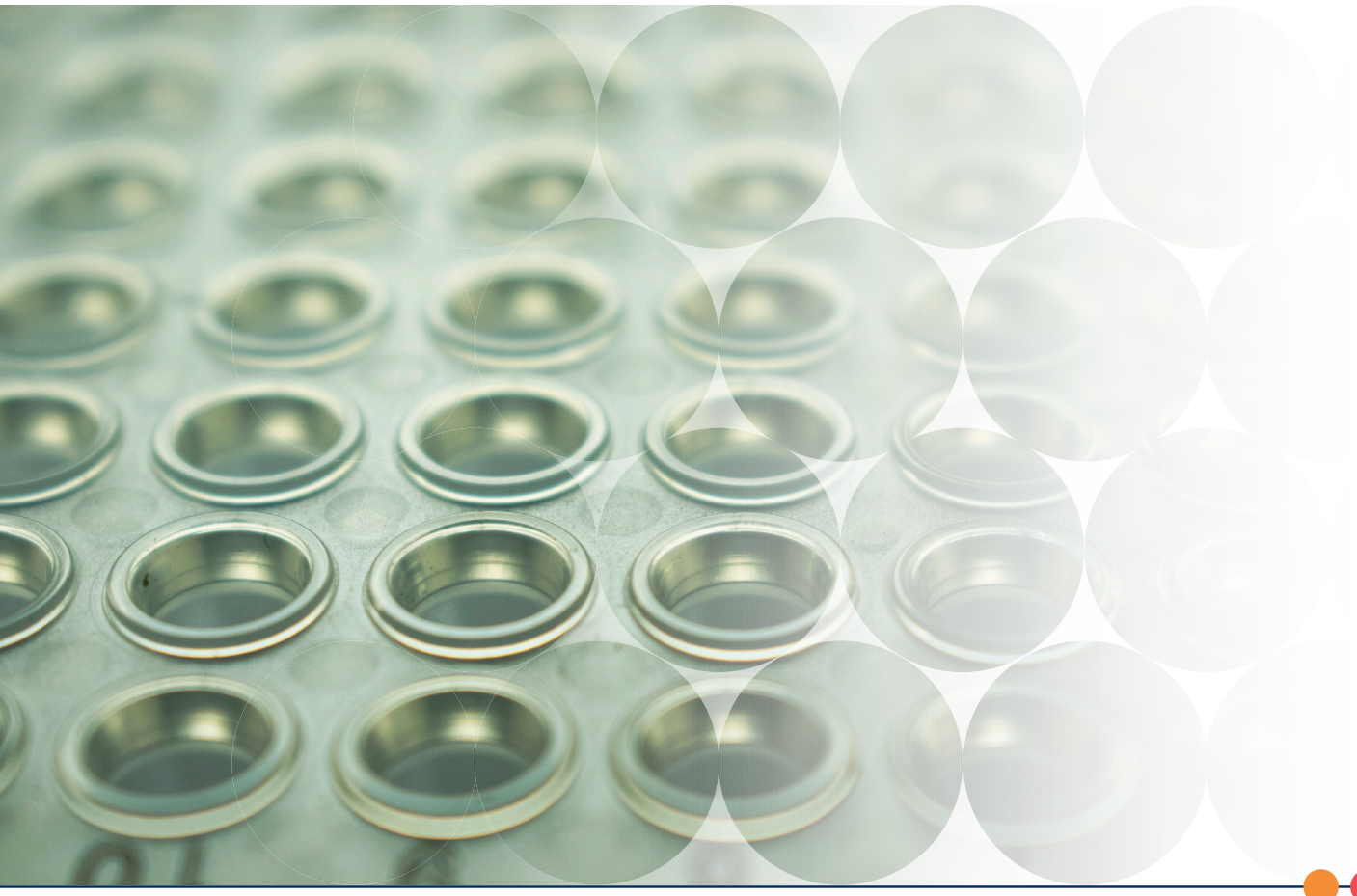


GI fx GI Effects Stool Profiles®

Interpretive Guide

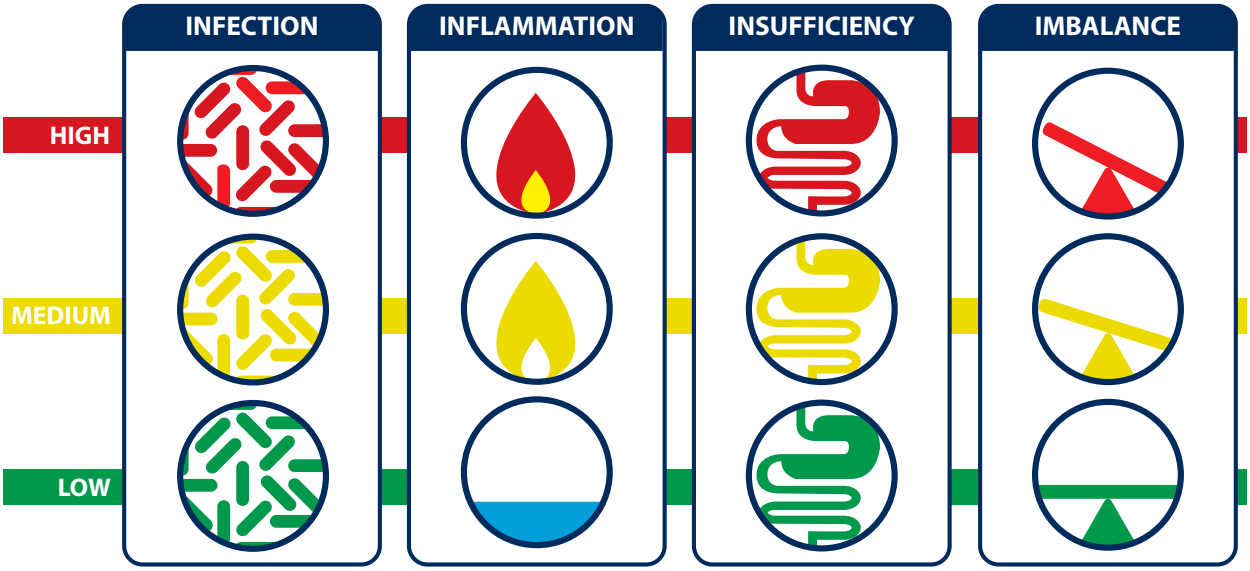
GENOVA DIAGNOSTICS®

Interpretive Guide

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

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

