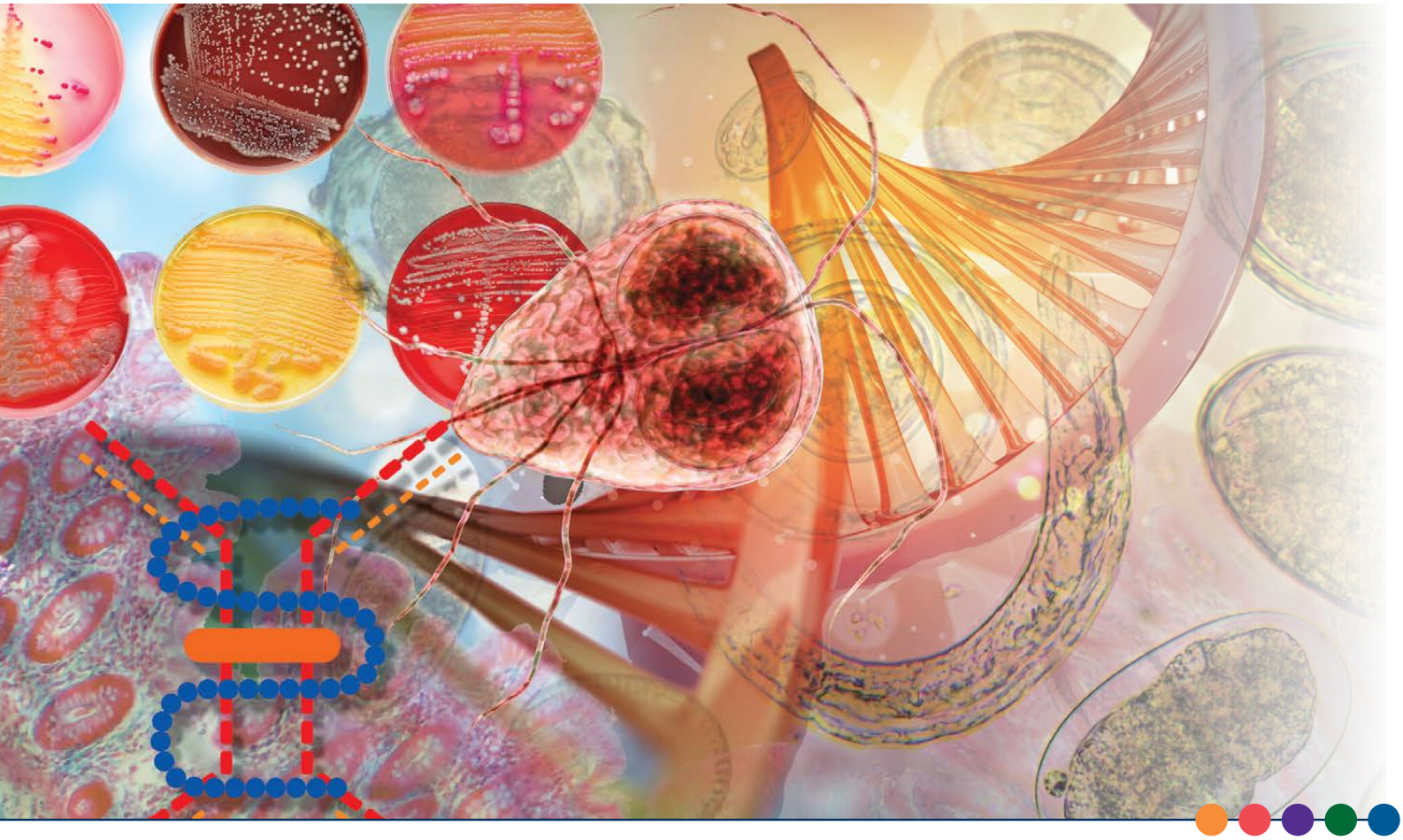

GI fx GI Effects® Stool Profiles

Support Guide

GENOVA DIAGNOSTICS®

Genova Diagnostics GI Effects Stool Support Guide

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GI fx GI Effects Stool Profiles

INTRODUCTION

Advances in research, combined with clinical insight, confirm the essential role of the gut in determining overall health and wellness. The **GI Effects 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.

The GI Effects 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 offer valuable insight into digestive function, intestinal inflammation, as well as the gastrointestinal microbiota. The GI Effects is designed to identify potential root causes of symptoms. It assists clinicians by providing targeted therapeutics that improve symptoms and overall gut health.

In addition to providing a comprehensive set of GI functional biomarkers, the GI Effects 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. These 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 a complex algorithm summarizing the results of these comprehensive stool profiles into an Interpretation-At-A-Glance page, providing convenient access to global evaluation of GI function. 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.

Gastrointestinal Profiles Biomarkers Comparison Table

BIOMARKERS REPORTED	2200*	2205*	2207*
*Not Available in New York			
Digestion and Absorption			
Pancreatic Elastase 1	•		
Products of Protein Breakdown (Total) (Valerate+Isobutyrate+Isovalerate)	•		
Fecal Fat (Total)	•		
Triglycerides	•		
Long Chain Fatty Acids	•		
Cholesterol	•		
Phospholipids	•		
Inflammation and Immunology			
Calprotectin	•		
Eosinophil Protein X (EPX)	•		
Fecal sIgA	•		
Metabolic			
SCFA (Total) (Acetate, n-Butyrate, Propionate)	•		
n-Butyrate Concentration	•		
n-Butyrate %	•		
Acetate %	•		
Propionate %	•		
Beta- glucuronidase	•		
Gastrointestinal Microbiome			
Commensal Bacteria (PCR)			
***Bacteroides-Prevotella* group**	•	•	
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	+	+	•

Interpretation-At-A-Glance

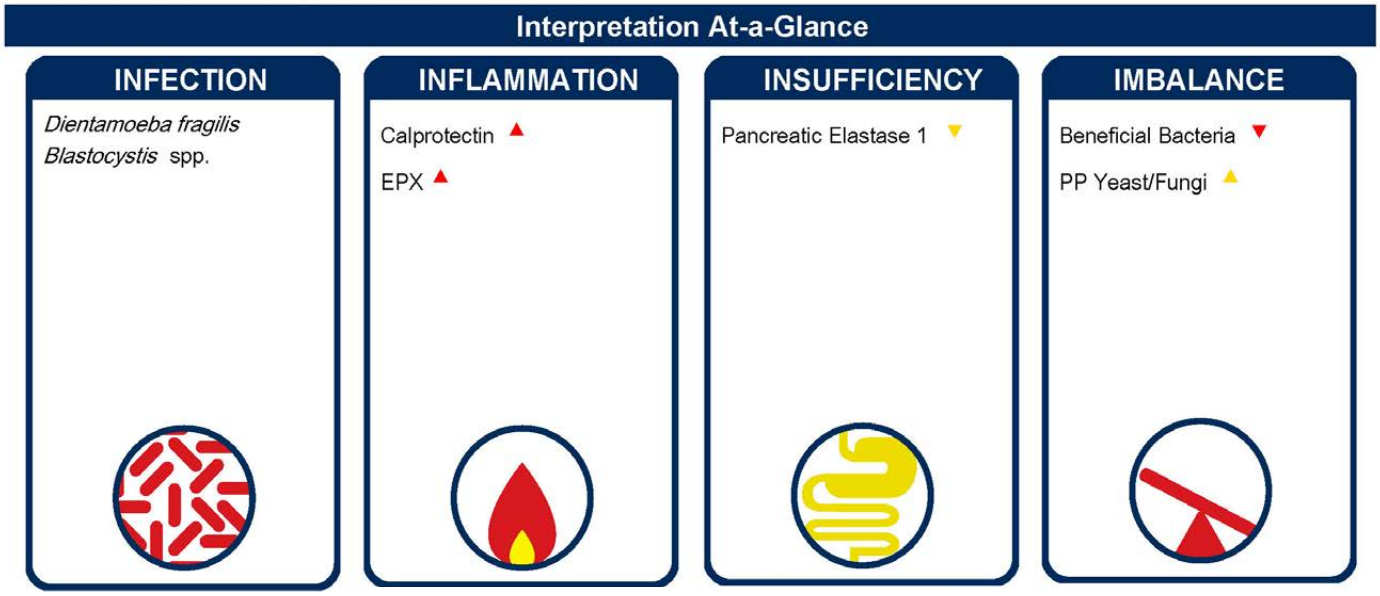
The GI Effects Stool Profile report is organized to guide the clinician through the results in a logical order to enhance clinical utility, beginning with the **Interpretation-At-A-Glance** page.

Using weighted algorithms, the page synthesizes patient results into key functional areas of clinical significance and provides a directional indication of potential next steps in patient management.

The page is divided into three major sections:

1. **Four Functional Pillars**
2. **Commensal Balance Graphic**
3. **Relative Abundance**

1



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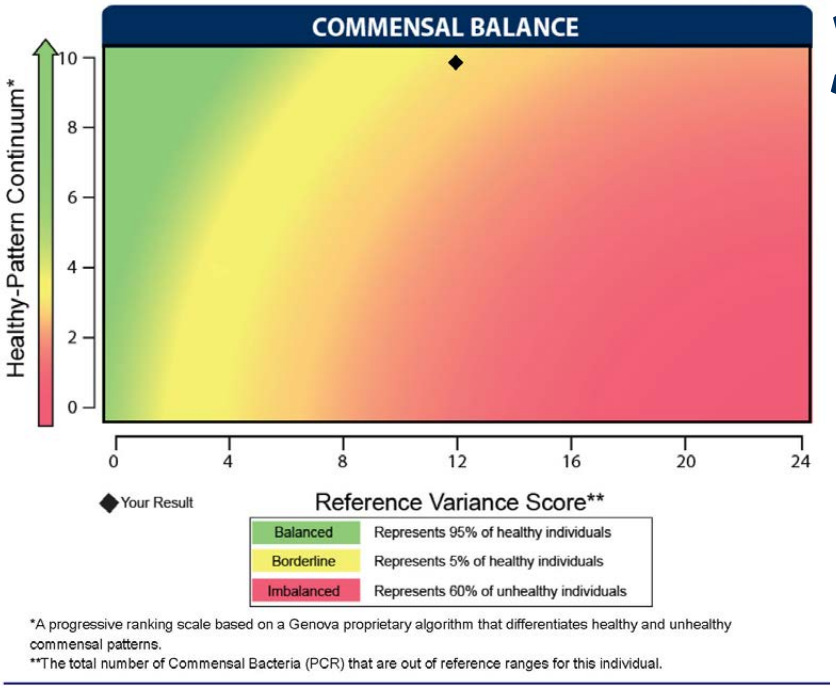
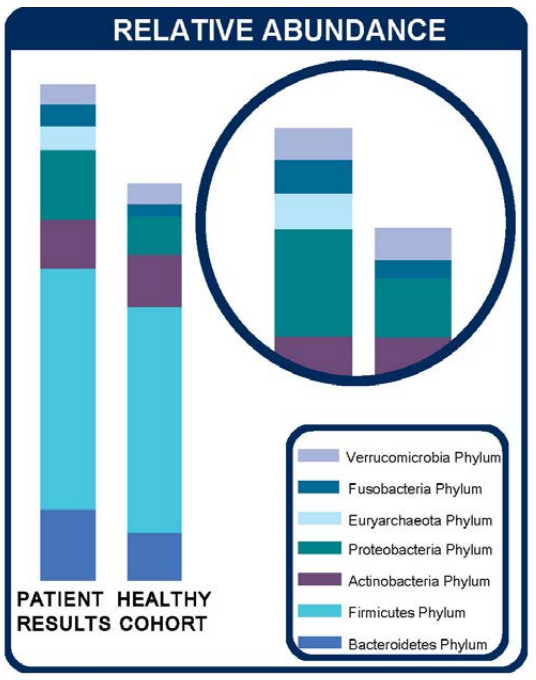
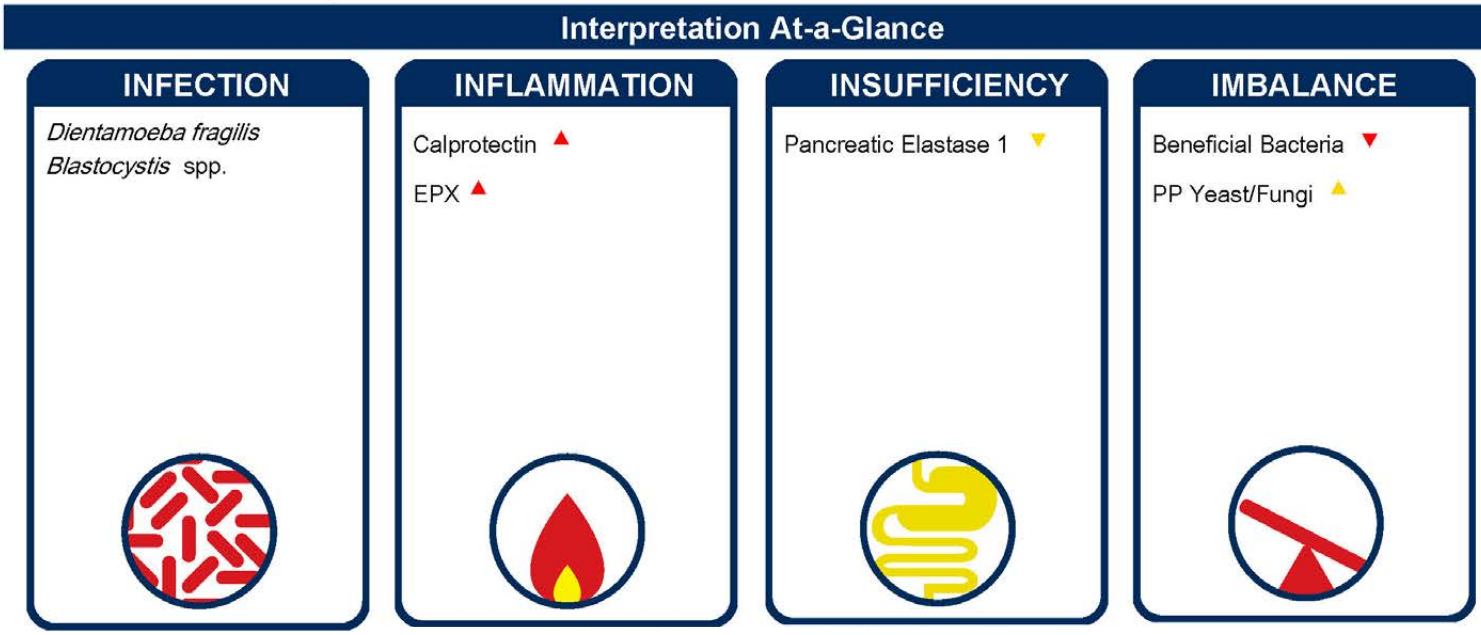


*A progressive ranking scale based on a Genova proprietary algorithm that differentiates healthy and unhealthy commensal patterns.
**The total number of Commensal Bacteria (PCR) that are out of reference ranges for this individual.

3





Four Functional Pillars:

The four functional pillars take into account the level of individual biomarkers throughout the test and their degree of clinical impact. An overall score of high, medium, or low is represented with color-coded icons and informational graphics.

