The GI Effects Interpretive Guide has been created to provide a high-level approach to the GI Effects profile, biomarker interpretation, and therapeutic considerations. It is divided into two major sections: an overview of the GI Effects Interpretation At-a-Glance page; and a more in-depth review of the biomarkers comprising each of the Four Functional Pillars.

**Interpretation At-a-Glance Overview**

Using evidence-based rules and weighted algorithms, the Interpretation At-a-Glance section on the first page of the GI Effects report synthesizes patient test results into key functional areas of clinical significance and provides a directional indication of potential next steps in patient management.

**Four Functional Pillars Biomarker Map**

<table>
<thead>
<tr>
<th>Infection Box</th>
<th>Inflammation Box</th>
<th>Insufficiency Box</th>
<th>Imbalance Box</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Any pathogenic organism present</td>
<td>• Calprotectin</td>
<td>• Pancreatic Elastase 1 (PE1)</td>
<td>• n-Butyrate</td>
</tr>
<tr>
<td></td>
<td>• Eosinophil Protein X (EPX)</td>
<td>• Total Fecal Fats</td>
<td>• Total SCFA</td>
</tr>
<tr>
<td></td>
<td>• Fecal Secretory IgA</td>
<td>• Products of Protein Breakdown (Total)</td>
<td>• Beta-glucuronidase</td>
</tr>
<tr>
<td></td>
<td>• Fecal Occult Blood</td>
<td></td>
<td>• Beneficial Bacteria</td>
</tr>
<tr>
<td></td>
<td>• Fecal Lactoferrin (if ordered)</td>
<td></td>
<td>Lactobacillus,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bifidobacterium, E. coli (PCR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Any potential pathogen (PP)</td>
</tr>
</tbody>
</table>

**Four Functional Pillars**

In this section, pertinent biomarkers have been grouped into four clinically actionable areas: Infection, Inflammation, Insufficiency, and Imbalance. The four functional pillars utilize a proprietary algorithm to evaluate key clinical markers in each of these four functional areas. The algorithm takes into account the level of each individual biomarker and its degree of clinical impact. As a result, an overall score of high, medium, or low is provided for each functional pillar. The score is represented by color-coded icons and informational graphics.

The specific biomarkers of concern that are utilized to establish the results for each functional pillar are listed above.
Commensal Balance and Relative Abundance

The **Commensal Balance** infographic has been designed to provide a more precise view of an individual patient’s commensal bacteria (PCR) results relative to a healthy cohort. It is a composite of two measures:

- **The Healthy-Pattern Continuum** (formerly known as the Diversity Association Index) is a progressive ranking scale based on a Genova proprietary algorithm that 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 and is 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.

**The Relative Abundance (RA)** graphic represents the proportional levels of selected phyla in an individual’s microbiome and is represented relative to similar measures derived from a healthy cohort of individuals.

*See page 7 for commensal imbalance therapeutic recommendations.*
FOUR FUNCTIONAL PILLARS BIOMARKER DETAIL

INFECTION

This pillar is where common infectious microorganisms are reported and includes pathogenic bacteria and intestinal parasites.

INFLAMMATION

This pillar is where biomarkers that indicate inflammatory changes in the GI tract are reported. Biomarkers of GI inflammation and immunology provide information about the GI tract’s interactions with, and responses to, the outside world. They indicate how well the GI tract is maintaining its role as a barrier, as well as whether the GI tract is undergoing pathological responses to external or internal challenges. The biomarkers are Calprotectin, a marker of neutrophil-driven inflammation; Eosinophil Protein X (EPX), a marker of eosinophil-driven inflammation and allergic response; Fecal Secretory IgA, a marker of gut secretory immunity and barrier function; and Fecal Occult Blood.

Calprotectin 50 to 120 mcg/g

Borderline, suggestive of GI inflammation

Address cause of inflammation:
• Infection (review Infection pillar)
• Suspected or hx of IBD
• Chronic NSAID use
• Colorectal cancer
• Polyps
Recheck Calprotectin in 4-6 weeks

Calprotectin > 120 mcg/g

Significant GI inflammation

• 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.
**Fecal Secretory IgA**

- **Fecal Secretory IgA <dL**
  - Suggestive of loss of resilience
  - Consider Selective IgA Deficiency in patients with frequent infections (i.e., ear and sinus infections, pneumonia, etc.) - test serum IgA
  - EPX also <dL - consider loss of resiliency and barrier defense predisposing to infection

