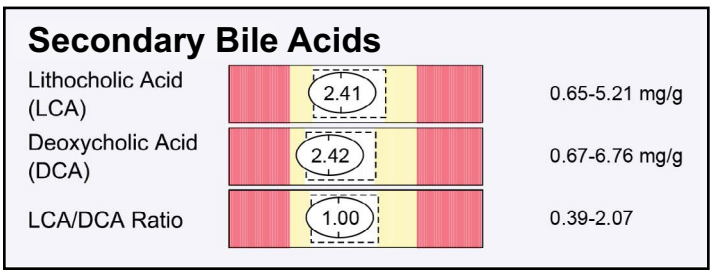
Biomarker Key Points

- Secondary bile acids have been shown to have carcinogenic and mutagenic properties.[170-176]
- The specific bacteria involved in primary bile acid deconjugation to secondary bile acids include *Clostridium, Enterococcus, Bacteroides* and *Lactobacillus*.[177]
- 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.[176,178]
- Secondary bile acids have been associated with increases of oxidative stress and DNA damage.[176,179,180]
- 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 cancer[181-184]
- Gallstone formation[185]
- Cholecystectomy[186,187]



Elevated secondary bile acids have also been associated with:

- Impaired gallbladder function or cholesterol gallstone formation[188,189]
- Inflammation within colonic mucosa, which is thought to also compromise intestinal permeability[180,190]

Low secondary bile acids may result from:

- Broad-spectrum antibiotics[191]
- Reduced cholesterol intake or absorption

Therapeutic Considerations 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.[192-194]
- Probiotics and prebiotics have been found to reduce the conversion of CDCA to LCA.[195]
- Lowering saturated fat and meat intake may decrease secondary bile acid levels.[176]

Commensal Bacteria

Gastrointestinal Microbiome

Commensal Bacteria (PCR)	Result CFU/g stool		QUINTILE DISTRIBUTION 1st 2nd 3rd 4th 5th	Reference Range CFU/g stool
Bacteroidetes Phylum				
Bacteroides-Prevotella group	2.4**E8**			3.4**E6** -1.5**E9**
Bacteroides vulgatus	1.2**E9**			<=2.2**E9**
Barnesiella spp.	3.6**E7**			<=1.6**E8**
Odoribacter spp.	7.1**E7**			<=8.0**E7**
Prevotella spp.	1.4**E8**	H		1.4**E5** -1.6**E7**
Firmicutes Phylum				
Anaerotruncus colihominis	3.4**E7**	H		<=3.2**E7**
Butyrivibrio crossotus	5.0**E7**	H		5.5**E3** -5.9**E5**
Clostridium spp.	2.1**E8**			1.7**E8** -1.5**E10**
Coprococcus eutactus	1.0**E8**			<=1.2**E8**
Faecalibacterium prausnitzii	7.5**E8**			5.8**E7** -4.7**E9**
Lactobacillus spp.	1.6**E8**			8.3**E6** -5.2**E9**
Pseudoflavonifractor spp.	3.0**E8**	H		4.2**E5** -1.3**E8**
Roseburia spp.	7.6**E7**	L		1.3**E8** -1.2**E10**
Ruminococcus spp.	1.9**E9**	H		9.5**E7** -1.6**E9**
Veillonella spp.	1.5**E8**	H		1.2**E5** -5.5**E7**
Actinobacteria Phylum				
Bifidobacterium spp.	1.5**E8**			<=6.4**E9**
Bifidobacterium longum	1.4**E8**			<=7.2**E8**
Collinsella aerofaciens	5.1**E8**			1.4**E7** -1.9**E9**
Proteobacteria Phylum				
Desulfovibrio piger	8.7**E7**	H		<=1.8**E7**
Escherichia coli	1.3**E8**	H		9.0**E4** -4.6**E7**
Oxalobacter formigenes	5.0**E7**	H		<=1.5**E7**
Euryarchaeota Phylum				
Methanobrevibacter smithii	1.4**E8**	H		<=8.6**E7**
Fusobacteria Phylum				
Fusobacterium spp.	2.3**E7**	H		<=2.4**E5**
Verrucomicrobia Phylum				
Akkermansia muciniphila	3.1**E7**			>=1.2**E6**
Firmicutes/Bacteroidetes Ratio				
Firmicutes/Bacteroidetes (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 10^6 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.[196] 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.[196]

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** assess 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: [197-199]

- 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.