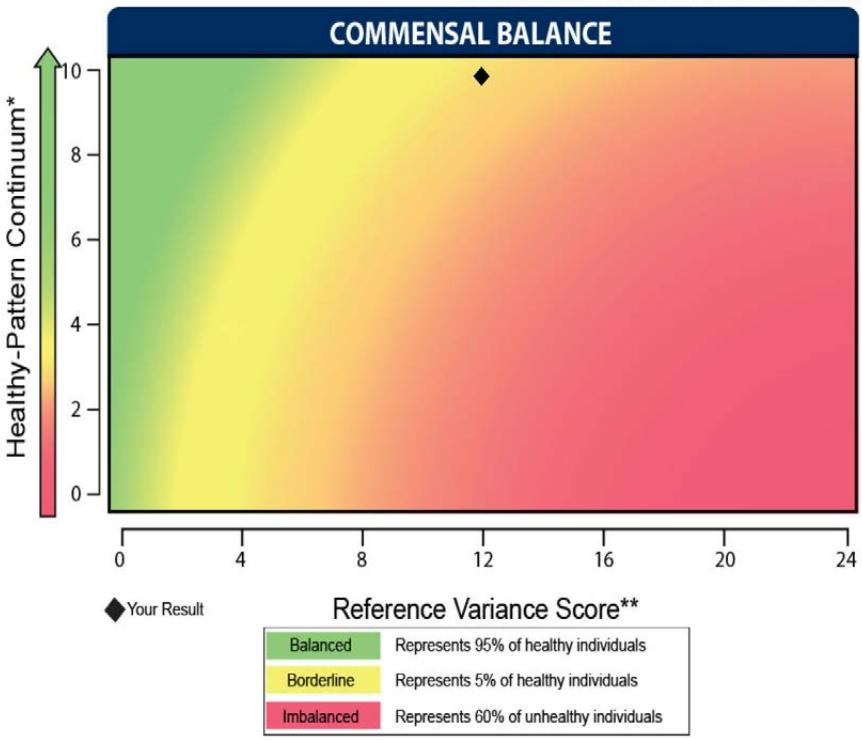
Commensal Balance Graphic:

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.

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.

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.

The patient's result on the **Commensal Balance infographic** is denoted by a black diamond 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 balanced commensal health status, yellow borderline, and red imbalanced.



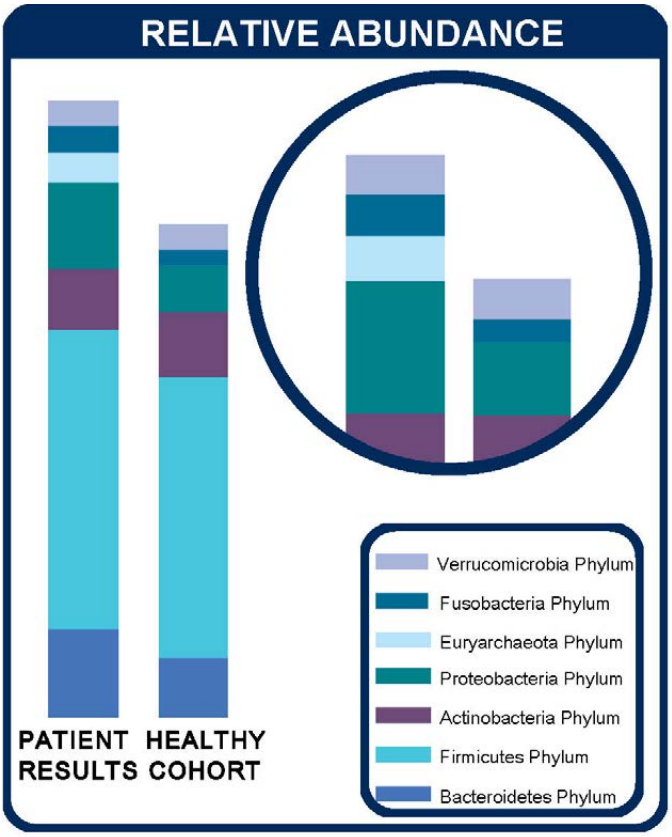
*A progressive ranking scale based on a Genova proprietary algorithm that differentiates healthy and unhealthy commensal patterns.

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

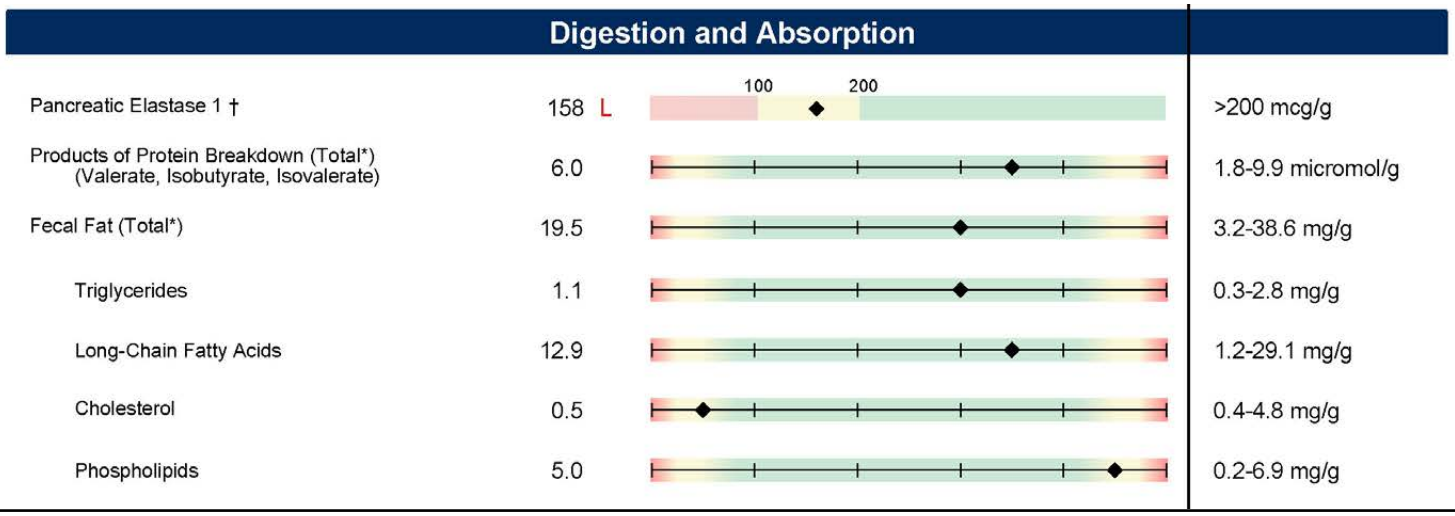
Relative Abundance:

The Relative Abundance (RA) graphic represents the proportional levels of selected phyla, as measured by PCR, in an individual's microbiome. Those results are then compared to similar levels derived from a healthy cohort.

Higher relative abundance can be seen in patients who take probiotics or who have a varied diet rich in fermented foods. It may also be seen in overgrowth syndromes such as **SIBO**. Lower abundance can be seen in patients who are currently (or had been) on antimicrobial therapy, those with limited diets, or in patients with mucosal inflammation.



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.[1] 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.[2] Genova utilizes the Schebo ELISA method using a monoclonal antibody which is highly specific for human PE-1. The monoclonal antibodies used in the test do not cross react with elastases of animal origin, which are contained in enzyme substitution preparations. Therefore, PE-1 should not be used to monitor PERT.

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

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

Reference values are adopted from an FDA-cleared kit and are based on correlation with the gold-standard testing for exocrine pancreatic insufficiency (EPI) as described in the literature. Since the reference range for PE-1 was evaluated using patients with severe EPI, the fecal PE-1 test does not have a high sensitivity for mild and moderate EPI. An optimal range of PE-1 may be higher than 200µg/g. Although the sensitivity and specificity of fecal PE-1 in EPI varied among studies, in several healthy cohorts, most individuals had average values ≥ 500µg/g.[3-6]

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.[7] Some patients can have mild to moderate EPI, which may not be associated with maldigestion and/or malabsorption signs and symptoms.[7]

Signs and symptoms of EPI include:

- Diarrhea
- Steatorrhea
- Foul-smelling stools
- Bloating
- Excess flatulence
- Abdominal discomfort
- Weight loss[8-10]

Causes of EPI:

Exocrine pancreatic insufficiency can occur secondary to:

- Cystic Fibrosis[11]
- Chronic pancreatitis (CP)[12]
- Pancreatic resection[13]
- Autoimmune pancreatitis[14]
- Gallstones[15]
- Pancreatic tumor/cancer.[16]
- GI surgery (i.e. gastric bypass, pancreatic resection).[17]

Other clinical factors associated with EPI through unknown mechanisms include:

- Celiac disease[18-22]
- Inflammatory Bowel Disease (IBD)[23]
- Zollinger-Ellison syndrome.[24,25]
- Aging[7,26]
- Excessive alcohol consumption[27]
- Small Intestinal Bacterial Overgrowth (SIBO).[7,28 29,30]
- Smoking[27]
- Obesity[31]
- Vegan/vegetarian diets[32]
- Diabetes[33,34]

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[9,10 35,36]
3. Consider small, frequent meals, smoking cessation, and reduced alcohol consumption[9,10]
4. Consider **SIBO testing** if there is an elevated Relative Abundance of commensal bacteria, high products of protein breakdown, high fecal fats, high short chain fatty acids, or high levels of *Methanobrevibacter smithii* via PCR.

Products of Protein Breakdown

(Putrefactive Short Chain Fatty Acids)

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

Biomarker Key Points:

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

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

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

- Colorectal cancer [37,38]
- Depression[39]
- Small Intestinal Bacterial Overgrowth (SIBO)[40]
- Antibiotics[41]
- Increased protein consumption[42]
- Diverticulosis[43]
- Celiac disease[44]
- Autism
- GI bleeding[45]
- Chronic pancreatitis, steatorrhea[46]

Causes of high fecal products of protein breakdown:

- Exocrine pancreatic insufficiency[47]
- High protein diet
- Small intestinal bacterial overgrowth (SIBO)[48]
- Low gastric HCL (hypochlorhydria, acid-blocking medications[49]
- Certain types of dysbiosis
- GI bleeding[45]

Causes of low fecal products of protein breakdown:

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

Therapeutic considerations for elevated PPB:

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

Therapeutic considerations for low PPB:

1. Evaluate dietary protein intake
2. Evaluate relative abundance of commensal bacteria
3. Consider prebiotics, probiotics, and fermented foods
4. 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, cholesterolphospholipids long chain fatty acids (LCFAs)Triglycerides,** 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.[50,51]
- **LCFAs**: Increased fecal LCFAs are often indicators of malabsorption.[50,51]
- **Cholesterol**: Fecal cholesterol can come from different sources: diet, bile, and intestinal secretion.[52] Our daily fecal cholesterol excretion may exceed cholesterol intake.[52] 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.[53] Elevations in fecal phospholipids can be due to mucosal cell turnover, malabsorption, or bile.

Causes of fat maldigestion:

1. **Exocrine pancreatic insufficiency (EPI)[54]**
2. **Bile salt insufficiency[54]**
3. **PPI usage and hypochlorhydria[55]**
   - PPI's increase the secretion of most pancreatic enzymes, but reduce the secretion of colipase.[56] 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)[55]
   - Bile acid deconjugation[48,57]
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)[58]

Causes of fat malabsorption

1. Intestinal dysbiosis and SIBO[59]
2. Intestinal parasites[60]
3. Gastric bypass, ileal resection, or other surgeries that limit absorptive surface area[61]
4. Irritable bowel syndrome (often as a symptom of pancreatic exocrine insufficiency or bile acid malabsorption)[54,62] – more likely with the constipation subtype
5. Inflammatory bowel disease[63]
6. Celiac disease[64]

Therapeutic considerations for elevated fecal fats:

Target evaluation and treatment for common etiologies of fat maldigestion:

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

Target evaluation and treatment for common etiologies of fat malabsorption:

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

Further Evaluation:

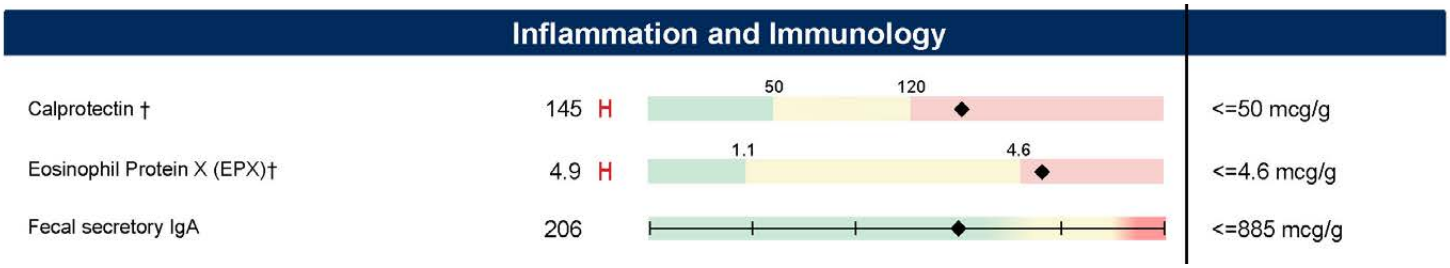
- Fat malabsorption or digestion may be associated with deficiencies in fat or fat-soluble nutrients
- Consider nutritional assessment of essential fatty acids and fat-soluble vitamins

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

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

PHOSPHOLIPIDS
LONG CHAIN
CHOLESTEROL
FATTY ACIDS (LCFAs)
TRIGLYCERIDES

INFLAMMATION AND IMMUNOLOGY



Calprotectin

Calprotectin is a calcium-binding protein with antimicrobial properties.[65] It accounts for 60% of neutrophil cytosolic content and is also found in monocytes and macrophages.[66] 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.[67]
- 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.[68]

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.[69]

Causes of elevated calprotectin:

- Age (children younger than 5 years old, and patients greater than 60)[70]
- IBD, not in remission[71]
- Colorectal cancer and polyps[71]
- Infection (bacteria and some parasitic organisms)[71]
- Non-steroidal anti-inflammatory medication use (NSAIDs) and NSAID enteropathy [72]
- 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.[71,73]

Therapeutic considerations for elevated calprotectin:

- Calprotectin 50 to 120 mcg/g
- Address cause of inflammation:
  - Infection
  - Suspected or history of IBD
  - Chronic NSAID use
- Recheck calprotectin in 4-6 weeks

Calprotectin >120 mcg/g

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

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

Eosinophil Protein X (EPX)

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

Biomarker Key Points:

Under steady-state conditions, the digestive tract's mucosa harbors a substantial number of eosinophils, which, if need be, are activated and exert several effector and immunoregulatory functions.[74]

While small-intestinal eosinophils are anti-inflammatory, large-intestinal eosinophils, when activated, secret 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.[74]

Causes of EPX elevation:

- **Immune-mediated food hypersensitivity, atopic dermatitis, and food allergies.**[75,76]
- **IBD.**[77]
- **Certain parasitic infections**[78]
  - 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.[79]
- **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.

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.[80-84]

Early studies of sIgA focused on immune exclusion (the prevention of pathological material and organisms from gaining entry into 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.[81,84]

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.[85]

In people with genetic immunodeficiency of systemic sIgA, GI symptoms such as diarrhea have been reported.[86] 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.[87]

Causes of elevated fecal sIgA:

- Any defective epithelial barrier[88-90]
- A defective epithelial barrier allows bacterial and microbial penetration, which is the strongest stimulator of sIgA production.
- Celiac disease[91]
- Colon cancer[92]
- Infections
- IBS (especially the diarrhea subtype)

Therapeutic considerations for elevated fecal sIgA:

- **Assess for and treat root causes of immune upregulation /inflammation**:
  - Infection (bacterial, parasitic, and/or viral pathogen, potential pathogen)
  - Compromised intestinal barrier function (i.e., intestinal permeability)
  - Heightened response to noninfectious stimuli (i.e., food sensitivity/allergy, etc.)
    - » Consider Food Antibody testing
      - – If positive, consider elimination diet

Considerations for low fecal sIgA:

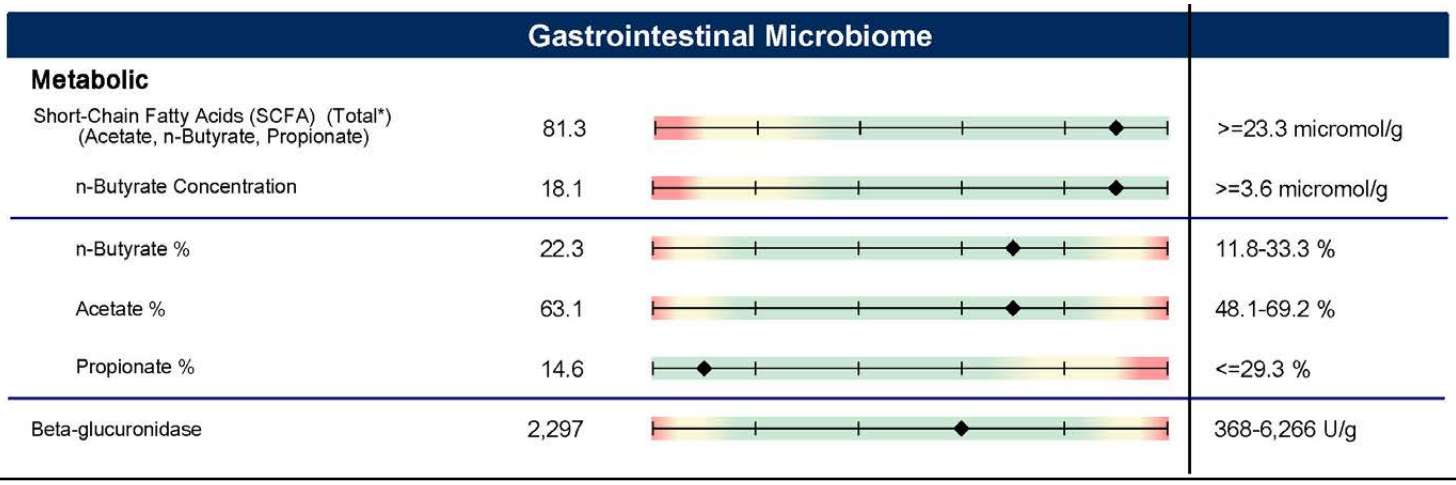
Because of the lack of clinical evidence, there is no clear cut-off value for low fecal sIgA.

