GI fx GI Effects® Stool Profiles

Support Guide

Introduction

This Support Guide is intended to help clinicians understand and use the *GI Effects® Comprehensive Profile*, a select set of fecal biomarkers aimed at identifying key processes that influence both gastrointestinal and overall health.

GENOVA DIAGNOSTICS®

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GI fx GI Effects® Stool Profiles

GENOVA DIAGNOSTICS®

Interpretation-At-A-Glance Synthesis

The test report is organized so that the clinician may move through the results in a logical order that enhances clinical utility, beginning with the Interpretation-At-A-Glance Synthesis Page.

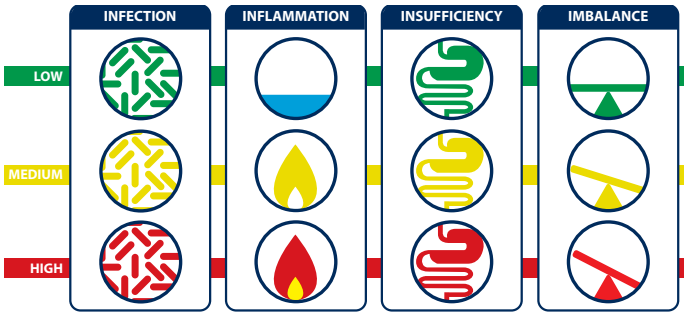
Using evidence-based rules and weighted algorithms, this page synthesizes patient test results into key functional areas of clinical significance and provides a directional indication of potential next steps in patient management.

The Interpretation-At-A-Glance page is divided into two major sections: 1) Four Funtional Pillars, and 2) Diversity and Abundance.

Four Functional Pillars

Pertinent biomarkers have been grouped into four clinically actionable areas:

- Infection
- Inflammation
- Insufficiency (Digestive)
- Imbalance (Metabolic)



The four functional pillars utilize a proprietary algorithm to evaluate key clinical markers in the 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 in the Four Functional Pillars Biomarker Map.

Four Functional Pillars Biomarker Map

Infection	Inflammation	Insufficiency	Imbalance
any parasite present	Calprotectin	PE1	n-Butyrate
any pathogen present	EPX	Total Fecal Fats	Total SCFA
	Fecal IgA	Total Protein Products	Beta-glucuronidase
			Lactobacillus
			Bifidobacterium
			E. coli

Diversity and Abundance

It is now known that the human GI tract is home to more than 1000 species of microbial organisms, almost all of them bacteria. These organisms – collectively known as the microbiome – far outnumber the human cells in any individual and fulfill many metabolic functions.[1-3]

It is becoming evident that many factors go into developing and maintaining what might be called a "healthy microbiome," but this emerging area of biology is vastly complex. Indeed, prior to very modern analytic developments, there was no way to understand either the number or the functions of the tremendous population of organisms making up the human GI microbiota. Recent developments rely on DNA or RNA patterns, and by comparing detected sequences with libraries of known organisms, laboratories can now detect a tremendous number and variety of organisms.

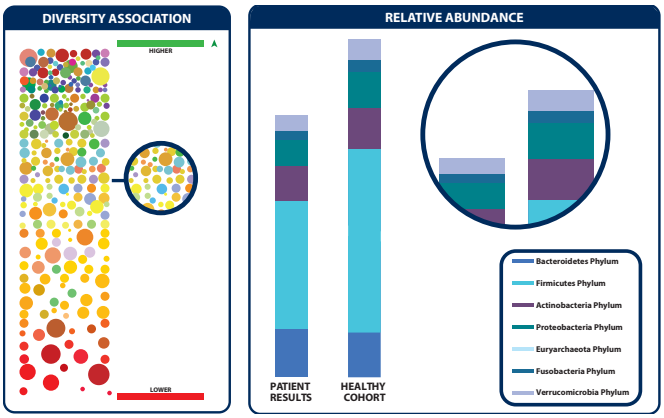
One of the first fruits of this new technology is the discovery that, while the entire pool of possible members of the microbiota is large (> 1000 species), a much smaller number, 150 to 170 species, is found to predominate in any given individual.