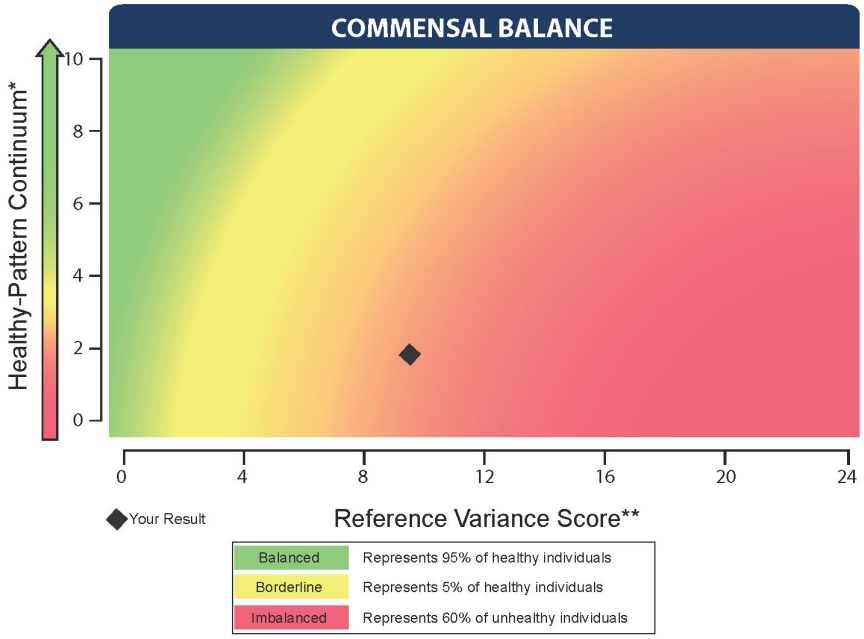
The **Commensal Balance** infographic has been designed to provide a more precise view of an individual patient's commensal bacteria (PCR) results relative to a healthy cohort. It is a composite of two measures:

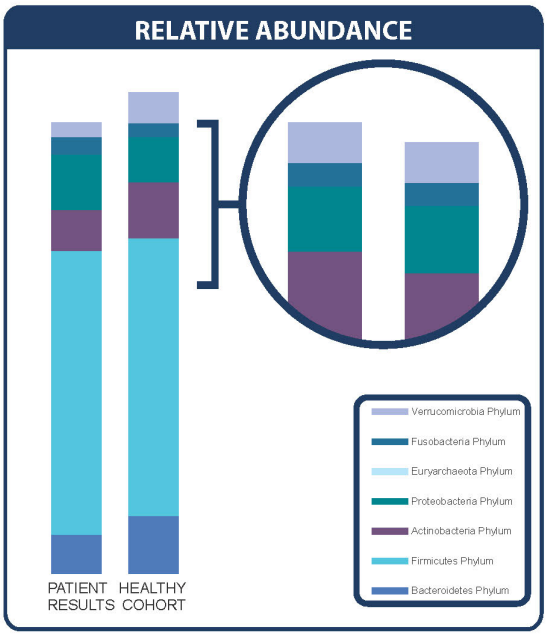
- The **Healthy-Pattern Continuum** (formerly known as the Diversity Association Index) is a progressive ranking scale based on a Genova proprietary algorithm that differentiates healthy and unhealthy commensal patterns. This algorithm is applied to an individual patient's GI Effects commensal bacteria (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.

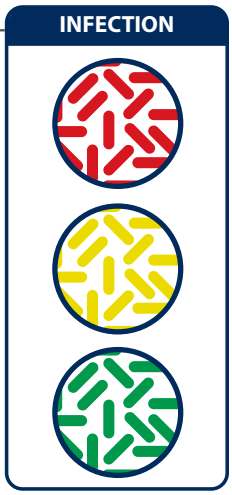




* A progressive ranking scale based on a Genova proprietary algorithm that differentiates healthy and unhealthy commensal patterns.
**The total number of Commensal Bacteria (PCR) that are out of reference range for this individual

FOUR FUNCTIONAL PILLARS BIOMARKER DETAIL

INFECTION

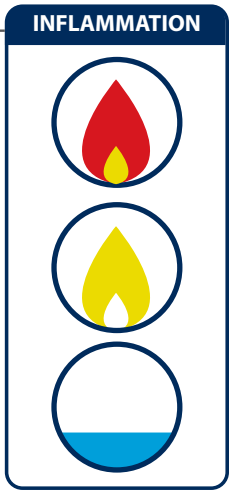


This pillar is where common infectious microorganisms are reported and includes pathogenic bacteria and intestinal parasites.

Pathogenic Bacteria Add-on (EIA)	Bacteriology (Culture)	Parasitology (Microscopy, PCR)
• *Clostridium difficile* toxin • *Helicobacter pylori* • *Campylobacter* spp. • Shiga toxin *E. coli*	• Known Pathogen (i.e., *Salmonella*, *Aeromonas*, all others)	• Pathogenic and potentially pathogenic parasites (i.e., *Cryptosporidium, Giardia, Entamoeba histolytica*, all others)
• Antibiotics (if appropriate*) • Natural Agents • Probiotics • Rehydration • see www.cdc.gov for the most current information		• Antiparasitics (if appropriate*) • Natural Agents • Consider probiotic *S. boulardii* in *Blastocystis* infection[1] • see www.cdc.gov/dpdx/az/html for the most current information

*Some patients may have colonization and treatment decisions must be considered on an individual basis.