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

Fecal sIgA may be low in severe/prolonged IBD patients due to a switch from intestinal IgA to IgG production as well as a deficiency in producing IgA dimers and polymers.[84]

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.[87] This test result should be considered together with the patient's medical condition, other biomarkers, and microbiome profiles when interpreting the data.

GASTROINTESTINAL MICROBIOME



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 and short chain fatty acids (butyrate, acetate, and propionate). These biomarkers reflect specific and vital metabolic functions performed by the microbiota.

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

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

KOH preparation 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 select GI Effects profiles also offer PCR detection. Six targets are chosen to detect common protozoan parasites. These include *Blastocystis* spp. with reflex subtyping 1-9, *Cryptosporidium* spp., *Cyclospora cayetenensis, Dientamoeba fragilis, Entamoeba histolytica,* and *Giardia*. PCR for pathogenic organisms has emerged as a preferred, highly sensitive method for infectious organism detection. 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.

GASTROINTESTINAL MICROBIOME METABOLIC INDICATORS

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.[93,94]

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.

SCFA production from fiber is dependent on the specific enzymes each gut bacteria possesses.[94] The table below lists the commensal bacteria listed on the GI Effects profile, and the type of short chain fatty acid they primarily produce, based on literature.

Butyrate:

Butyrate is the primary fuel source for colonocytes. Inadequate levels are associated with disordered colonic health.[95,96]

Based on the literature, the three major butyrate-producers are *Faecalibacterium*, *Eubacterium*, and *Roseburia*.[93]

Low butyrate indicates low anaerobic bacteria or low dietary sources of fiber, fermentable carbohydrates, and resistant starch.

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.[97]

Acetate is recognized as a volatile signal for biofilm formation.[98]

Inulin supplementation has been shown to increase acetate levels.[99]

Propionate:

Propionate is a minor energy source for the colonocytes, though it has anti-inflammatory effects.[100]

Propionate acts as a precursor for gluconeogenesis in the liver.[97]

Systemic propionate inhibits acetate incorporation into cholesterol.[99]

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* *Odoribacter* *A. colihominis* *Clostridium* spp *C. eutactus* *Lactobacillus* spp *Ruminococcus* *Veillonella* spp *Bifidobacterium* *A. muciniphila*	*Prevotella* *Odoribacter* *Clostridium* spp *Veillonella* spp. *A. muciniphila* *Clostridium* spp *Veillonella* spp *A. muciniphila*
Butyrate Utilizer	Acetate Utilizer	Propionate Utilizer
Clostridium	*Roseburia*	*Clostridium*

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[101-103]
- 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) and/or butyrate supplementation
- Arabinogalactans and β-glucan, as found in whole-grains[104]
- Inulin supplementation[99]
- Probiotics and fermented foods to balance the microbiome

Causes of elevated SCFAs:

- Elevated commensal bacteria abundance or bacterial overgrowth[105]
- High dietary intake of fiber and resistant starches

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

Therapeutic considerations for high SCFAs:

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

Beta-glucuronidase

Beta-glucuronidase is an enzyme which is produced by colonocytes and by some intestinal bacteria (particularly *E. coli*, but also *Ruminococcus, Bacteroides, Eubacterium, Peptostreptococcus, Staphylococcus*, and *Clostridium*).[106]

Biomarker Key Points:

Beta-glucuronidase breaks down complex carbohydrates and increases the bioavailability and reabsorption of plant polyphenols (lignans, flavonoids, ceramides, and glycyrrhetinic acid).[107]

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.[106]

Limited research suggests an association between elevated fecal beta-glucuronidase and cancer risk, primarily colorectal and breast cancer.[108-111]

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[106,112]

Therapeutic considerations for elevated beta-glucuronidase:

- Probiotics[113,114]
- Dietary fiber, prebiotics[113-116]
- 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).[117]
- Oral supplementation inhibits the enzymatic activity of beta-glucuronidase[117]
  - Milk thistle[118,119]
  - Low-calorie and vegetarian diets[106,120]

Causes of low beta-glucuronidase:

- Dysbiosis
- Antibiotic use[121,122]

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.

GASTROINTESTINAL MICROBIOME 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.

Commensal Bacteria

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.[123] 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.[123]

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

The commensal gut microbiota interacts extensively with the host, influencing multiple metabolic and physiological functions, such as: [124,125]

- regulating the gut's development
- facilitating digestion
- producing SCFAs
- shaping the immune system
- preventing the growth of harmful microflora species
- synthesizing nutrients (such as biotin 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.

Dysbiosis:

The term 'dysbiosis' is often used to describe altered microbiome patterns as compared to a healthy cohort.[136] Others define dysbiosis as the changes in gut microbiota composition associated with disease.[137] 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.

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.[138,139]

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.[126]	**Acetate Producer (C2:0)** The majority of acetate is produced by most enteric bacteria from carbohydrate fermentation. One-third of acetate comes from acetogenic bacteria, which synthesize acetate from hydrogen and carbon dioxide or formic acid.[93]	**Propionate Producer (C3:0)** The majority of acetate is produced by most enteric bacteria from carbohydrate fermentation. One-third of acetate comes from acetogenic bacteria, which synthesize acetate from hydrogen and carbon dioxide or formic acid.[93]
• *F. prausnitzii* • *B. crossotus* • *Colihominis* • *Clostridium* • *C. eutactus* • *Roseburia* spp.	• *Prevotella* • *Odoribacter* • *Colihominis* • *Clostridium* • *C. eutactus* • *Lactobacillus* • *Ruminococcus* • *Veillonella* • *Bifidobacterium* • *A. muciniphila*	• *Prevotella* • *Odoribacter* • *Prevotella* • *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.[127]	**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.[128] H2 is used by intestinal bacterial methanogens, acetogens, and SRB.	**Sulfate reducing bacteria (SRB)** H2S Producer: AA metabolism utilizes sulfate (SO42–) and reduces it to hydrogen sulfide (H2S). SRB are part of a normal gut microbiota, though increased levels may contribute to disease. Excess is not absorbed and is available for rapid exogenous H2S production by the SRB.[129,130]
• *B. crossotus* • *Lactobacillus* • *Bifidobacterium* • *B. longum*	• *Odoribacter* • *Clostridium* • *E. coli*[128]	• *Odoribacter* • *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.[131]	**H2-using (hydrogenotrophic)** H2 consumers include reductive acetogens, methanogenic archaea, and sulphate-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.[132]
• *Roseburia spp.* • *Ruminococcus* • *Veillonella spp.*	• Rumin*ococcus* • *D. piger* • *M. smit*hii	• *M. smithii*

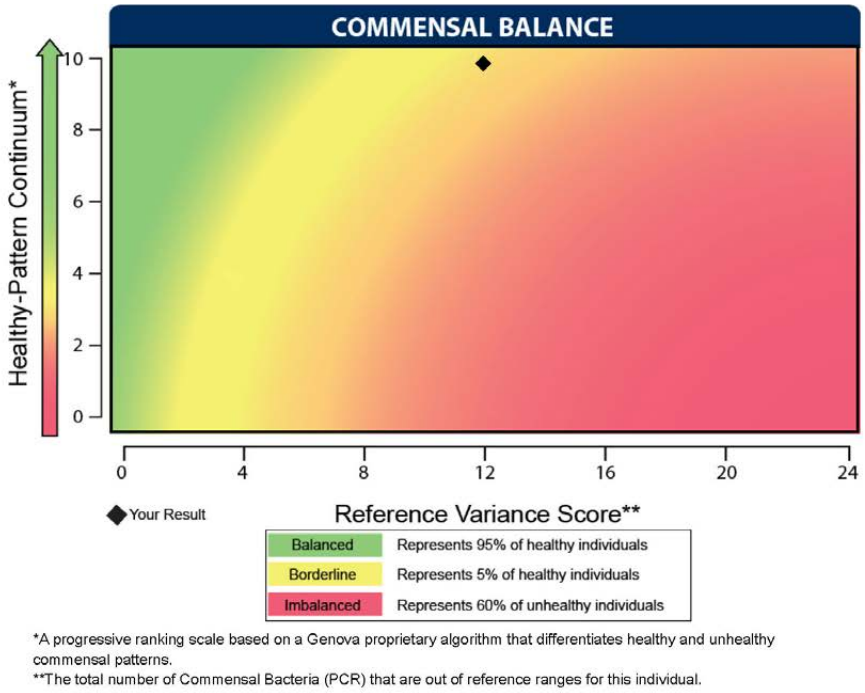
Firmicutes/Bacteroidetes (F/B) Ratio

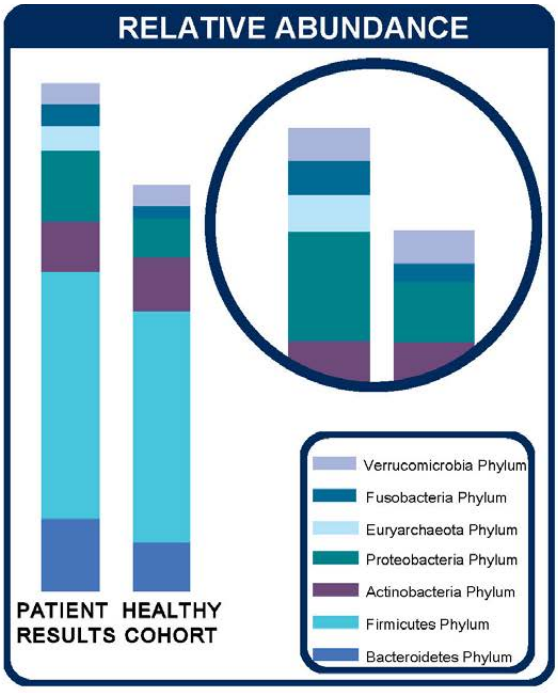
Literature suggests that a high **Firmicutes/Bacteroidetes (F/B) ratio** may be associated with a greater risk of metabolic syndrome, diabetes, and obesity.[133-135] However, the literature is mixed on this subject. Additionally, not all sources calculate the ratio using the same methodology.

At Genova, the Firmicutes/Bacteroides ratio calculation is made by adding the abundance of *Anerotruncus colihominis, Butyrivibrio crossotus, Clostridium* spp., F*aecalibacterium 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, *Barnsiella*, 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.

On the GI Effects Comprehensive and Microbial Ecology profiles, two graphics are used to assess dysbiosis using the 24 commensal bacteria PCR findings: Commensal Balance graphic and Relative Abundance. These graphics were outlined previously as part of the Interpretation-At-A-Glance page. (Please see page 3)





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. In addition, these charts feature patient results marked as high (H) or low (L) compared to the reference range for each commensal bacteria or biomarker.

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						↑↓			↑↓

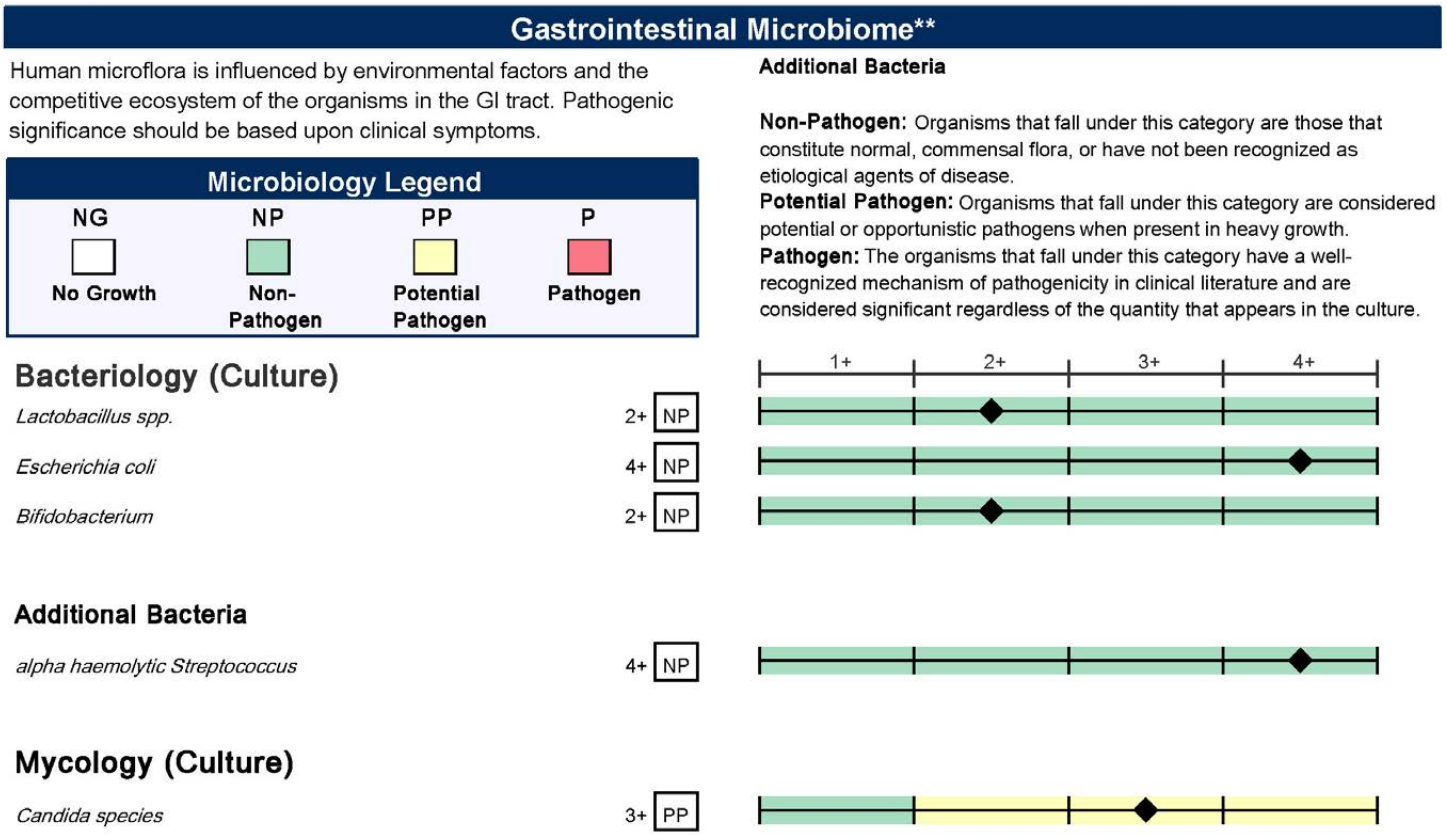
***Information derived from GDX results data comparing a healthy cohort to various clinical condition cohorts. The chart above showing a comparison of patient results to clinical conditions is meant for informational purposes only; it is not diagnostic, nor does it imply that the patient has a specific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

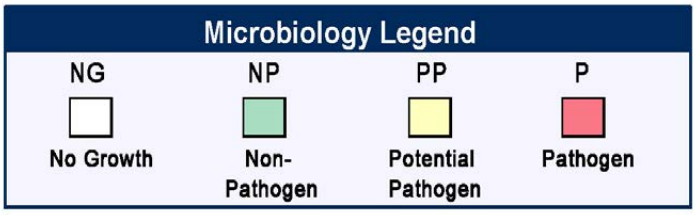
GASTROINTESTINAL MICROBIOME BACTERIOLOGY AND MYCOLOGY CULTURE WITH SENSITIVITIES



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.