Butyrate Producer (C4:0) Increases with fermentation of starch and inulin-type fructans.[200]	**Acetate Producer (C2:0)** 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.[127]	**Propionate Producer (C3:0)** Increases with fermentation of oat bran and β-glucan, pectin, pulses, wheat dextrin, and pyrodextrins.[200]
F. prausnitzii *B. crossotus* *A. colihominis* *Clostridium* spp. *C. eutactus* *Roseburia* spp.	*Prevotella* spp. *Odoribacter* spp. *A. colihominis* *Clostridium* spp. *C. eutactus* *Lactobacillus* spp. *Ruminococcus* spp. *Veillonella* spp. *Bifidobacterium* spp. *A. muciniphila*	*Prevotella* spp. *Odoribacter* spp. *Clostridium* spp. *Veillonella* spp. *A. muciniphila*
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.[201]	**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.[202] H2 is used by intestinal bacterial methanogens, acetogens, and SRB.	**Sulfate reducing bacteria (SRB)** H2S Producer: AA metabolism utilizes sulfate (SO^{2-}_4) 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.[203,204]
B. crossotus *Lactobacillus* spp. *Bifidobacterium* spp. *B. longum*	*Odoribacter* spp. *Clostridium* spp. *E. coli*	*Odoribacter* spp. *D. piger*
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.[205]	**H2-using (hydrogenotrophic)** H2 consumers include reductive acetogens, methanogenic archaea, and sulfate-reducing bacteria [SRB].	**Methane Producer – Methanogens** Methane producers produce methane by utilizing hydrogen and carbon dioxide. Approximately 30% to 62% of individuals harbor methane-producing bacteria.[206]
Roseburia spp. *Ruminococcus* spp. *Veillonella* spp.	*Ruminococcus* spp. *D. piger* *M. smithii*	*M. smithii*

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.[207-209] However, the literature is mixed on this subject. Additionally, not all sources calculate the ratio using the same methodology.

At Genova, the Firmicutes/Bacteroides 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.[210] Others define dysbiosis as the changes in gut microbiota composition associated with disease.[211] 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.[212-216]

On the GI Effects Comprehensive profile, the Commensal Microbiome Analysis section assesses dysbiosis. These graphics were outlined previously.

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.[217,218]

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									
Bacteroides-Prevotella group		↑	↑	↑	↑	↑	↑	↑	↑
Bacteroides vulgatus		↑			↑	↑		↑	↑
Barnesiella spp.									
Odoribacter spp.									
Prevotella spp.	H	↑		↑	↑	↑		↑	↑
Firmicutes Phylum									
Anaerotruncus colihominis	H	↑	↑	↑	↑	↑	↑	↑	↑
Butyrivibrio crossotus	H								
Clostridium spp.									
Coprococcus eutactus		↑			↑	↑		↑	↑
Faecalibacterium prausnitzii		↑				↑			↑
Lactobacillus spp.									
Pseudoflavonifractor spp.	H	↑	↑	↑	↑	↑	↑	↑	↑
Roseburia spp.	L		↓						
Ruminococcus spp.	H	↓↑	↓	↓	↓	↓↑	↓↑	↓↑	↓↑
Veillonella spp.	H	↑	↑	↑	↑	↑	↑		↑
Actinobacteria Phylum									
Bifidobacterium spp.									
Bifidobacterium longum									
Collinsella aerofaciens		↓↑	↓↑	↓	↓↑	↓↑	↓↑	↓↑	↓↑
Proteobacteria Phylum									
Desulfovibrio piger	H								↑
Escherichia coli	H	↑	↑	↑	↑	↑	↑	↑	↑
Oxalobacter formigenes	H	↑		↑	↑				↑
Euryarchaeota Phylum									
Methanobrevibacter smithii	H	↑				↑			↑
Fusobacteria Phylum									
Fusobacterium spp.	H	↑	↑	↑	↑	↑	↑	↑	↑
Verrucomicrobia Phylum									
Akkermansia muciniphila		↓	↓	↓	↓	↓	↓	↓	↓

***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.

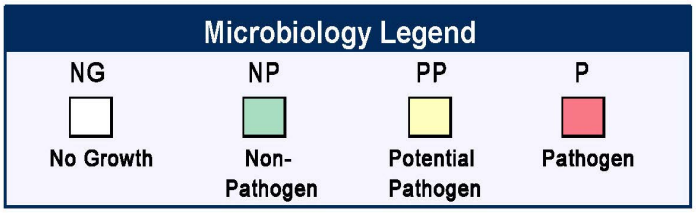
Cells with bolded arrows indicate Genova's clinical condition cohort had more test results falling above versus below **↓↑** or more below versus above **↓↑** the reference range compared to that of Genova's healthy cohort.