INFLAMMATION

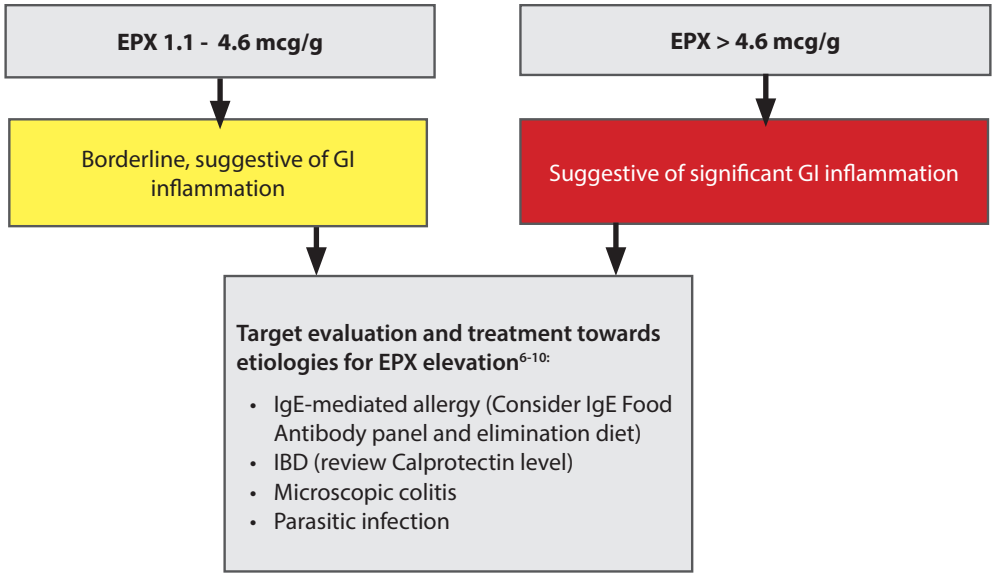


This pillar is where biomarkers that indicate inflammatory changes in the GI tract are reported. Biomarkers of GI inflammation and immunology provide information about the GI tract's interactions with, and responses to, the outside world. They indicate how well the GI tract is maintaining its role as 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[2]; Eosinophil Protein X (EPX), a marker of eosinophil-driven inflammation and allergic response[3]; Fecal Secretory IgA, a marker of gut secretory immunity and barrier function[4]; and Fecal Occult Blood.

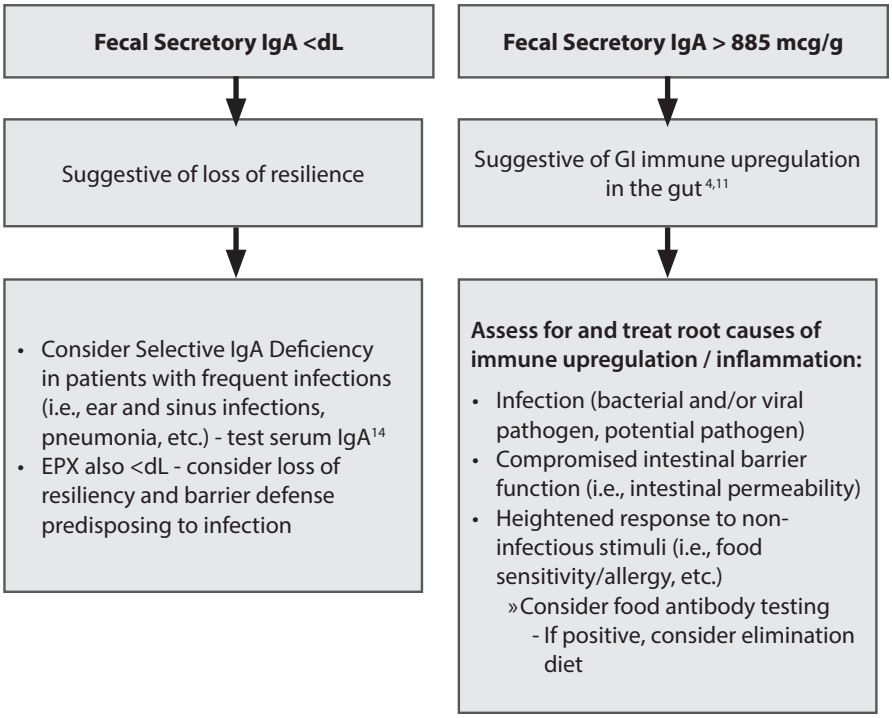
Calprotectin 50 to 120 mcg/g	Calprotectin > 120 mcg/g
Borderline, suggestive of GI inflammation	Significant GI inflammation
Address cause of inflammation[5]: • Infection (review Infection pillar) • Suspected or hx of IBD • Chronic NSAID use • Colorectal cancer • Polyps **Recheck Calprotectin in 4-6 weeks**	• 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.