Bacteria Sensitivity

Prescriptive Agents

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

Natural Agents

Citrobacter amalonaticus	LOW INHIBITION	HIGH INHIBITION
Berberine		
Oregano		
Plant Tannins		
Uva-Ursi		

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.[140-143]
- Lower levels of these beneficial bacteria have been associated with disease.[144,145]

Additional Bacteria and Mycology Culture:

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

**Please see the Appendix for a clinical information chart 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.

Sensitivities

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.

GASTROINTESTINAL MICROBIOME PARASITOLOGY

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	

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 GI Effects offers the most comprehensive parasitology assessment available including:

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

When clinical suspicion for a parasitic infection is high, a three-day sample collection is recommended. This traditional recommendation in textbooks and lab manuals to collect at least three samples has been challenged, in an attempt to reduce cost and improve patient ease of use.[146-148] 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*.[149] However, older studies demonstrated that in at least 90% of cases, examination of only one stool sample was sufficient to detect an enteric parasite.[146]

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.

Microscopic Ova & Parasites (O&P):

Microscopic examination of stool specimen 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.[149]

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 is capable of detecting any and all parasites, some parasites are more difficult to detect due to their small size, irregular shedding schedules, etc. Additional testing methods are recommended to enhance sensitivity, such as PCR or EIA.
A negative O&P microscopy result is reported as "Not Detected." A positive finding is reported as the amount of that organism (rare, few, moderate, many), followed by the organism's morphology characteristics (trophozoites, cysts, ova.)

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

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.[150,151] They are observed more commonly in the sputum of asthmatics, but are rarely found in the stool.[152] Studies show that Charcot-Leyden crystals can be present with *E. histolytica* and *Blastocystis* infections.[153] 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.[152,154]

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*.[155]

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.

GASTROINTESTINAL MICROBIOME POLYMERASE CHAIN REACTION (PCR)

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

***Blastocystis* spp. Reflex Subtyping**

Type 1:	Not Detected	Type 4:	Not Detected	Type 7:	Not Detected
Type 2:	Detected	Type 5:	Not Detected	Type 8:	Not Detected
Type 3:	Not Detected	Type 6:	Not Detected	Type 9:	Not Detected

Polymerase Chain Reaction (PCR):

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.[156]

Genova's 6 parasite targets include *Cryptosporidium* spp., *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* spp.

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.[157]

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

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

Positive PCR, negative microscopy

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

Negative PCR, positive microscopy

PCR testing is performed on the third-day vial, while microscopy is performed using a homogenized sample mixing all three days of stool. If a parasite intermittently sheds, it may be possible to miss in PCR since only one stool sample is tested.

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

percentage of inhibition, however, for those samples continuing to exhibit inhibition we will not report results. This is due to the fact that further dilution will adversely impact the limit of detection and may result in false negatives. Additionally Genova will not increase the number of amplification cycles in order to compensate for the reduced sensitivity due to dilution. This approach may result in amplification of artifact and thus generate false positives.

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

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

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

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 see the Appendix for a clinical information charts regarding specific parasitic organisms.**

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.[158] 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).[157,159]

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.[148] https://www.semanticscholar.org/paper/Beyond-O%26P-Times-Three-Mohapatra-Mbbs/f27b8716181ce0263a20e732e8da37bce403d032
- 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.[150]
- 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.[160] Retesting PCR should not be used to document cure.[161,162]

Reflex *Blastocystis* Subtyping 1-9:

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

Biomarker Key Points:

Although most individuals host only a single ST, it is possible to have more than one subtype, or a mixed-ST infection.[163,164]

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

ST1-ST4 are the most prevalent, representing approximately 90% of all isolates. ST3 is the most common, with a predominance of around 60% of all isolates.[159,163,165]

There is regional distribution of specific *Blastocystis* subtypes - each with differing zoonotic transmissions.[153,159,163,166-171]

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

Blastocystis has known treatment resistance, with multiple causes:

- Morphologic states (vacuolar, amoeboid, granular, cyst) vary in sensitivity to treatment (i.e., the cyst stage is known to be resistant to metronidazole).[172]
- Different subtypes vary in sensitivity to treatment due to different genetic makeup and thus mechanism of pathogenicity.
    - Studies show that the pathogenic potential of ST3 is enhanced when isolates were treated with metronidazole, suggesting a mechanism to produce higher numbers of viable cells to ensure survival during stressed conditions.[173-175]
    - *Blastocystis*-derived proteases found in ST4 and ST7 have been shown to degrade luminal secretory IgA (sIgA), leading to an ineffective immune response, favoring a chronic, symptomatic *Blastocystis* infection.167,176 In-vitro studies using cysteine protease inhibitors show efficacy against *Blastocystis* isolates, however there are no commercially available cysteine protease inhibiting drugs.[172,177]
    - In vitro studies show that certain ST may be more sensitive to drugs that affect nitric oxide (NO), and that ST-7 actually has the ability to modulate the antiparasitic host NO defense for its own survival.[178,179]
    - The microbiome may influence treatment outcomes.[178]
- Reinfection may be mistaken for treatment failure or resistance.
- *Blastocystis* isolates from differing geographical regions have different degrees of metronidazole resistance.[178]

Blastocystis Subtype Treatment

Research on individual treatments for *Blastocystis* subtypes is in its early stages, and evidence is inconclusive. There is no single drug that is effective across all isolates of different *Blastocystis* subtypes.[178] Pre-clinical, in-vitro studies have mixed results.[180,166]

In-vitro studies are difficult to translate clinically because the isolates are outside of the human microbiome environment, and *Blastocystis* is known to feed on bacteria.[172]

Human studies on treating *Blastocystis* subtypes are limited mainly to case studies. Large-scale clinical outcome studies regarding effective treatments for *Blastocystis* subtypes are lacking; however, this presents an opportunity for Genova and clinicians to correlate patient data as the literature evolves.

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

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

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

Treatment selection is at the discretion of the clinician.

Table 1: In-vitro sensitivities (including conventional and experimental agents) for treating *Blastocystis* spp. subtypes.[172,173,175,177,178,180-183]

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

	Sensitive	Resistant
ST1	• TMP/SMX (most effective) • MTZ (effective ↑ concentrations, but not total clearance) • Albendazole (effective ↑ concentrations) • Garlic • *Achillea millefolium* (Yarrow) • Egyptian propolis • Note: pathogenic potential may be enhanced by NAC, producing higher numbers of viable cells for survival	• Antifungals (fluconazole, nystatin, itraconazole ketoconazole) • TMP/SMX (lower doses) • Ginger, black pepper, cumin
ST2	*Achillea millefolium* (Yarrow)	N/A
ST3	• MTZ (effective ↑ concentrations, but not total clearance; pathogenic potential may be enhanced, producing higher numbers of viable cells for survival) • TMP/SMX (effective ↓ concentrations) • Garlic • *Ferula asafetida* • *Achillea millefolium* (Yarrow) • *Eurycoma longifolia* (Tongkat Ali) • Egyptian propolis	• Antifungals (fluconazole, nystatin, itraconazole, ketoconazole) • NTZ (lower doses) • Ginger, black pepper, cumin
ST4	• TMP/SMX (most effective; 1:2 combination more effective versus 1:5) • MTZ (effective ↑ concentrations, but not total clearance) • Albendazole (effective ↑ concentrations) • Ronidazole • Ornidazole • Nitazoxanide • Furazolidone • Mefloquine • Quinicrine • Quinine • Iodoacetamide • Note: pathogenic potential may be enhanced by NAC, producing higher numbers of viable cells for survival	• Antifungals (fluconazole, nystatin, itraconazole) • MTZ • Emetine (literature mixed) • Paromomycin • Chloroquine • Doxycycline • Ampicillin • Pyrimethamine
ST5	• MTZ	• Ketoconazole
ST6	• N/A	• N/A

ST7	• TMP/SMX (1:2 combination more effective versus 1:5) • Emetine (literature mixed; limited use clinically due to severe side effects) • Ronidazole • Ornidazole, Nitazoxanide • Furazolidone • Mefloquine • Quinicrine • Quinine • Iodoacetamide • Note: pathogenic potential may be enhanced by NAC, producing higher numbers of viable cells for survival	• MTZ • Paromomycin • Chloroquine • Doxycycline • Ampicillin • Pyrimethamine
ST8	TMP/SMX (most effective) MTZ (effective ↑concentrations, but not total clearance)	Antifungals (fluconazole, nystatin, itraconazole)
ST9	N/A	N/A

Table 2: Human studies for treating *Blastocystis* spp. subtypes.[184-191]
TMP/SMX = Trimethoprim/Sulfamethoxazole; MTZ = Metronidazole; NTZ = Nitazoxanide; TAB = triple antibiotic therapy

Author, year	Study Type	Subtype(s)	Outcomes
Angelici, 2018	Case study; 41 y.o. male with chronic GI symptoms after acute GI symptoms drinking contaminated well water; infection became chronic and patient refused conventional therapies	ST1	*Saccharomyces boulardi*i was effective whereas a probiotic w/ *Lactobacillus* & *Bifidobacterium* was not effective
Nagel, 2014	Prospective, longitudinal case study; n=10 IBS-D patients treated with 14 days of TAB (diloxanide furoate, TMP/SMX, secnidazole); follow up testing day 15 and 4 weeks following TAB	ST1, ST3, ST4, ST7, ST8	Successful eradication occurred in 60% of patients, however no correlations could be made between subtype and eradication or subtype and clearance of symptoms
Nagel, 2012	Prospective, longitudinal case study; n=11 symptomatic patients treated with 14 days of either MTZ or TMP/SMX; follow up testing day 15, 28 and 56	ST1, ST3, ST4, ST6	No patient cleared the organism following either monotherapy
Stensvold, 2013	Case study; 40 y.o. female hospitalized with severe traveler's diarrhea and fever, and later developed chronic post-infectious GI symptoms of bloating, flatulence and abdominal pain	ST8	Patient treated with 2 rounds of 10 days of MTZ with little symptom relief, and later 10 days of TMP/SMX with marked symptom improvement and negative stool testing

Roberts, 2013	Prospective, longitudinal case study; n=18 patients with diarrhea, abdominal cramps and bloating treated with multiple regimens including MTZ, iodoquinol, doxycycline, NTZ, furazolidone, secnidazole, ciprofloxacin, tinidazole, norfloxacin, and paromomycin	ST1, ST3, ST4, ST5	Paromomycin was the only therapy that resulted in eradication and symptom resolution in 3 patients with either ST3 or ST5
Katsarou-Katsari, 2008	Case study; 19 y.o. male with 3 week hx of urticaria, soft stools and 2.5 month hx abdominal pain	ST3	Patient treated with MTZ for 10 days and experienced resolution of urticaria and GI symptoms
Vogelberg, 2010	Case study; 20 y.o. male with chronic urticaria and flatulence	ST2	Patient treated with MTZ with no symptom relief, and later TMP/SMX with no symptom relief; finally treated with combo of MTZ and paromomycin with symptom resolution and negative stool testing
Jones, 2008	Cross-sectional observational study; n=21 symptomatic patients with fatigue, depression, skin rash, joint pain, constipation, abdominal pain, diarrhea; 9/21 patients positive for *Blastocystis* with six having ST3 and one having ST1	ST1, ST3	Most patients reported failure with MTZ

Additional Tests:

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

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

Add-on Testing:

There are several optional add-on tests available to the GI Effects Profiles.

- **Enzyme Immunoassay (EIA) to evaluate four pathogenic bacteria:**
  - *Clostridium difficile* (*C. difficile*) (Toxin A/B)
  - Shiga toxin-producing *Escherichia coli* (E.coli)
  - *Campylobacter* spp.
  - *Helicobacter pylori* (*H. pylori*)
- **Fecal Lactoferrin**
- **Zonulin Family Peptide**
- **KOH Preparation for Yeast** (standard on the Gut Pathogen Profile)
- **Macroscopic Examination for Worms** (standard on the Gut Pathogen Profile and previously outlined)

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 see the Appendix for a clinical information chart regarding specific 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 takes the shape of the container.
  - Genova's EIA kit measures antibodies to both toxin A and B. Clinical relevance is determined by the presence of toxin A/B. When these toxins are present, correlation with patient symptoms is recommended.

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

- ***Campylobacter* spp.:**
  - *Campylobacter* is bacterial pathogen associated with a wide range of symptoms and gastrointestinal conditions. It can cause watery or bloody diarrhea, fever, nausea, and abdominal pain. It is also associated with IBD, Barrett's esophagus, colorectal cancer, and reactive arthritis.[192]
  - 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.[193]
  - 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.[193]
  - Genova's enzyme immunoassay uses an enzyme-immunoassay platform that utilizes antibodies to detect *H. pylori* antigen present in the stool sample.

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.194

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.194

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.

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.[195]

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

The Scheffler paper suggests that the kits may detect properdin, a protein involved in the alternative complement pathway and inflammation. Preliminary study results from an external investigator suggest that properdin may be structurally and functionally similar to zonulin.

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.

Because of the lack of information on the mechanism of action and clinical utility of zonulin family peptide, Genova will not provide support on interpreting the test results. Genova will continue monitoring this issue and provide clients with new information as it becomes available.

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.

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APPENDIX
MICROORGANISM CHARTS

Parasitic Organisms

NEMATODES – ROUNDWORMS

Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
Ancylostoma -Necator *Ancylostoma duodenale* *Necator americanus*	Hookworms Soil-transmitted nematodes (P)	Found in tropical and subtropical climates, as well as in areas where sanitation and hygiene are poor.[1] Infection occurs when individuals come into contact with soil containing fecal matter of infected hosts.[2]	*Necator* can only be transmitted through penetration of the skin, whereas *Ancylostoma* can be transmitted through the skin and orally. *Necator* attaches to the intestinal mucosa and feeds on host mucosa and blood.[2] *Ancylostoma* eggs pass from the host's stool to soil. Larvae can penetrate the skin, enter the lymphatics, and migrate to heart and lungs.[3]	Some are asymptomatic, though a heavy burden is associated with anemia, fever, diarrhea, nausea, vomiting, rash, and abdominal pain.[2] During the invasion stages, local skin irritation, elevated ridges due to tunneling, and rash lesions are seen.[3] *Ancylostoma* and *Necator* are associated with iron deficiency anemia.[1,2]
Ascaris lumbricoides	Soil-transmitted nematode Most common human worm infection (P)	Common in Sub-Saharan Africa, South America, Asia, and the Western Pacific. In non-endemic areas, infection occurs in immigrants and travelers. It is associated with poor personal hygiene, crowding, poor sanitation, and places where human feces are used as fertilizer. Transmission is via the fecal-oral route.[4]	*Ascaris* eggs attach to the small intestinal mucosa. Larvae migrate via the portal circulation into the pulmonary circuit, to the alveoli, causing a pneumonitis-like illness. They are coughed up and enter back into the GI tract, causing obstructive symptoms.[5]	Most patients are asymptomatic or have only mild abdominal discomfort, nausea, dyspepsia, or loss of appetite. Complications include obstruction, appendicitis, right upper quadrant pain, and biliary colic.[4] Intestinal ascariasis can mimic intestinal obstruction, bowel infarction, intussusception, and volvulus. Hepatic and pancreatic ascariasis can mimic biliary colic, acute acalculous cholecystitis, hepatic abscess, acute pancreatitis, and ascending cholangitis. Appendicular ascariasis can mimic appendicular colic, appendicitis, appendicular gangrene. Gastric ascariasis can mimic pyloric obstruction.[6]
Capillaria philippinensis	Fish-borne nematode (P)	Although rare in the US, it is more common in Asia (Thailand and the Philippines)[4] Infection occurs from eating raw or undercooked fish containing larvae.	Ingested larvae reside in the human small intestine, where the female deposits eggs, which then develop, causing autoinfection and hyperinfection.[4]	Diarrhea, anorexia, malaise, and vomiting.[4] Capillariasis can mimic IBD and other causes of protein losing enteropathy.[6]
Enterobius vermicularis	Pinworm The most common worm infection in children ages 5-10 in the US (P)	Compared to other intestinal parasites, the transmission of pinworm is limited because their eggs are unable to survive in the environment. The main routes of infection are autoinfection from eggs or larvae deposited on the anus, contamination from bed sheets, clothing, door handles, and inhalation of eggs from	Eggs are deposited around the anus by the worm. Autoinfection occurs due to scratching the perineal area, then thumb-sucking or nail-biting. Pinworms reside in the intestine but can migrate to distant organs.[4]	Some infections are asymptomatic. Symptoms may include itching and irritation. Occasional migration of the worm to distant organs can cause dysuria, vaginal discharge, enuresis, and peritoneal granulomas.[4] Enterobiasis can mimic hemorrhoids and IBD.[6]