Utilizing a molecular assay platform optimized for stool analysis, *GI Effects* assesses a set of clinically relevant 24 genera/species that map to 7 major phyla. *GI Effects* utilizes 16S rRNA gene polymerase chain reaction (PCR) amplification techniques. The improved method offers an enhanced DNA extraction method, updated primer sequences, and optimized thermal cycling for an enhanced reportable range. After detection of these organisms, a computer algorithm is used to map them into a graphic representations of diversity association and relative abundance.

Diversity Association is a proxy measure of gut biodiversity, which is defined as the number and abundance of distinct types of organisms present in the gut.[4] The clinical utility of biodiversity of the gut microbiome is not yet fully defined, but in general a high diversity of GI organisms has been associated

with states of relatively good health, while low diversity has been associated with states of disease or chronic dysfunction in the scientific literature.

Relative Abundance represents the levels of selected phyla in an individual's microbiome and is represented relative to similar measures derived from a healthy cohort of individuals.



An algorithm is also used to graphically represent the microbial diversity and relative abundance of the commensal bacteria. The Diversity Association is a proxy measure of the diversity level of organisms in the gut. The impact of each commensal genera/species is weighted based on its association with high and low bacterial diversity. The Relative Abundance represents the size of each of the phyla as calculated by the assessed commensal organisms and is shown in comparison to the levels seen in a defined healthy cohort.

Measuring diversity association and relative abundance serve as global measures of an individual's gut health. Monitoring these markers over time provides insight into the impact of medications, supplements, diet, and lifestyle interventions on current and future health. Specific treatments, as directed by the results of the four functional pillars, would be expected to produce positive responses in the diversity and abundance of gut bacteria.

Biomarker Review

Modern technology allows the use of a growing number of biomarkers found in stool to supplement, and often supplant, more invasive and generally more expensive tests of GI function.

While the most obvious role of the human gastrointestinal (GI) tract is the incorporation of nutrients and energy from the diet, and the elimination of waste products and toxins, it is now clear that functions of the GI tract influence, not only GI health, but that of the entire human organism.

For these reasons, the results from the *GI Effects Comprehensive Profile* are reported using the "DIG" framework, which provides information on the three main categories of GI function:

- **D**igestion and Absorption reports on the effectiveness of GI breakdown and absorption of nutrients from ingested food. This category contains:
  - Pancreatic Elastase-1 (PE1), a marker of exocrine pancreatic function
  - Products of Protein Breakdown identifies bacterial fermentation of proteinaceous material
  - Fecal Fat, a marker of fat maldigestion and malabsorption

- **I**nflammation and Immunology reports on the functioning of the inflammatory response and the secretory immune system in the GI tract. This category contains:
  - Calprotectin, a marker of neutrophil activity and inflammation
  - Eosinophil Protein X, a marker of eosinophil activity and inflammatory, allergic, and parasitic influences
  - Fecal secretory IgA (fsIgA), a marker of secretory immune function, GI mucosal defense, and the maintenance of gut barrier function

- **G**astrointestinal Microbiome reports on the status and function of the hundreds of microbial species (chiefly bacteria and fungi) that constitute the non-host living contents of the human GI tract. This category contains the following subcategories and their constituents:
  - **Metabolic**, a series of biomarkers that indicate the microbial production of beneficial molecules including metabolites of incompletely-digested nutrients, as well as enzymes involved in the trafficking of biliary conjugate molecules. This subcategory contains:
    - Short-chain fatty acids (SCFAs), markers of colonic fermentation of carbohydrates into SCFAs required for colonocyte health and signaling
    - Beta-glucuronidase, an enzyme involved in carbohydrate digestion and lysis of bonds linking bioactive molecules to their glucuronide conjugates
  - **Commensal Bacteria (Polymerase Chain Reaction – PCR)**, a listing of the levels of 24 major bacterial genera/species in 7 major phyla of commensal organisms, i.e., organisms known to exist symbiotically with humans, sharing and exchanging metabolic functions. Increasing evidence suggests that the makeup and metabolic functions of the commensal bacteria in the human microbiome are essential to maintenance of general homeostasis and health of the host organism.