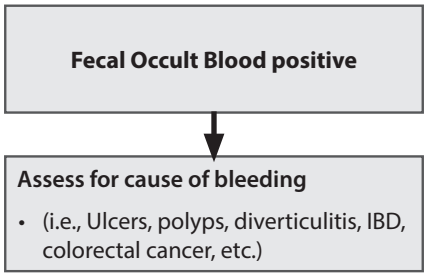
EPX



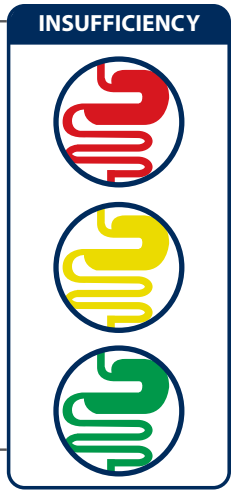
Fecal Secretory IgA



Fecal Occult Blood



INSUFFICIENCY



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[12]; Products of Protein Breakdown, markers of undigested protein reaching the colon[13]; 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)

Evaluate and address lifestyle issues:

- Small, frequent meals for rapid absorption
- Reduce alcohol intake
- Smoking cessation

Pancreatic Elastase (PE1) < 100 mcg/g

Severe exocrine pancreatic insufficiency

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 maldigestion and malabsorption resulting in colonic protein fermentation, and/or excessive delivery of protein to the colon