Pathogenic Bacteria & Yeast

Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Bacillus species	*Bacillus* species are Gram-positive aerobic (or facultatively aerobic) rods.[8] Most human non-anthracis *Bacillus* spp. infections are caused by *B. cereus*. Not all isolates are associated with disease. Many *Bacillus* species are used in spore- and soil-based probiotics, such as *B. subtilis, B. coagulans,* and *B. licheniformis.*[9] (PP)	*Bacillus* organisms are widely distributed in the environment, though the primary habitats are soil and water. Many *Bacillus* species are beneficial and used in probiotics and in biocidal environmental insecticides.[9,10]	Different *Bacillus* species produce various extracellular products, including antimicrobial substances, enzymes, pigments, and toxins. Except for a select few species, most *Bacillus* species have no pathogenic potential and are not associated with disease.[8]	*Bacillus* infection is not always pathogenic and often asymptomatic. Infections caused by the *Bacillus* species include self-limiting gastroenteritis (*B. cereus*), localized infections due to trauma, ocular infections, and rarely systemic illness as seen in *B. anthracis.*[8]
Campylobacter spp. *Campylobacter jejuni* *Campylobacter coli*	*Campylobacter* species are non-spore-forming, Gram-negative, helical, rod-shaped, or curved bacteria.[11] *Campylobacter* genus belongs to the family *Campylobacteraceae.*[12] (P)	*Campylobacter* has a world-wide distribution and international travel is a risk factor for infection. *Campylobacter* is a confirmed foodborne bacterial pathogen. Infection occurs after consumption of contaminated food, particularly poultry, unpasteurized milk, and water.[12,13]	*Campylobacter*'s helical shape and flagella are thought to be responsible for their ability to colonize the intestinal tract, and for adhesion and invasion into epithelial cells.[11] Additionally, cytotoxin production leads to cell death, damage to mucosal surfaces, and subsequent diarrhea.[14] The onset of symptoms usually occurs 24-72 hours following ingestion.[12]	*C. jejuni* and *C. coli* are established causes of gastroenteritis world-wide. *C. jejuni* can also lead to autoimmune conditions like Guillain-Barre' syndrome and Miller Fischer syndrome. Patients with *C. jejuni* or *C. coli* experience acute watery or bloody diarrhea, weight loss, and abdominal cramping.[12] Many *Campylobacter* species are known pathogens associated with a wide range of gastrointestinal conditions, including inflammatory bowel disease, Barrett's esophagus, and colorectal cancer. They have also been known to cause extra-gastrointestinal manifestations, including bacteremia, lung infections, brain abscesses, meningitis, and reactive arthritis.[12]

Pathogenic Bacteria & Yeast

Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Candida spp. *Candida albicans* *Candida* species, not *albicans* *Candida auris* *Candida dubliniensis* *Candida famata* *Candida glabrata* *Candida guilliermondii* *Candida krusei* *Candida lusitaniae* *Candida parapsilosis* *Candida pseudotropicalis* *Candida rugosa* *Candida stellatoidea* *Candida tropicalis* *Candida zeylanoides*	*Candida* spp. have commonly been identified as part of the healthy human mycobiome. Host defense interruption, or immunocompromise, is required for them to act as pathogens.[15] *Candida albicans* is the most prevalent among the *Candida* spp.[15] (PP)	Fungi, including *Candida*, are ubiquitous in our environment and are part of natural foods and industrial processes, including antibiotic production, bread, cheese, alcoholic beverages, decomposing natural debris, fruits, and soil nutrients.[16] *Candida* is present in the gut of up to 70% of healthy adults, but certain factors, including diabetes, antibiotics, antacid, and steroid inhaler use, promote overgrowth.[17] *Candida* growth in the GI tract is positively correlated with carbohydrate consumption.[18]	*Candida* pathogenesis depends on virulence factor expression, like germ tube formation, adhesions, phenotypic switching, biofilm formation, and hydrolytic enzyme production. Most *Candida* disease processes are primarily due to biofilm formation.[15] During overgrowth, *Candida* produces pseudohyphae that push their way into the intestinal lining, destroying cells and brush borders, and may eventually send toxic metabolic by-products through the intestinal wall into the blood.[19] High-level *Candida* colonization is frequently observed in ulcer and IBD patients. This may in part reflect common treatments for these conditions. In addition, the presence of *Candida* delays healing and exacerbates disease.[20]	As noted, most patients are asymptomatic, and *Candida* is considered a commensal organism. Depending on the host's immune status and comorbidities, symptoms will vary. *Candida* overgrowth in the GI tract has been shown to cause diarrheal illness.[21] Other GI symptoms sometimes seen include thrush, bloating, gas, intestinal cramps, rectal itching, and altered bowel habits.[22] Some generalized symptoms of patients with yeast infections include chronic fatigue, mood disorders, and malaise.[22]

Pathogenic Bacteria & Yeast

Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Citrobacter spp. *Citrobacter amalonaticus* *Citrobacter braakii* *Citrobacter freundii* *Citrobacter youngae* *Citrobacter koseri/diversus*	*Citrobacter* are Gram-negative, non-spore-forming, facultatively anerobic bacilli. *Citrobacter* fall within the *Enterobacteriaceae* family.[23] *Citrobacter* is considered a commensal bacteria; however, depending on the clinical picture, it is also known to be an opportunistic pathogen.[24] (PP)	*Citrobacter* species are found in water, soil, food, and commonly in the human intestinal tract.[23] *Citrobacter* infections can also be nosocomial.[23]	Although considered a commensal, some *Citrobacter* isolates have virulent toxins, such as Shiga-like toxins, heat-stable toxins, and cholera B toxin B subunit homologs.[25]	*Citrobacter* is most often asymptomatic but can cause diarrhea.[24]
Clostridium difficile	*C. difficile* is an anaerobic, Gram-positive, spore-forming, toxin-producing bacillus.[26] (P/PP) *See GI Symptoms column Genova measures *C. difficile* toxin via EIA. A prerequisite for *C. difficile* EIA toxin testing is a stool consistency of 7 on the Bristol stool scale, whereby the sample takes the shape of the container. Clinical relevance is determined by the presence of toxin A/B. When these toxins are present, correlation with patient symptoms is recommended.[27]	*C. difficile* spores are frequently found in healthcare facilities, and are found in lower levels in the environment and food supply. Infection can be nosocomial or community transmitted.[26]	*C. difficile* spores are resistant to heat, acid, and antibiotics. They colonize the large intestine and release two protein exotoxins (A, B). These exotoxins cause colonocyte death, barrier function loss, and neutrophilic colitis.[26] Colonization is prevented by barrier properties of the microbiota; weakening of this barrier by antibiotics is the major risk factor for disease.[26,28]	Not all colonized patients develop symptoms.[27] A majority of infants are colonized with C. *difficile* and are asymptomatic.[26] When present, *C. difficile* infection presents with bloody and non-bloody diarrhea, fever, abdominal pain, vomiting, ileus, and dehydration. Toxic megacolon and peritonitis are significant complications of advanced infections.[26] Of note, many successfully treated patients will continue to test positive for weeks or months after symptom resolution; additional treatment is neither required nor effective.[26]

Pathogenic Bacteria & Yeast

Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Cryptococcus albidus *Cryptococcus gattii* *Cryptococcus humicolus* *Cryptococcus laurentii* *Cryptococcus luteolus* *Cryptococcus neoformans*	*Cryptococcus* is a fungus. Although there are more than 30 species of *Cryptococcus*, only two commonly affect humans and animals: *C. neoformans and C. gattii*.[29] 95% of cryptococcal infections are caused by *C. neoformans*.[30] (PP)	Cryptococcosis has a worldwide distribution. Cryptococcosis occurs through the inhalation of fungal cells from soil, plants, and decaying natural materials, though zoonotic transmission is possible. The yeast may incidentally enter the gastrointestinal tract, though this is less likely.[30]	There are prominent virulence factors attributed to *Cryptococcus,* including capsule formation, thermotolerance, and melanin pigment production, which protects the yeast from host oxidative stresses. An effective host immune response is common, using helper T cell reactions; therefore, any weakening of that response allows *Cryptococcus* to survive and thrive.[30]	Cryptococcal infection primarily affects the lungs or central nervous system, though GI tract infection causing diarrhea is increasing among immunocompromised patients (HIV/AIDS).[29]
Edwardsiella tarda	*E. tarda* is a Gram-negative, facultatively anaerobic rod.[31] It is a member of the *Enterobacteraceae* family. (PP)	*E. tarda* exists widely in nature and is isolated from lakes, streams, seawater, and aquatic animals/fish.[31] Infection results from the consumption of contaminated meat/fish, though human infection is rare.[32]	Pathogenicity of *E. tarda* is associated with many virulence factors, such as hemolysins, which enable the bacteria to have access to essential nutrient elements in order to colonize.[33]	Gastroenteritis, with fever and vomiting, is the most common symptom of *E. tarda* infection, ranging from mild secretory enteritis to chronic enterocolitis. Symptoms can be self-limiting; however, extraintestinal manifestations can include systemic abscesses and septicemia.[32,33]
Enterobacter cloaceae	*E. cloaceae* is a Gram-negative, non-spore-forming, enteric bacilli belonging to the *Enterobacteriaceae* family. *Enterobacteriaceae* are not considered primary human pathogens, but are capable of causing opportunistic infections.[34] (PP)	*Enterobacter* have a ubiquitous environmental distribution (trees, plants, crops, soil, water, and foods). They are also part of the normal flora of the GI tract.[34] It can also be a common nosocomial infection.[35]	*Enterobacter's* ability to form biofilms and to secrete various cytotoxins, such as enterotoxins and hemolysins, contribute to its pathogenicity.[35]	Most patients with an *E. cloaceae* infection are asymptomatic. However, when present, symptoms can include nausea, vomiting, diarrhea, and abdominal cramps.[36]

Pathogenic Bacteria & Yeast

Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Escherichia coli 0157:H7 Shiga toxin producing	*E. coli* is a Gram-negative, rod-shaped, facultative anaerobe. Most *E. coli* harmlessly colonize the GI tract as normal flora. However, some strains have evolved and acquired virulence factors, which are characterized by serotypes. *E.coli 0157:H7* has become one of the most virulent foodborne pathogens.[37] (P)	*E. coli 0157* is transmitted to humans through contaminated food and water, directly between persons, and through contact with animals. The most common reservoir is cattle, and the most frequently identified mode of transmission is through ground beef consumption.[38]	*E. coli 0157's* ability to induce injury is a result of its ability to produce Shiga toxin, which is cytotoxic. Additionally, it produces other proteins which aid in the attachment and colonization in the intestinal wall and can lyse red blood cells to liberate iron to support its own metabolism. It should be noted that there are other organisms which can also produce Shiga-like toxin. The characteristic histopathological lesions caused by *E. coli 0157:H7* are called attaching and effacing (A/E) lesions. Microvilli are effaced and bacteria adhere to the epithelium.[37]	Signs and symptoms associated with Shiga-toxin producing *E. coli 0157* include bloody diarrhea, stomach cramping, and vomiting. This can progress to hemolytic uremic syndrome and death.[38]
Geotrichum species *Geotrichum candidum* *Geotrichum capitum*	*Geotrichum* is a eukaryotic, aerobic, Gram-positive, non-capsulated fungus. *Geotrichum* is considered a common commensal in the human GI tract, though opportunistic infections are seen in immunocompromised patients.[39] (PP)	*Geotrichum* is ubiquitous and is commonly found on fruits, vegetables, cheeses, mil, soil, water, air, and in the human digestive tract.[40] Transmission is through inhalation of fungal cells or ingestion of contaminated foods.[39]	*Geotrichum* infection is rare and, in general, *Geotrichum* has low virulence. In patients with normal immunity, it is not pathogenic.[41]	Clinical manifestations are very similar to candidiasis. Many patients are asymptomatic; when present, symptoms include diarrhea, abdominal pain, and mucus in the stool.[39]
Hafnia alvei	*H. alvei* is a Gram-negative, facultatively anaerobic bacillus that belongs to the *Enterobacteriaceae* family. Though rare, it is considered an opportunistic pathogen.[42] (PP)	*H. alvei* is most commonly isolated from vacuum-packed meat, raw milk, raw fish, and other foods. Transmission is via ingestion of contaminated foods, but nosocomial infections have been seen.[42]	*H. alvei* pathogenicity is in biofilm formation and cellulose production; this aids in colonization and mediates cell-cell interaction. It also produces adhesins and toxins which contribute to symptoms and antimicrobial resistance.[42]	*H. alvei*'s clinical relevance is not clear. It has been isolated from feces in asymptomatic patients, yet is also known to cause gastroenteritis, necrotizing enterocolitis, and extra-intestinal illnesses.[43]

Pathogenic Bacteria & Yeast

Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Hansenula anomala Also known as *Pichia anomala* and *Wickerhamomyces anomalus*	*H. anomala/W. anomalus* is an ascomycete yeast.[44] Although useful in food processing, it has been shown to be a very rare opportunistic and nosocomial pathogen in humans, mainly neonates and immunocompromised patients.[44,45] (PP)	*H. anomala/W.anomalus* is frequently found in natural environments (plants, soil, fruit, animals) and is useful in wine fermentation.[45] It also has antimicrobial properties and has been used as a biocontrol agent. It can be found on the skin and as normal flora in the human gastrointestinal tract.[44]	*H. anomala/W. anomalus* are classed as biosafety level 1 by the European Food Safety Authority, and there are no reports in the literature regarding hazardous mycotoxin formation or allergic reactions to spores from this yeast. However, rare isolates from immunocompromised patients are emerging with no clear specific pathogenicity.[44]	*H. anomala/W. anomalus* are considered normal flora and very rarely cause disease, but they have been known to cause sepsis, fungal arthritis, pneumonia, and endocarditis in immunocompromised patients.[46]
Helicobacter pylori	*H. pylori* is a Gram-negative, aerophilic bacterium. *H. pylori* infection is one of the most common chronic bacterial infections affecting humans.[47] (P) Genova uses an enzyme immunoassay platform that utilizes antibodies to detect *H. pylori* antigen present in the stool sample.	*H. pylori* infection is chronic and is usually acquired in childhood. The exact means of infection is not clear.[47]	After entering the host stomach, *H. pylori* uses its urease activity to neutralize the acidic environment. It has a flagella-mediated motility to help it move toward the gastric epithelium. Specific bacterial adhesin proteins lead to colonization and persistent infection. It finally releases effector proteins and toxins causing host tissue damage.[48]	*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).[47] According to the American College of Gastroenterology, the indications to test for *H. pylori* 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, and patients with ITP, should be tested.[47] 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.[47]