- **Fecal Secretory IgA > 885 mcg/g**
  - Suggestive of GI immune upregulation in the gut
  - Assess for and treat root causes of immune upregulation / inflammation:
    - Infection (bacterial and/or viral pathogen, potential pathogen)
    - Compromised intestinal barrier function (i.e., intestinal permeability)
    - Heightened response to non-infectious stimuli (i.e., food sensitivity/allergy, etc.)
    - Consider food antibody testing
    - If positive, consider elimination diet

**Fecal Occult Blood**

- **Fecal Occult Blood positive**
  - Assess for cause of bleeding
    - (i.e., Ulcers, polyps, diverticulitis, IBD, colorectal cancer, etc.)
This pillar is where biomarkers that indicate digestive function are noted. Biomarkers of digestion and absorption provide information about nutrient breakdown and entry into the circulation. They ultimately indicate how well the GI tract is performing its basic digestive functions. The biomarkers are Pancreatic Elastase 1, a marker of exocrine pancreatic function; Products of Protein Breakdown, markers of undigested protein reaching the colon; and Fecal Fat (Total), a marker of fat digestion/absorption.

**Pancreatic Elastase 1 (PE1)**

- **Pancreatic Elastase (PE1) >200 mcg/g**
  - No pancreatic exocrine insufficiency; most healthy people are >500 mcg/g
  - In patients with levels between 200-500, consider:
    - Aging
    - Vegan/vegetarian diet
    - See list of primary causes of exocrine pancreatic insufficiency (see right)
    - Assess Products of Protein Breakdown and Fecal Fats; if elevated, consider other causes (see below) and a trial of PERT

- **Pancreatic Elastase (PE1) 100 to 200 mcg/g**
  - Mild-to-moderate exocrine pancreatic insufficiency
  - Initiate pancreatic enzyme replacement therapy (PERT)

- **Pancreatic Elastase (PE1) < 100 mcg/g**
  - Severe exocrine pancreatic insufficiency
  - Evaluate and address lifestyle issues:
    - Small, frequent meals for rapid absorption
    - Reduce alcohol intake
    - Smoking cessation

  Seek primary cause(s) of exocrine pancreatic insufficiency
  - Chronic Pancreatitis
  - Gallstones
  - Diabetes
  - Celiac Disease
  - Inflammatory Bowel Disease (IBD); see inflammatory markers
  - Excessive alcohol consumption
  - Cystic Fibrosis

**Products of Protein Breakdown (Total)**

- **Total Protein Products < 1.8 micromol/g**
  - May indicate decreased protein intake, imbalanced bacterial levels, or intestinal inflammation

- **Total Protein Products > 7.12 micromol/g**
  - May indicate protein malabsorption resulting in colonic protein fermentation, and/or excessive delivery of protein to the colon

  Assess for and treat root causes of insufficient protein digestion:
  - Hypochlorhydria
    - Assess for/reduce use of acid-blocking medications (as clinically indicated)
    - Consider addition of betaine HCl (as clinically indicated)
  - Pancreatic exocrine insufficiency
    - Evaluate fecal PE1 and support with PERT as clinically indicated

  Assess for excessive delivery of protein to the colon:
  - High-protein diet
  - Bacterial overgrowth
  - GI irritation/inflammation, bleeding
  - Review, evaluate, and treat any abnormal inflammatory biomarkers and/or infection
Fecal Fat (Total)

<table>
<thead>
<tr>
<th>Fecal Fat (Total) &lt; 5 mg/g</th>
<th>Fecal Fat (Total) &gt; 28.38 mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-fat diet</td>
<td>Suggestive of fat maldigestion and/or malabsorption</td>
</tr>
<tr>
<td>Consider amount of dietary intake of fat</td>
<td></td>
</tr>
</tbody>
</table>

**Target evaluation and treatment for common etiologies of fat maldigestion:**

- **Pancreatic exocrine insufficiency\(^\text{15}\)**
  - If PE1 is less than 200, consider PERT
  - Low fecal fat concentration does not exclude exocrine pancreatic insufficiency

- **Small Intestinal Bacterial Overgrowth (SIBO)**
  - Consider SIBO breath testing if: increased relative abundance, increased products of protein breakdown, increased SCFAs, or the presence of *Methanobrevibacter smithii*

- **Hypochlorhydria\(^\text{16}\)**
  - Assess for/reduce use of acid-blocking medications (as clinically indicated)
  - Consider a betaine HCl challenge test, and treat as indicated

- **Bile Salt Insufficiency\(^\text{15}\)**
  - Assess for causes including liver damage, impaired gallbladder function
  - Consider addition of bile salts and/or cholagogues