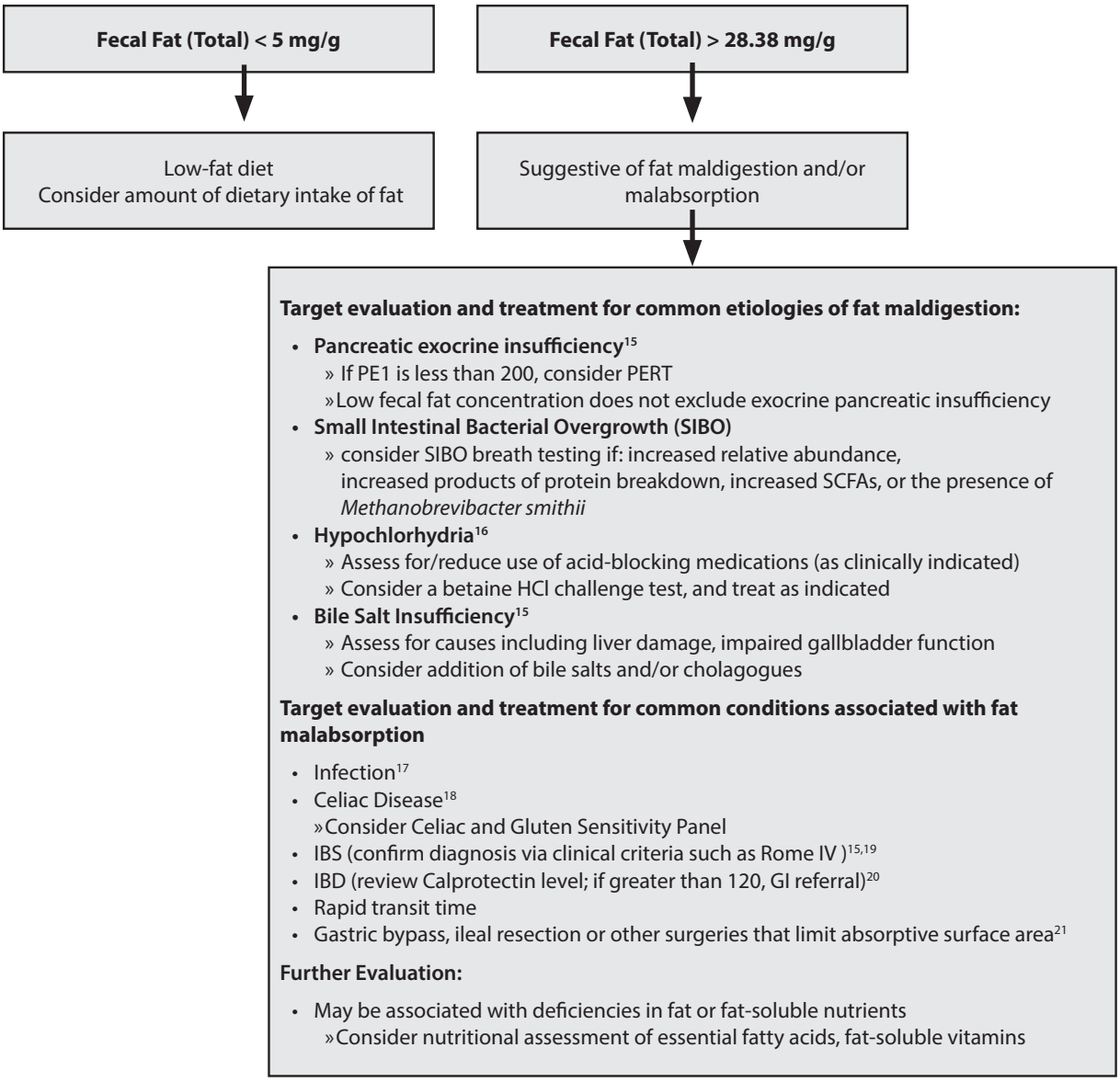
Assess for and treat root causes of insufficient protein digestion:

- 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:

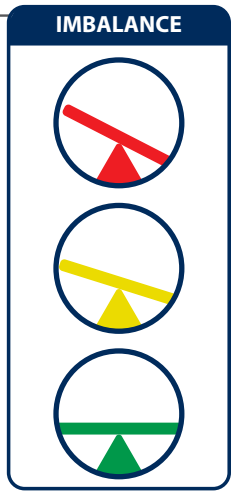
- High-protein diet
- Bacterial overgrowth
- GI irritation/inflammation, bleeding
- Review, evaluate, and treat any abnormal inflammatory biomarkers and/or infection

Fecal Fat (Total)



IMBALANCE

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[22], 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.[23]



Metabolic Indicators
n-Butyrate Concentration
Total SCFA
Beta-glucuronidase

↓

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[24,25]
- Consider adding multi-strain probiotic [25]
- For elevated beta-glucuronidase also consider calcium-d-glucarate[26]

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

Bacterial and Mycologic Culture
Potential pathogens (PP) on culture

- First treat abnormalities in the Infection, Inflammation, and & Insufficiency pillars
- For potential pathogens, consider pharmaceutical or non-pharmaceutical (botanical) antibiotic and/or antifungal agents (based on organism sensitivity)
  - »Therapeutic intervention is warranted if the practitioner has a high clinical suspicion that the PP organism is causing the patient's symptoms.
- Consider multi-strain probiotic
- Consider addition of prebiotics
- Consider addition of fermented foods (i.e., kefir, yogurt, kim chee and other fermented vegetables, miso, etc.)

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.[27,28]
- 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. [27,28]
- Physical activity can increase gut microbial diversity, as well as health-promoting bacterial species.[29]

OVERALL PATTERN RECOGNITION

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.

Biomarker pattern, along with associated symptoms suggest	Biomarkers	Next steps
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

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