Pathogenic Bacteria & Yeast

Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Klebsiella oxytoca *Klebsiella pneumoniae*	*Klebsiella* are non-motile, Gram-negative rods that belong to the *Enterobacteriaceae* family. *Klebsiella* bacteria are considered commensal but act as opportunistic bacteria in the GI tract. *Klebsiella* is a leading cause of hospital-acquired infections.[49] (PP)	*Klebsiella* is part of the normal intestinal flora. The environment likely acts as a reservoir for human acquisition, either as colonization or infection. It is frequently found in water, sewage, soil, and plant surfaces.[50]	*Klebsiella* possesses virulence factors, such as a capsule, lipopolysaccharides, and pili. *Klebsiella* translocates across the intestinal epithelium via a transcellular mechanism by active bacterial invasion. This allows it to penetrate the intestinal barrier and enter systemic circulation causing extraintestinal disease.[51] Cytotoxins produced by *Klebsiella oxytoca* are associated with antibiotic-associated hemorrhagic colitis (AAHC).[52] Ankylosing spondylitis and Crohn's disease have been shown to be triggered by *Klebsiella pneumoniae*. Increased starch consumption by genetically susceptible patients (HLA-B27 allelotypes) could trigger disease by enhancing the growth of *Klebsiella* in the gut. The cross-reactive antibodies between *Klebsiella* and AS/Crohn's trigger inflammatory cascades, such as the complement system, as well as producing various cytokines causing pathologic changes .[53]	*Klebsiella* can asymptomatically colonize the GI tract. However, depending on host factors and immunocompetence, it may cause diarrhea and systemic illnesses.[49,50]
Listeria monocytogenes	*Listeria* is a Gram-positive, facultative intracellular bacterium.[54] (P)	*Listeria* is ubiquitous in the environment. It is the causative agent of Listeriosis, a rare but fatal foodborne disease.[54,55]	*Listeria* can cross several physiological barriers, including the intestinal epithelium and placenta, and survive in multiple cell types. Following internalization into the host cell, the bacterium escapes its membrane-bound vacuole using the toxin listeriolysin. It then replicates within the cytosol and can multiply and spread from cell to cell.[55]	Ingestion of *L. monocytogenes*-contaminated food by immune-competent individuals is often limited to gastroenteritis that resolves in a few days, with pathogenic clearance from the intestine.[54] Severe complications include systemic dissemination causing septicemia, meningitis, and chorioamnionitis; all are associated with high mortality.[54]

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Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Moellerella wisconsensis	*Moellerella wisconsensis* is a Gram-negative bacilli from the *Enterobacteriaceae* family.[56] (PP)	*M. wisconsensis* has been recovered from various sources, such as water, food, and animals.[56] Isolation of this bacteria in clinical samples is very rare. The majority of *M. wisconsensis* isolates from human clinical samples have been from stool, though bronchial aspirates, biliary samples, and peritoneal exudates have been seen.[56,57]	Pathogenicity is unclear due to the scarcity of human infection.	Though rare, isolated case reports show that *M. wisconsensis* has been associated with diarrhea.[56,57]
Morganella morganii	*M. morganii* is a facultative anaerobic, Gram-negative, enteric bacterium which belongs to the *Enterobacteriaceae* family. *M. morganii* is an opportunistic pathogen often isolated as a cause of nosocomial infections in adults.[43] (PP)	*M. morganii* is found in the environment and colonizes the human intestinal tract as part of the normal flora.[58]	*Morganella* produces a urease that predisposes to encrustation of urinary catheters. It may also produce a hemolysin, which enhances virulence by lysing erythrocytes.[58]	Although *Morganella* is part of the normal intestinal flora, it has been implicated in various diseases, including diarrhea, urinary tract infections, and wound infections. Serious infections, like meningitis in AIDS patients, have been reported.[43]
Pichia ohmeri More recently known as *Kodamaea ohmeri*	*K. ohmeri* is a fungus that belongs to the *Saccharomycetes* family, which acts as a very rare opportunistic pathogen.[59] (PP)	*K. ohmeri* is widely used in the food industry for the fermentation of fruits, pickles, and rinds.[59] In the past, *Kodamaea ohmeri* was considered a food contaminant, but is now recognized as an emerging opportunistic pathogen in immunocompromised patients.[60]	Pathogenicity is not yet clearly defined due to the rarity of human infection.	*K. ohmeri* infection is rarely reported to cause human infection, with only isolated case reports seen in the literature; these are primarily in infants and immunocompromised patients.[60-62] Systemic fungemia has been rarely seen in association with indwelling catheters, phlebitis, wound infections, endocarditis, and outbreaks in intensive care units.[60]
Plesiomonas shigelloides	*P. shigelloides* is an anerobic, Gram-negative bacillus, belonging to the *Enterobacteriaceae* family. (P)	*Plesiomonas* is a global pathogen with worldwide distribution. It is most often isolated in aquatic environments. Infection occurs primarily by undercooked freshwater fish consumption.[63]	*Plesiomonas* contains a Shigella phase I antigen, cholera-like toxins, hemolysins, and cytotoxic lipopolysaccharides.	*P. shigelloides* causes gastroenteritis, which ranges from a secretory enteritis to a cholera-like diarrhea. Extraintestinal manifestations can occur with bacteremia and sepsis.[63]

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Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Providencia alcalifaciens	*P. alcalifaciens* is a Gram-negative rod that belongs to the *Enterobacteriaceae* family. It is usually considered to be a commensal bacteria, but can also be an opportunistic pathogen and a cause of traveler's diarrhea.[64] (PP)	*P. alcalifaciens* is found throughout the environment, and as a commensal bacteria in the large intestine. Food contamination and human transmission has been shown to be via the fecal-oral route, lack of sanitation, and poor food storage.[64]	*P. alcalifaciens* has lipopolysaccharides that cause epithelial barrier dysfunction and endothelial apoptosis.[65]	Although often considered a commensal bacteria, *P. alcalifaciens* has been shown to cause diarrhea.[64]
Proteus mirabilis *Proteus penneri* *Proteus vulgaris*	*Proteus* is Gram-negative bacteria belonging to the *Enterobacteriaceae* family. *Proteus* spp. are considered opportunistic pathogens, isolated from urine, stool, and wounds.[66,67] *Proteus* are a common cause of nosocomial infections in patients with impaired immunity. (PP)	*Proteus* is widespread in the environment and considered part of the normal GI flora. *Proteus* spp. are found in soil or water habitats and are often regarded as indicators of fecal contamination.[67]	The chemical structure of *Proteus'* lipopolysaccharides plays an important role in how it adapts to the environment and its pathogenicity. In impaired immunity, *Proteus* bacteria become opportunistic. Cross infection with the urinary tract is common.[67]	*Proteus* species in the stool are considered normal flora, but have been shown to cause diarrheal illness.[67]
Pseudomonas aeruginosa	*P. aeruginosa* is a Gram-negative aerobic bacilli. Although seen as part of the normal healthy intestinal flora, it is considered a potential pathogen. It is generally not a common cause of infectious diarrhea in a healthy host. Patients with chronic disease, chronic antibiotic use, or immunocompromise are at highest risk for infection.[68] (PP)	*Pseudomonas aeruginosa* is readily found in the environment (soil and water) and in the healthy gastrointestinal tract.	*P. aeruginosa* induces pro-inflammatory responses and anti-microbial peptides within intestinal epithelial cells. It also has cytotoxic activity. Disruption of the intestinal epithelial protective mechanisms allow for disease progression.[69]	Most patients are asymptomatic, though *P. aeruginosa* can cause mild diarrhea. A rare complication is Shanghai Fever, which is characterized by fever, diarrhea, and sepsis. *P. aeruginosa* has also been associated with antibiotic-related diarrhea.[68]

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Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Pseudomonas pseudomallei Also known as *Burkholderia pseudomallei*	*P. pseudomallei* is a Gram-negative, aerobic, saprophytic bacillus. It causes the rare, often fatal disease, melioidosis.[70] There are fears that *P. pseudomallei* can be used as a biological weapon.[71] (P)	*Pseudomonas/Burkholderia pseudomallei* is widespread in South, Central, and North America; it is also common in Southeast Asia. Infection occurs through contact with soil and water in endemic areas through inhalation, skin inoculation, or ingestion.[70]	*P. pseudomallei* possesses several secretion systems essential for its dissemination. Pathogenicity is due to its endotoxins inducing apoptosis. Most infections occur in the lung, though systemic disease is possible.[71] It is likely to be consumed in water and food in settings where the organism is present in the environment. It can colonize the gastrointestinal tract without clinical features for months or years.	Most symptoms of melioidosis are pulmonary, though colonization, shedding, and carriage through the GI tract are possible. Systemic disseminated abscesses are common.[71]
Rhodotorula spp. *Rhodotorula glutinis* *Rhodotorula rubra*	*Rhodotorula* is a saprophytic yeast. Previously considered non-pathogenic, it has emerged as an opportunistic pathogen. (PP)	*Rhodotorula* is a common, ubiquitous yeast that is found in air, soil, lakes, ocean water, food, and beverages.[72]	It has been shown that *Rhodotorula* species are able to form biofilms which may play a role in its pathogenicity. Antibiotics and cytotoxic agent exposure increases intestinal colonization and mucosal damage.	Isolation from non-sterile sites, like skin and stool, are more commonly contaminant or colonization. Specific gastrointestinal symptoms are not well studied. Systemic infections and fungemia are possible in immunocompromised patients.[73]
Saccharomyces cerevisiae	*Saccharomyces cerevisiae* and *Saccharomyces boulardii* are two closely related strains of non-spore-forming yeast which are nearly identical at the molecular level. Classically considered a safe, nonpathogen, *S. cerevisiae* can cause disease in immunocompromised patients.[74] (PP)	*S. cerevisiae* commonly colonizes the human respiratory, gastrointestinal, and urinary tracts. *S. cerevisiae* is found in many niches in the environment, but is commonly known as baker's yeast, and is frequently used in the industrial fermentation of bread, beer, and wine.[74] *S. cerevisiae* is also commercially available as a nutritional supplement and is used to treat antibiotic-related diarrhea and IBS.[75]	*S. cerevisiae* uses adhesin proteins to penetrate disrupted epithelial or endothelial barriers. Most fungal pathogens display resistance to the reactive oxygen species used by human cells to resist infection.[74]	Immunosuppression can lead to *S. cerevisiae* infection, though indwelling catheters, chronic antibiotic therapy, and nosocomial spread are common risk factors. *S. cerevisiae* infection can cause a wide variety of clinical syndromes, such as fungemia, pneumonia, abscess, esophagitis, and fever. It has been associated with Crohn's disease and ulcerative colitis.[76]

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Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Salmonella typhii *Salmonella* species *S. arizonae* *Salmonella group A, B, C, D, E, E+G, C+D* *S. paratyphi A, B, C*	*Salmonella* is a facultative intracellular, Gram-negative bacteria within the *Enterobacteriaceae* family. It is the causative agent of human typhoid fever.[77,78] (P)	Humans are typically infected with *Salmonella* after consuming food or drinking water contaminated with bacteria, and transmission is often fecal-oral.[77]	After oral ingestion, *Salmonella* invades the epithelial cells in the distal ileum and invades Peyer's patches. *Salmonella* travels via the afferent lymphatics to gain access to the blood and systemic tissues.[77]	Depending on the serotype, *Salmonella* symptoms can vary from a self-limiting gastroenteritis and diarrhea, to systemic infection with fever, respiratory distress, hepatic and splenic complications, and neurologic damage.[78]
Serratia marcescens	*Serratia* are non-spore-forming, Gram-negative rods, and are part of the *Enterobacteriaceae* family. *S. marcescens* is an opportunistic pathogen, which is generally thought not to be pathogenic in the intestine, but is emerging as a frequent nosocomial infectious agent.[79,80] (PP)	*Serratia* species are ubiquitous in the environment, and found in water, soil, plants, insects, humans, and other animals.[81] Infection is acquired through ingestion of contaminated food or contact with hospital equipment and personnel.[80]	*S. marcescens* has the potential for adhesion, invasion, cytotoxicity, perturbation of intestinal barrier function, cytokine release, and alteration of cellular morphology.[80]	Patients most at risk for *S. marcescens* infection include those with immunocompromise, patients on broad spectrum antibiotics, or hospitalized patients subjected to invasive instrumentation/catheters. Most patients are asymptomatic carriers, though *S. marcescens* infection symptoms may include diarrhea and rarely necrotizing enterocolitis.[80]
Shigella species *Shigella boydii* *Shigella dysenteriae* *Shigella flexnari* *Shigella sonnei*	*Shigella* are Gram-negative pathogenic bacteria that belong to the Enterobacteriaceae family.[82] *Shigella* is the causative organism of Shigellosis, accounting for the majority of dysentery worldwide.[82] (P)	*Shigella* species are transmitted via the fecal-oral route. They are easily transmitted by personal contact with an infected person or consumption of contaminated food or water.[83] *Shigella* species are geographically stratified based on the level of economic development in a given country. *S. flexneri* is the primary infectious species in the developing world, whereas *S. sonneri* rates increase with economic development. *S. boydii* is restricted to Bangladesh and Southeast Asia. S. *dysenteriae* occurs sporadically worldwide.[82]	The *Shigella* bacteria invades colonic mucosa, then can multiply causing epithelial cell death, and spread laterally to cause mucosal ulcers, bleeding, and inflammation.[83]	Symptoms of shigellosis include fever, bloody diarrhea, and abdominal cramping. Infection is usually restricted to the gastrointestinal tract, though extra-intestinal manifestations (reactive arthritis, hemolytic-uremic syndrome, and neurologic complications) can be seen.[83]

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Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Staphylococcus aureus	*S. aureus* in the GI tract is a commensal Gram-positive bacterium, which can be responsible for opportunistic toxigenic infections.[84] (PP)	*S. aureus* is a common cause of food-borne disease. Though ubiquitous in the environment, and a commensal found on the skin, nasopharynx, and gastrointestinal tract, it can be transmitted via contaminated food or water consumption.[85] Fecal carriage is considered an important risk factor for hospital- and community-acquired infections.[86]	*S. aureus* produces varying enterotoxins and contains several virulence genes.[87]	Asymptomatic fecal *S. aureus* carriage is common. However, *S. aureus* GI infection symptoms include nausea, vomiting, and abdominal cramping, with or without diarrhea.[85] The foodborne illness can be self-limiting with resolution after 24-48 hours. Severe disease often requires hospitalization.[87] Colonization with *S. aureus* increases the risk of systemic infection and bacteremia.[84]
Trichosporon species *Trichosporon beigelii* *Trichosporon pullulans*	*Trichosporon* are amorphic fungi. Though considered a commensal yeast, they are increasingly recognized as opportunistic pathogens in immunocompromised individuals.[88] (PP)	*Trichosporon* fungi are commonly found in nature and can reside harmlessly as commensals on the skin and in healthy individuals' gastrointestinal tracts.[88]	*Trichosporon's* ability to invade the skin and other tissues includes several virulence factors, including yeast-to-hyphae transition, biofilm formation, lipases and proteases, and cell wall plasticity.[88]	*Trichosporon* is a commensal yeast in the GI tract and is usually asymptomatic. Changes in nutrient availability may influence *Trichosporon* spp. abundance and diversity and underlie gut mycobiome dysbiosis. This can potentially lead to inflammatory pathologies, such as inflammatory bowel disease. Invasive and systemic trichosporonosis is seen in immunocompromised hosts.[88]
Vibrio cholerae	*Vibrio cholerae* is a Gram-negative, facultative anaerobic bacterium that is responsible for epidemic cholera, a severe diarrheal disease.[89,90] (P)	*V. cholerae* naturally inhabits aquatic environments. Epidemic cholera is transmitted to humans by contaminated water and food consumption.[89] Cholera is associated with unsanitary conditions and countries with poor infrastructure.[90]	*V. cholerae* are ingested and colonize the intestinal mucosa using adhesin proteins and mucinase enzymes. The incubation period is between 12 hours and 5 days. Once a certain concentration of cells is reached, enterotoxin cascades are produced. After being shed, cells can be found in a hyperinfectious state, which make secondary infection to others prevalent.[89]	When mild, cholera symptoms are often indistinguishable from other diarrheal causes. However, more commonly, patients develop severe dehydration or die due to acute watery diarrhea.[90]
Vibrio fluvialis	*V. fluvialis* is a Gram-negative rod known to be pathogenic in humans.[91] (P)	*V. fluvialis* occurs widely in the aquatic environment. It is one of the emerging foodborne pathogens throughout the world. *V. fluvialis* is often associated with raw or undercooked fish consumption.[92]	Upon ingestion into the GI tract, the prevalent virulence factors in *V. fluvialis* infection are hemolysin and cytotoxins.[92]	*V. fluvialis* is found to be associated with cholera-like diarrhea. Rare complications include biliary tract infection, suppurative cholangitis, peritonitis, and other extraintestinal manifestations.[92]