Bacteriology and Mycology

Bacteriology and Mycology Culture with Sensitivities

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:

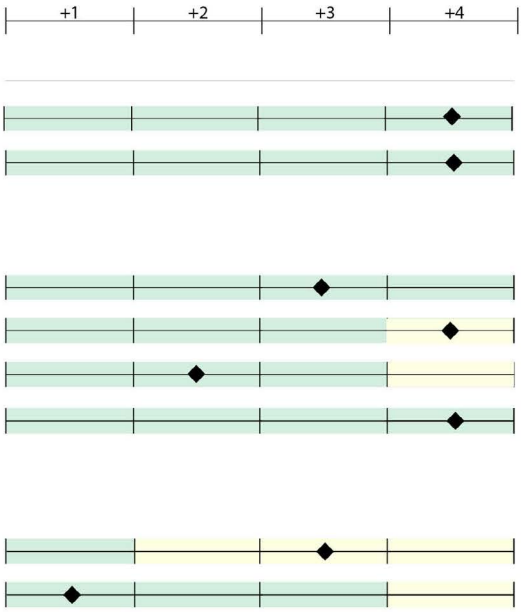
- *Lactobacillus, Escherichia coli*, and *Bifidobacterium* are cultured to offer a more complete microbiome assessment. They are also measured via PCR for quantification.
- *Lactobacillus, Escherichia coli*, and *Bifidobacterium* are known to exert positive local and systemic effects in the microbiome.[219-222]
- Lower levels of these beneficial bacteria have been associated with disease.[223,224]

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 can identify a limitless number of organisms. Any organism identified will be reported.

Gastrointestinal Microbiome

Bacteriology (Culture)



Mycology (Culture)

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

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

Klebsiella oxytoca	R	I	S-DD	S	NI
Ampicillin	R				
Amox./Clavulanic Acid				S	
Cephalothin				S	
Ciprofloxacin				S	
Tetracycline				S	
Trimethoprim/Sulfa				S	

Natural Agents

Klebsiella oxytoca	LOW INHIBITION	HIGH INHIBITION
Berberine		
Oregano		
Uva Ursi		

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.

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 samples take 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.[225]
  - » 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.[226]
  - » 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.[226]
  - » 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**
- **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, to reduce cost and improve patient ease of use.[227-229] 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*.[230] However, older studies demonstrated that in at least 90% of cases, examination of only one stool sample was sufficient to detect an enteric parasite.[227]

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.[230]

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 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	
Ancylostoma/Necator (Hookworm)	Not Detected
Ascaris lumbricoides	Not Detected
Capillaria philippinensis	Not Detected
Enterobius vermicularis	Not Detected
Strongyloides stercoralis	Not Detected
Trichuris trichiura	Not Detected
Cestodes - tapeworms	
Diphyllobothrium latum	Not Detected
Dipylidium caninum	Not Detected
Hymenolepis diminuta	Not Detected
Hymenolepis nana	Not Detected
Taenia spp.	Not Detected
Trematodes - flukes	
Clonorchis/Opisthorchis spp.	Not Detected
Fasciola spp./ *Fasciolopsis buski*	Not Detected
Heterophyes/Metagonimus	Not Detected
Paragonimus spp.	Not Detected
Schistosoma spp.	Not Detected
Protozoa	
Balantidium coli	Not Detected
Blastocystis spp.	**Rare Detected**
Chilomastix mesnili	Not Detected
Cryptosporidium spp.	Not Detected
Cyclospora cayetanensis	Not Detected
Dientamoeba fragilis	**Moderate Detected**
Entamoeba coli	Not Detected
Entamoeba histolytica/dispar	Not Detected
Entamoeba hartmanii	Not Detected
Entamoeba polecki	Not Detected
Endolimax nana	Not Detected
Giardia	Not Detected
Iodamoeba buetschlii	Not Detected
Cystoisospora spp.	Not Detected
Trichomonads (e.g. *Pentatrichomonas*)	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.[231,232] They are observed more commonly in the sputum of asthmatics, but are rarely found in the stool.[233] Studies show that Charcot-Leyden crystals can be present with *E. histolytica* and *Blastocystis* infections.[234] 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.[233,235]

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*.[236]

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.

Polymerase Chain Reaction (PCR) - Protozoa

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.[237]

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.[238]

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

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.

Parasitology

PCR Parasitology - Protozoa**

Methodologies: DNA by PCR, Next Generation Sequencing

Organism	Result	Units		Expected Result
Blastocystis spp.	6.00e2	femtograms/microliter C&S stool	**Detected**	Not Detected
Cryptosporidium spp.	<4.87e2	genome copies/microliter C&S stool	Not Detected	Not Detected
Cyclospora cayetanensis	<2.65e2	genome copies/microliter C&S stool	Not Detected	Not Detected
Dientamoeba fragilis	6.40e2	genome copies/microliter C&S stool	**Detected**	Not Detected
Entamoeba histolytica	<1.14e3	genome copies/microliter C&S stool	Not Detected	Not Detected
Giardia	<1.57e2	genome copies/microliter C&S stool	Not Detected	Not Detected

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 because 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 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.

	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."

Therapeutic Considerations for Parasitology

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

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.[239] 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).[238,240]

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.[229]

- 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.[231]
- 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.[241] Retesting PCR should not be used to document cure.[242,243]

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.

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.

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 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.[244]

To the best of Genova's knowledge, the Scheffler paper has impacted the zonulin assay across the United States, including Genova's stool zonulin test. 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.[245]

Several papers have been published using the IDK kit and clinical associations range 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.[246,247]
- 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.

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