**Target evaluation and treatment for common conditions associated with fat malabsorption**

- **Infection\(^\text{17}\)**
- **Celiac Disease\(^\text{18}\)**
  - Consider Celiac and Gluten Sensitivity Panel
- **IBS** (confirm diagnosis via clinical criteria such as Rome IV\(^\text{15,19}\))
- **IBD** (review Calprotectin level; if greater than 120, GI referral\(^\text{20}\))
- **Rapid transit time**
- **Gastric bypass, ileal resection or other surgeries that limit absorptive surface area\(^\text{21}\)**

**Further Evaluation:**

- May be associated with deficiencies in fat or fat-soluble nutrients
  - Consider nutritional assessment of essential fatty acids, fat-soluble vitamins
This pillar is where microbiome imbalances are noted. Biomarkers of the GI Microbiome provide information about the health, function, and abundance of the trillions of microbial cells in the GI tract. They indicate how well the microbiome is performing its shared metabolic functions with the human host. Abnormal results in this pillar may be associated with a large number of conditions and symptoms. The biomarkers which assess gut microbial imbalance are Metabolic Indicators, which demonstrates the microbiome's specific and vital metabolic function; these include Short Chain Fatty Acids that are produced by bacterial fermentation of fiber, and Beta-glucuronidase, an enzyme produced by bacteria; Commensal Bacteria, a PCR evaluation of 24 key bacterial groups/species; and Bacterial and Mycologic Culture, which identifies potentially pathogenic (PP) organisms.

### Metabolic Indicators
- n-Butyrate Concentration
- Total SCFA
- Beta-glucuronidase

### Commensal Bacteria (PCR)
- ↓ Lactobacillus (Beneficial)
- ↓ Bifidobacterium (Beneficial)
- ↓ E. coli (Beneficial)

### Bacterial and Mycologic Culture
- Potential pathogens (PP) on culture

**Suggestive of microbial imbalance**
- Treat abnormalities in Infection, Inflammation & Insufficiency pillars
- Dietary modifications to increase SCFAs, to include prebiotic food sources (i.e., FOS, inulin, psyllium, oat bran, xylooligosaccharide, beta-glucan, arabinogalactan), increased intake of fiber and whole, complex carbohydrate and resistant starch
- Consider adding multi-strain probiotic
- Consider addition of calcium-d-glucarate

**Commensal Bacteria and Diet and Lifestyle Influences**
- In general, a high-fat and high-sugar diet, stress and antibiotics shift the gut microbial ecology to contain more pro-inflammatory species and a lower bacterial gene count.
- A healthy diet rich in plants and fiber, prebiotics and probiotics produce a favorable shift towards more anti-inflammatory species and increased bacterial gene count.
- Physical activity can increase gut microbial diversity, as well as health-promoting bacterial species.
One of the benefits of ordering a comprehensive stool panel like GI Effects is the ability to observe trends across different groups of biomarkers. In addition to patterns discussed within the 4 functional pillars, clinicians commonly observe other patterns including Small Intestinal Bacterial Overgrowth (SIBO), Intestinal Permeability, Immune Dysregulation/ Loss of Resilience, and Inflammation. These patterns are not diagnostic, but rather suggestive of these common conditions and further workup may be appropriate.

<table>
<thead>
<tr>
<th>Biomarker pattern, along with associated symptoms suggest</th>
<th>Biomarkers</th>
<th>Next steps</th>
</tr>
</thead>
</table>
| Small Intestinal Bacterial Overgrowth (SIBO)             | Relative Abundance  
|                                                          | Products of Protein Breakdown  
|                                                          | SCFA  
|                                                          | n-butyrate  
|                                                          | Fecal Fat (total)  
|                                                          | Methanobrevibacter smithii | • Confirm with SIBO Breath Test |
| Intestinal Permeability                                  | sIgA  
|                                                          | EPX  
|                                                          | Akkermansia muciniphila | • Confirm with Intestinal Permeability Assessment  
|                                                          |                                            | • Determine root cause of permeability  
|                                                          |                                            | • Consider GI repair/ support  
|                                                          |                                            | • Consider IgG and/or IgE Food Antibody testing |
| Immune Dysregulation/ Loss of Resilience                 | <DL sIgA  
|                                                          | <DL EPX  
|                                                          | PP bacteria/yeast  
|                                                          | Beneficial bacteria  
|                                                          | Bifidobacterium spp.  
|                                                          | Methanobrevibacter smithii and Desulfovibrio piger  
|                                                          | High probability of Blastocystis hominis | • Evaluate root cause of immune dysregulation (i.e., Adrenocortex Stress Profile)  
|                                                          |                                            | • Consider prebiotics/probiotics  
|                                                          |                                            | • Consider SIBO testing |
References