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Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Vibrio furnissii	*V. furnissii* is a Gram-negative rod. Initially it was assigned and named as a subgroup of *V. fluvialis*, but it is now considered a separate species. It is considered pathogenic, but rare.[93] (P)	*V. furnissii* is ubiquitous in aquatic marine environments. Infection is associated with ingestion of contaminated seafood, or exposure to coastal waters.[93]	Flagellum are one virulence factor in *Vibrio* infections, in addition to proteases, hemagglutinins, and hydrolytic exoenzymes.[93]	*V. furnissii* has been associated with gastroenteritis in humans.[93]
Vibrio hollisae Now reclassified as *Grimontia hollisae*	*G. hollisae* is a Gram-negative, aerobic, rod-shaped bacteria, which belongs to the Vibrionaceae family.[94] (P)	Infection usually follows the ingestion of raw, undercooked, or contaminated seafood.[94]	*G. hollisae* shares a pathogenic gene cluster with the entire *Vibrio* genus. It releases a thermostable hemolysin toxin, which is absorbed in the intestines after ingestion.[95]	*G. hollisae* causes severe gastroenteritis, hypovolemia, and septicemia. It is associated with hepatotoxicity.[95]
Vibrio metschnikovii	*V. metschnikovii* is a Gram-negative rod. It is a very rare species with only a small number of cases reported.[96] (P)	Nonhuman sources include shrimp, crab, birds, water, sewage, and other seafood.[96]	As with other members of the *Vibrio* genus, hemolysin and cytotoxins contribute to pathogenicity.[96]	Presentation includes diarrhea and vomiting, though infections with *V. metschnikovii* can be fatal in patients with significant comorbidities.[96]
Vibrio mimicus	*V. mimicus* is a Gram-negative rod closely related to *V. cholerae*.[97] (P)	The natural habitat of *V. mimicus* is similar to *V. cholera*—the aquatic ecosystem. Infection usually occurs from the consumption of infected seafood.[98]	Many *V. mimicus* virulence factors have been identified, including enterotoxin, hemolysin, proteases, and hemagglutinin.[97]	*V. mimicus* gastroenteritis is characterized by diarrhea, nausea, vomiting, abdominal cramping, and fever. However, unlike *V. cholerae*, it is not associated with cholera epidemics since most isolates do not produce cholera toxin.[98]
Vibrio parahaemolyticus	*V. parahaemolyticus* is a Gram-negative rod belonging to the Vibrionaceae family. (P)	*V. parahaemolyticus* grows in warm, low salinity marine water and is the most prevalent food poisoning bacterium associated with seafood consumption.[99]	The vast majority of *V. parahaemolyticus* strains have hemolysin, causing hemolysis in the initiation of disease.[99]	Infection usually causes acute gastroenteritis and is generally self-limiting. Common characteristics include abdominal cramps, nausea, headaches, diarrhea, fever, and chills.[99]

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Genus/Organism	Description	Habitat/Sources of Isolation	Pathogenicity	GI Symptoms
Yersinia enterocolitica *Yersinia pseudotuberculosis*	*Yersinia* is a Gram-negative bacillus belonging to the *Enterobacteriaceae* family. Genus *Yersinia* includes three bacteria that cause human pathology: *Y. enterocolitica, Y. pseudotuberculosis,* and *Y. pestis.* *Y. pestis* causes plague and is transmitted via flea bites. *Y. enterocolitica* and *Y. pseudotuberculosis* cause gastroenteritis and are mainly transmitted via contaminated food and water.[100] (P)	Yersiniosis has been detected on all continents. *Yersinia enterocolitica* has been associated with contamination of a variety of foods, including milk and milk products, raw meats, poultry, eggs, vegetables, seafood, and others. *Yersinia* species are able to propagate in vacuum-packed foods and at refrigeration temperatures.[101]	Following ingestion, approximately 10% of bacteria survive the acidic gastric environment and translocate the gut barrier, which compromises the Peyer's patches in the small bowel and lymphoid follicles in the large bowel. *Yersinia* then drains to neighboring lymph nodes and possibly the portal blood stream.[101] It has been postulated that *Yersinia* species contribute to the occurrence or persistence of gut inflammation in Crohn's disease.[101]	*Y. enterocolitica* and *Y. pseudotuberculosis* can both cause acute watery or bloody diarrhea and gastroenteritis. Although gastroenteritis from *Yersinia* is often self-limiting, some patients develop chronic infections, such as reactive arthritis, erythema nodosum, glomerulonephritis, or myocarditis.[100]

TREATMENT RESOURCES:

The decision to treat potentially pathogenic organisms should be based on the patient's clinical presentation.
The following resources provide valuable insight into the clinical management of pathogenic and potentially pathogenic bacteria and yeast:

- Sanford Guide – infectious disease treatment guidelines: https://www.sanfordguide.com/
- Johns Hopkins Antibiotic Guide – subscription service for in depth information on pathogens, treatment, and clinical implications: https://www.hopkinsguides.com/hopkins/index/Johns_Hopkins_ABX_Guide/All_Topics/A
- PubMed – literature search engine for up to date clinical and treatment information: https://www.ncbi.nlm.nih.gov/pubmed/
- Mayo Clinic – conditions search engine: https://www.mayoclinic.org/
- Merck Manual – treatment and clinical implications of infectious diseases: https://www.merckmanuals.com/professional

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Parasitic Organisms

NEMATODES – ROUNDWORMS

Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
Ancylostoma -Necator *Ancylostoma duodenale* *Necator americanus*	Hookworms Soil-transmitted nematodes (P)	Found in tropical and subtropical climates, as well as in areas where sanitation and hygiene are poor.[1] Infection occurs when individuals come into contact with soil containing fecal matter of infected hosts.[2]	*Necator* can only be transmitted through penetration of the skin, whereas *Ancylostoma* can be transmitted through the skin and orally. *Necator* attaches to the intestinal mucosa and feeds on host mucosa and blood.[2] *Ancylostoma* eggs pass from the host's stool to soil. Larvae can penetrate the skin, enter the lymphatics, and migrate to heart and lungs.[3]	Some are asymptomatic, though a heavy burden is associated with anemia, fever, diarrhea, nausea, vomiting, rash, and abdominal pain.[2] During the invasion stages, local skin irritation, elevated ridges due to tunneling, and rash lesions are seen.[3] *Ancylostoma* and *Necator* are associated with iron deficiency anemia.[1,2]
Ascaris lumbricoides	Soil-transmitted nematode Most common human worm infection (P)	Common in Sub-Saharan Africa, South America, Asia, and the Western Pacific. In non-endemic areas, infection occurs in immigrants and travelers. It is associated with poor personal hygiene, crowding, poor sanitation, and places where human feces are used as fertilizer. Transmission is via the fecal-oral route.[4]	*Ascaris* eggs attach to the small intestinal mucosa. Larvae migrate via the portal circulation into the pulmonary circuit, to the alveoli, causing a pneumonitis-like illness. They are coughed up and enter back into the GI tract, causing obstructive symptoms.[5]	Most patients are asymptomatic or have only mild abdominal discomfort, nausea, dyspepsia, or loss of appetite. Complications include obstruction, appendicitis, right upper quadrant pain, and biliary colic.[4] Intestinal ascariasis can mimic intestinal obstruction, bowel infarction, intussusception, and volvulus. Hepatic and pancreatic ascariasis can mimic biliary colic, acute acalculous cholecystitis, hepatic abscess, acute pancreatitis, and ascending cholangitis. Appendicular ascariasis can mimic appendicular colic, appendicitis, appendicular gangrene. Gastric ascariasis can mimic pyloric obstruction.[6]
Capillaria philippinensis	Fish-borne nematode (P)	Although rare in the US, it is more common in Asia (Thailand and the Philippines)[4] Infection occurs from eating raw or undercooked fish containing larvae.	Ingested larvae reside in the human small intestine, where the female deposits eggs, which then develop, causing autoinfection and hyperinfection.[4]	Diarrhea, anorexia, malaise, and vomiting.[4] Capillariasis can mimic IBD and other causes of protein losing enteropathy.[6]
Enterobius vermicularis	Pinworm The most common worm infection in children ages 5-10 in the US (P)	Compared to other intestinal parasites, the transmission of pinworm is limited because their eggs are unable to survive in the environment. The main routes of infection are autoinfection from eggs or larvae deposited on the anus, contamination from bed sheets, clothing, door handles, and inhalation of eggs from	Eggs are deposited around the anus by the worm. Autoinfection occurs due to scratching the perineal area, then thumb-sucking or nail-biting. Pinworms reside in the intestine but can migrate to distant organs.[4]	Some infections are asymptomatic. Symptoms may include itching and irritation. Occasional migration of the worm to distant organs can cause dysuria, vaginal discharge, enuresis, and peritoneal granulomas.[4] Enterobiasis can mimic hemorrhoids and IBD.[6]

NEMATODES – ROUNDWORMS

Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
		hands, bed mattresses, or dust. As a result, infections tend to be limited to families and individuals in close proximity, like nurseries and boarding schools.[7] Spread by overcrowding and poor hygiene.		
Strongyloides stercoralis	Soil-transmitted nematode (P)	Endemic to the tropics and temperate subtropics where poor sanitation facilitates fecal contamination. Also found in poorer areas of the US: Appalachian mountain communities, Kentucky, and rural Tennessee.[1] Transmission is from contaminated soil.[8]	Infection occurs from skin penetration where the organism then travels systemically (blood, lung, GI tract).[8] *Strongyloides stercoralis* is unique among nematodes infections in humans because larvae passing in the feces can give rise to a free-living generation of worms. The potential for autoinfection exists if larvae attain infectivity while in the host.[4]	Most patients have subclinical or asymptomatic infections. They are commonly chronic and longstanding due to the autoinfective lifecycle.[4] Irritation, edema, and urticaria at the site of skin penetration.[8] Diarrhea, constipation, abdominal pain, anorexia. Dry cough, tracheal irritation, recurrent asthma.[8] Strongyloidiasis can mimic IBD and eosinophilic enterocolitis.[6]
Trichuris trichiura	Whipworm Soil-transmitted nematode The third most common roundworm in humans[4] (P)	Found in areas where human feces is used as fertilizer. Found in the tropics and places with poor sanitation. Transmitted via the fecal-oral route.[4]	A human host consumes eggs, sometimes in food. Once the eggs are ingested, the larvae hatch in the small intestine. From there they migrate to the large intestine, where the anterior ends lodge within the mucosa. This leads to cell destruction and activation of the host immune system, recruiting eosinophils, lymphocytes, and plasma cells. This causes the typical symptoms of rectal bleeding and abdominal pain.[9]	Mild infections are usually asymptomatic. Heavy worm burden causes painful defecation with mucus, water, and blood (Trichuris dysentery syndrome). Rectal prolapse is also seen.[9] Children develop iron deficiency anemia, growth retardations, and impaired cognitive development.[4] Trichuriasis can mimic IBD, bacillary dysentery and acute intestinal amebiasis.[6]

Parasitic Organisms

CESTODES – TAPEWORMS

Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
Dipylidium caninum	Dog (or cat) tapeworm (P)	Human infection is rare but can occur in those who kiss or are licked by their infected pets.[10]	Fleas ingest *D. caninum* eggs. Adult fleas are ingested by pets and establish in the small intestine where the eggs develop into the adult tapeworm. The tapeworm sheds proglottids, which are found in the stool. Humans are infected by accidental ingestion of infected dog or cat fleas.[10]	Most are asymptomatic. When present, symptoms include weight loss, colic, and vomiting.[11]
Diphyllobothrium latum	Fish tapeworm (P)	*D. latum* occurs in freshwater fish throughout much of the northern hemisphere; intermediate hosts include bears, pigs, cats, dogs, foxes, and wolves. Humans become infected after eating raw or undercooked fish.[11]	After ingestion, in humans the adult helminth can live up to 20 years in the small intestine. It adheres to the mucosa and can eliminate millions of eggs each day. Diagnosis is made by the demonstration of eggs or proglottids in the stool.[12]	Mostly asymptomatic, but signs and symptoms can include nausea, vomiting, diarrhea, abdominal pain, and weight loss.[11] Can cause megaloblastic anemia.[6]
Hymenolepis diminuta	Rat tapeworm (P)	Human infection with *H. diminuta* is rare with only a few hundred cases reported, mainly in children. *H. diminuta* is prevalent worldwide in temperate to tropical conditions with poor sanitation.[13] *H. diminuta* infection requires an intermediate host (usually rodents, but also insects). Humans become infected by ingesting food contaminated with larvae, or by direct hand contact.[14]	Once ingested, *H. diminuta* grows to adult form and sheds eggs through the stool. It attaches to the mucosal surface of the intestine and grows to approximately 20-50 cm. in length.	Infection is usually asymptomatic, though may cause abdominal pain, diarrhea, and irritability.[14]
Hymenolepis nana	Dwarf tapeworm (P)	*H. nana* is one of the most common parasitic tapeworm infections worldwide, found mainly in children. It does not require an intermediary host and can be transmitted human to human, though rodents can also carry *H. nana*.[15] It has fecal-oral transmission from food and water in areas of poor sanitation.[16]	*H. nana* eggs are immediately infective when passed through the stool and cannot last more than 10 days in the environment. Once ingested, larvae penetrate intestinal villi and develop into adults that measure 15-40 cm. in length. Eggs pass into stool or can reside within the intestinal villi and cause continual autoinfection.[17]	Symptoms include abdominal pain, diarrhea, anorexia, weight loss, malnutrition, and anemia.[18]

Parasitic Organisms

CESTODES – TAPEWORMS

Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
Taenia spp. *Taenia saginata* *Taenia solium*	Tapeworm (P)	This tapeworm is found in people who have traveled outside of the US where the infection is endemic, or in Latin American immigrants. Locally acquired infections are rare but have been diagnosed in Los Angeles, New York, Chicago, and Oregon. Infection occurs upon ingestion of raw or undercooked meat.[4]	Ingested parasite cysts reach the intestine and develop into adult tapeworms, releasing motile segments and/or eggs in the stool.[19] One adult tapeworm can expel a minimum of 100,000 eggs per day. The enclosed larvae penetrate the intestinal wall and are transported via the bloodstream to various tissues where they undergo multiple development stages to become cysticerci.[20]	The adult tapeworm stage is relatively innocuous and does not have human pathogenic effects. However, some species' intermediate stages can develop in human brains causing neurocysticercosis, a major cause of neurologic disease in developing countries. Cysticercosis can develop in other organs causing intramuscular, ocular, subcutaneous, and spinal cystericoses.[19] Taeniasis can mimic IBS.[6]

TREMATODES – FLUKES

Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
Clonorchis - *Opisthorchis* spp.	Liver flukes (P)	*Clonorchis* and *Opisthorchis* infections have been reported in many parts of east Asia, Thailand, Laos, Cambodia, and Japan.[21] These flukes live in freshwater snails and fish. Humans are infected by eating raw or partially cooked, infected fish.	Adult flukes attach to bile ducts where they feed for as long as 10-30 years, resulting in chronic inflammation, epithelial hyperplasia, fibrosis, and granuloma.[22] Both are classified as Group 1 carcinogens. Infection by these parasites is frequently asymptomatic and rarely diagnosed during early exposure. Persistent infection is associated with parasite-associated cancer. The mechanism of this transformation is yet to be fully defined.[23]	Early infection is often asymptomatic. Chronic infection is associated with cholangitis, obstructive jaundice, biliary fibrosis, cholecystitis, and cholangiocarcinoma.[24]
Fasciola spp.- *Fasciolopsis buski* ova	Plant-borne intestinal fluke *F. buski* is known as the giant intestinal fluke, and is one of the largest flukes to infect humans.[25] *F. hepatica* is a liver fluke. (P)	Largely confined to Asian countries, including China.[25] Humans are infected by ingesting eggs adhering to the surface of edible water plants.[25]	After ingestion, gastric juices aid the release of the worm. The worm migrates to the small intestine, and produces eggs, which are passed in feces.[26]	Light infections are often asymptomatic. Moderate to heavy infection causes abdominal pain, diarrhea, nausea, vomiting, and fever. Extensive intestinal inflammation, erosions, ulceration, abscess, and hemorrhage are possible.[26]
Heterophyes - *Metagonimus* ova	Fish-borne intestinal fluke (P)	These are mostly seen in Far East and Asian countries.[27] These flukes are exclusively fish-borne and are contracted by humans by ingesting raw or improperly cooked freshwater or brackish fish.	Mechanical irritation is caused by movement of the worms causing mucosal villous atrophy. Chemical excretory/secretory proteins acts as active antigens and toxins, provoking a systemic immune response.[27]	Mucosal changes lead to nutrient malabsorption, intestinal permeability, and watery diarrhea.[27] Abdominal pain, weight loss, and anorexia are also seen.[27]

Parasitic Organisms

TREMATODES – FLUKES

Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
Paragonimus spp.	Lung fluke (P)	There are roughly 9 species that cause clinical disease in humans, but only *P. kellicotti* is endemic to North America, where it is found in streams and rivers in the Mississippi River Basin, including the central United States west to the Rocky Mountains.[11] Infection is caused by ingestion of raw or undercooked crabs or crayfish.[28]	After ingestion of infected crabs or crayfish, the fluke resides in the small intestine and migrates through the intestinal wall into the peritoneal space and eventually into the pleural space.[11]	Patients are often asymptomatic after ingestion and during the initial migration phase. Some patients may develop abdominal pain and diarrhea. After migration to the pleural space, inflammatory pulmonary symptoms begin.[11] Typical features of pulmonary paragonimiasis include cough, hemoptysis, chest pain, and dyspnea.[28]
Schistosoma spp. *Schistosoma mansoni* *Schistosoma japonicum* *Schistosoma haematobium* *Schistosoma mekongi*	Blood fluke S. *mansoni*, S. *japonicum*, and *S. mekongi* cause intestinal disease[29] S. *haematobium* causes urinary disease (P)	This organism is prevalent in the tropics and subtropics where poor sanitation is common.[30] *S. mekongi* is primarily limited to the Mekong River Basin stretching from Laos to Cambodia.[29] Humans contract the infection via water sources.[30]	Humans acquire the infection by direct contact with water sources containing infectious larvae. The larvae penetrate skin and enter the circulation via the capillaries and lymphatics.[30]	Schistosome egg deposition and fluke burden can occur in any ectopic site, giving rise to site-specific symptoms and disease. These include dermatitis, abdominal pain, diarrhea, ascites, GI bleeding, and urinary obstructive symptoms.[30] Intestinal schistosomiasis can mimic diverticulitis and IBD. Hepatic schistosomiasis can mimic alcoholic liver disease and liver cirrhosis.[6]

Parasitic Organisms

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Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
Balantidium coli	Ciliate protozoan (PP)	*Balantidium* is reported worldwide, but is more prevalent in temperate and tropical regions. Human infections are related to poor sanitation and drinking water contaminated with human and animal (swine) feces.[4]	Trophozoites inhabit the intestine, feeding on bacteria and other intestinal contents. In most cases, infections are asymptomatic and the infected host shows no clinical signs, suggesting that this ciliate is an opportunistic parasite that could take advantage of the host's weakened status caused by other infections or diseases. In such cases, the parasite could invade the intestinal wall, causing the disease known as balantidiasis or balantidial dysentery.[31]	Often asymptomatic, but in the acute form symptoms may include mucus and blood in feces. In severe cases, hemorrhages and perforation could occur. Chronic infection may present with unspecific abdominal disorders (diarrhea, abdominal pain), cramping rectal pain, nausea, and vomiting.[31] Balantidiasis can mimic traveler's diarrhea, invasive amebiasis, bacterial dysentery and IBD.[6]
Blastocystis spp.	Although there are 17 different Blastocystis subtypes, subtypes 1-9 are the only subtypes found in humans. Subtypes 1-4 make up 90%. Subtype 3 is most common. Subtypes have geographic distribution. (PP)	*Blastocystis* is one of the most common parasites, affecting 1.5-30% of those in industrialized countries and 30-76% in developing countries.[32,33] *Blastocystis* transmission is via the fecal-oral route by ingesting contaminated food or water, exposure to daycare environments, and exposure to domestic and wild animals.[34,35] Various subtypes have different zoonotic transmissions.	*Blastocystis* resides in the ileum and cecum, adheres to mucus' outer layer, and uses certain bacteria as a nutritional source. (30) Its pathogenicity is controversial.[32] It is associated with higher microbial diversity, and may be regarded as a commensal organism.[33] Literature-based conclusions about disease associations and subtype pathogenicity is conflicting, but evolving.	*Blastocystis* is often asymptomatic. When present, symptoms include nausea, anorexia, abdominal pain, flatulence, acute/chronic diarrhea, constipation, anal itching, fatigue, joint pain, and urticaria. It is associated with irritable bowel syndrome (IBS), and is three times higher in patients with IBS-D.[36] Blastocystosis can mimic acute viral enteritis, and traveler's diarrhea.[6]
Chilomastix mesnili	Non-pathogenic parasite (NP)	*C. mesnili* is found in about 3.5% of the US population.[37] Transmission is fecal-oral via the ingestion of mature cysts from contaminated water or food.	*C. mesnili* lives in the cecum and colon, but is noninvasive and nonpathogenic.[37]	Although *C. mesnili* is nonpathogenic, and causes no symptoms, it often occurs with other parasitic infections.[37]
Cryptosporidium spp.	Coccidian parasite (P)	*Cryptosporidium* is endemic to North, Central, and South America, Africa, and Australia. Infection is spread via the fecal-oral route and indirectly through contaminated water. *Cryptosporidium* is a common cause of food and water-borne outbreaks.[4]	The parasite adheres to intestinal epithelial cells. The intestinal epithelium releases cytokines to incite an immune response and causes cell apoptosis and villous atrophy. *Cryptosporidium* has developed ways to slow this protective mechanism; therefore, host immune competency determines pathogenicity.[38]	In immunocompetent patients, infection is self-limiting with 2 weeks of watery diarrhea. Other symptoms include fever, nausea, vomiting, and abdominal pain. Symptoms can be cyclical.[39,40] It can be life threatening in immunocompromised patients.[4,41] Enteric cryptosporidiosis can mimic malabsorption syndrome, Giardiasis, and viral diarrhea. Biliary cryptosporidiosis can mimic acute cholangitis.[6]

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Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
Cyclospora cayetanensis	Food- and waterborne coccidian parasite (P)	*Cyclospora* is endemic to Nepal, Haiti, Peru, and Guatemala, but it has been found as a cause of traveler's diarrhea worldwide. There have been many US outbreaks related to imported fruits and vegetables.[4,42] In the US, increased cases are reported during the spring and summer months. Transmission is fecal-oral via ingestion of contaminated water or food.	Individuals with *Cyclospora* infection excrete unsporulated oocysts in their feces. These oocysts require 7 to 15 days to sporulate and become infectious to a susceptible host. When food or water contaminated with infectious oocysts is ingested by a susceptible host, the oocysts excyst and sporozoites are released and infect epithelial cells of the duodenum and jejunum.[43]	Cyclosporiasis is marked by profuse, non-bloody, watery diarrhea, anorexia, fatigue, weight loss, nausea, flatulence, abdominal cramping, myalgias, vomiting, and low grade fever. Symptoms start approximately 7 days after ingestion. If left untreated, it can last weeks to months, with remitting and relapsing symptoms.[4] Cyclosporiasis can mimic acute viral enteritis and traveler's diarrhea.[6]
Cystoisospora spp. *Cystoisospora belli*	Coccidian parasite Previously referred to as *Isospora*[44] (P)	*Cystoisospora* is an uncommon human intestinal parasite. It has a worldwide distribution. *Cystoisospora* is frequently found in tropical and subtropical regions.[45] Transmission is through the fecal-oral route by the ingestion of contaminated water and food.[46]	*Cystoisospora* releases sporozoites that penetrate the small intestinal columnar epithelium.[47]	Some *Cystoisospora* infections are asymptomatic. When present, infections are usually mild and self-limiting, consisting of diarrhea and abdominal pain.[45] It can cause severe chronic diarrhea in immunocompromised AIDS patients. It has also been reported to be a cause of traveler's diarrhea in the normal host and can mimic giardiasis or cryptosporidiosis.[48]
Dientamoeba fragilis	Flagellate protozoan parasite (P)	*D. fragilis* has a worldwide distribution, and is transmitted via the fecal-oral route. Transmission via helminth eggs (Ascaris, Enterobius) has also been postulated, but is still being investigated.	The role of *D. fragilis* as a pathogen is controversial because the trophozoites are not invasive and patients are commonly asymptomatic.[4]	Although many patients are asymptomatic, *D. fragilis* has been associated with diarrhea, abdominal pain, nausea, weight loss, anorexia, and flatulence.[4] Dientamebiasis can mimic eosinophilic colitis and IBD.[6]
Entamoeba coli	Non-pathogenic amoeba (NP)	*E. coli* is cosmopolitan in distribution and has been postulated to occur in approximately 50% of the population.[49] Transmission is fecal-oral via the ingestion of contaminated water or food.[49]	*E. coli* lives inside the large intestine but never enters the mucosa or sub-mucosal intestinal layers.	The presence of *E. coli* is not cause to seek treatment and is harmless. However, when a patient is infected with this benign amoeba, introduction of other pathogenic organisms is possible and may cause symptoms.[37]
Entamoeba dispar	Non-pathogenic amoeba *E. dispar* is morphologically and genetically similar to the virulent *E. histolytica;* therefore, other laboratory methods are necessary to distinguish the two.[50] (NP)	It is speculated that this species is responsible for most infections that were previously considered to be *E. histolytica*. *E. dispar* has a high worldwide prevalence.[50] Transmission is fecal-oral via the ingestion of contaminated water or food.[51]	*E. dispar* is noninvasive and considered non-pathogenic.[50]	*E. dispar* infection is not associated with clinical symptoms.

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Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
Entamoeba hartmanni	Non-pathogenic amoeba *E. hartmanni* can be distinguished from the virulent *E. histolytica* by the much smaller cyst size.[52] (NP)	*E. hartmanni* has a worldwide distribution, but is most common in developing countries with poor sanitation.[53] Transmission is fecal-oral via the ingestion of mature cysts from contaminated water or food.[52]	*E. hartmanni* is non-pathogenic.[53]	*E. hartmanni* infection is not associated with clinical symptoms.
Entamoeba histolytica	The leading parasitic cause of mortality globally,[52] and the third most common parasitic infection in the US.[4] Erythrophagocytosis has been used as a diagnostic indicator of invasive *E. histolytica* by microscopy.[54] (P)	*E. histolytica* has a worldwide distribution, but is most common in developing countries with poor sanitation.[53] Transmission is fecal-oral via ingestion of mature cysts from contaminated water or food, or contaminated individuals.[53]	Cysts are ingested and excystation occurs. Colonization usually happens in the large bowel, but the cecum is most common. It penetrates the endothelium causing ulceration. Host factors appear to promote a more invasive, systemic disease, leading to dysentery, liver abscess, pleuropulmonary involvement, and many other systemic complications.[53]	Although many cases are asymptomatic, it is likely that misdiagnosis is the cause (failure to identify *E. dispar/hartmanni*). Symptoms of *E. histolytica* infection (amoebiasis) include hemorrhagic diarrhea, fatigue, nausea, fever, weight loss.[55] Intestinal amebiasis can mimic infectious diarrhea, IBD, ischemic colitis, diverticulitis, AV malformation. Amoeboma can mimic colon carcinoma. Amebic strictures can mimic lymphogranuloma venereum (chlamydia) and malignancy. Hepatic amebiasis can mimic pyogenic liver abscess, necrotic hepatoma and echinococcal cyst.[6]
Entamoeba polecki	Non-pathogenic amoeba *E. polecki* can be distinguished from other Entamoeba species by microscopy. It is the only uninucleated species.[56] *E. polecki* comprises four subtypes, all of which are found in humans.[56] (NP)	Infection with *E. polecki* is rare, though its prevalence and distribution are often confused with those of the other Entamoeba species.[56,57] E. polecki, much like all Entamoeba species, is transmitted through the fecal-oral route by the ingestion of contaminated food or water.[56]	*E. polecki* is non-pathogenic.	*E. polecki* infection is not associated with clinical symptoms.
Endolimax nana	Non-pathogenic protozoa (NP)	*E. nana* has a global distribution. *E. nana* is transmitted through the fecal-oral route through ingestion of contaminated food or water.[58]	*E. nana* inhabits the colon and has been found in the appendix. *E. nana* feeds on bacteria and is non-invasive.[58]	*Endolimax* is an indicator of fecal contamination, which often entails co-infection by other organisms capable of causing diarrhea. There are rare cases of associations with urticaria, polyarthritis, and diarrhea. However, there is too little evidence to support pathogenicity.[58]

Parasitic Organisms

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Organism	Description	Epidemiology/Transmission	Pathogenicity	Symptoms
Giardia	Flagellate protozoa (P)	*Giardia* has a worldwide distribution. In the US, it is more frequently reported in children aged 1-9.[59] *Giardia* is transmitted through the fecal-oral route by the ingestion of contaminated water and food.[59]	*Giardia* (via secretory and excretory proteases) may alter the structure and composition of human intestinal microbiota biofilms. Bacteria from these dysbiotic microbiota in turn can cause epithelial and intestinal abnormalities after the enteropathogen has been cleared.[60]	*Giardia* is a leading cause of diarrhea worldwide. Some cases may be asymptomatic; when present symptoms include diarrhea, bloating, malabsorption, nausea, vomiting, and abdominal cramping. Infections are normally self-limiting, but chronic diarrhea may occur in children.[59] It is emerging as a prominent precursor to post-infectious irritable bowel syndrome and a variety of chronic extra-intestinal disturbances, such as reactive arthritis and chronic fatigue.[60] Acute giardiasis can mimic acute viral enteritis, bacillary dysentery, acute intestinal amebiasis and IBD.[6]
Iodamoeba butschlii	Non-pathogenic amoeba *Iodamoeba* gets its name from its appearance when stained with iodine.[37] (NP)	*Iodamoeba* has a worldwide distribution. Humans have a low prevalence of *Iodamoeba butschili*. Transmission is through the fecal-oral route by the ingestion of contaminated water and food.[37]	*Iodamoeba* is usually found in the large intestine and are non-invasive.[37]	*Iodamoeba* is not associated with symptoms. However, it is an indicator of fecal contamination, which often entails co-infection by other organisms capable of causing diarrhea.[37]
Trichomonads- *Pentatrichomonas* *Pentatrichomonas hominis* *Enteromonis hominis* *Retortamonas intestinalis*	Flagellate parasite *Trichomonas tenax* is usually found in oral/periodontal infections and cannot survive intestinal passage. *Pentatrichomonas hominis* (also known as *Trichomonas hominis*) will not survive in the oral cavity or genitourinary tract. It is considered a non-pathogenic parasite.[37] *Trichomonas vaginalis* is confined to the urogenital system. Among trichomonads, there is a habitat restriction: each can survive only in its site-specific location.[37] (NP)	Trichomonads have worldwide distribution. *E. hominis, P. hominis, and R. intestinalis* are transmitted via the fecal-oral route by the ingestion of contaminated water, food, and flies.[37]	*P. hominis, R. intestinalis, and E. hominis* are considered non-pathogenic commensals found in the cecum and colon.[37]	Trichomonads in the stool are not related to gastrointestinal illness.[37] The presence of trichomonad trophozoites in the stool can be an indicator of fecal contamination, and therefore doesn't rule out other infections as a cause of symptoms.[37]

Parasitic Organisms

TREATMENT RESOURCES:

The decision to treat parasitic organisms should be based on the patient's clinical presentation.
The following resources provide valuable insight into the clinical management of parasitic infections:

- Center for Disease Control – monographs on individual parasites and treatment protocols: https://www.cdc.gov/parasites/
- Sanford Guide - infectious disease treatment guidelines: https://www.sanfordguide.com/
- 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
- Mayo Clinic – conditions search engine: https://www.mayoclinic.org/
- PubMed – literature search engine: https://www.ncbi.nlm.nih.gov/pubmed/

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