- PCR can evaluate anaerobic targets and provides quantification of each target, giving a semi-quantitative result.
- A 25th biomarker is also provided in this section, the Firmicutes/Bacteroidetes Ratio (F/B Ratio). The F/B ratio provides an estimate of the predominance of two major phyla of commensal organisms, which has been associated with a number of metabolic disorders.

› **Bacteriology (Culture)**, a listing of both commensal and additional bacteria grown in traditional culture media. This familiar suite of tests complements targeted commensal bacterial detection by PCR methods by identifying the presence of hundreds of additional bacterial species, including opportunistic/potentially pathogenic bacteria, which may be indicators of imbalance in the gut microbiome.

› **Mycology (Culture)** detects fungal organisms using traditional culture techniques.

- Cultured organisms, both bacteria and mycology, are definitively identified using MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-of-Flight) technology. The MALDI-TOF mass spectrometry platform utilized for the rapid identification of bacteria and yeast from pure cultures on the *GI Effects Comprehensive Profile* report relies on the most extensive FDA-cleared library of microbial targets available on the market.
- Culture identifies all viable cultivable organisms, and is the accepted standard for assessment of aerobic organisms.

› **Parasitology** detects intestinal parasites by means of two complementary techniques:

- Ova and Parasites (O&P); microscopic examination using light microscopy
- Parasitology Enzyme ImmunoAssay (EIA) tests, which can detect specific pathogenic organisms of interest; this test panel detects *Cryptosporidium* species, *Giardia lamblia, and Entamoeba histolytica.*

› **Bacteria Sensitivity** lists cultured bacterial pathogens and their relative susceptibility to both prescription and natural antimicrobial agents.

› **Mycology Sensitivity** lists cultured fungal pathogens and their relative susceptibility to both prescription and natural antimicrobial agents.

The *GI Effects Comprehensive Profile* report concludes with a section on Additional Results. These include time-honored characteristics of fecal specimens such as occult blood, color, and consistency, as well as the option to add on specific EIA testing for certain known pathogens, including *Helicobacter pylori* stool antigen, *Campylobacter* species, *Clostridium difficile,* and Shiga toxin-producing strains of *E. coli*.

Organization of the Biomarker Review

In the following sections,

- Each ***biomarker*** is first identified and described
- The candidate ***patient population*** for the biomarker is described
- The ***comparator*** or existing gold standard for the biomarker is presented (if established), along with performance characteristics of the fecal biomarker when appropriate
- The ***interpretation of the fecal biomarker*** is discussed, including the significance of out-of-range results
- Desirable ***outcomes and therapeutic recommendations*** are discussed, indicating how the specific test might benefit patients in a variety of clinical settings

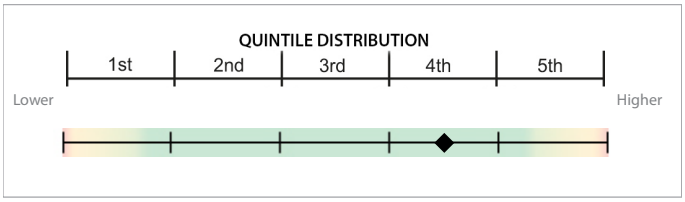
Graphical Representation and Color Coding for Biomarkers

In addition to a numeric result and a stated reference range for each result, all biomarker results on the *GI Effects* report are graphically represented and color coded in the context of a specific reference population by means of ***quintile reporting*** or ***medical decision point reporting***.

Quintile reporting permits the clinician to recognize at a glance where each individual patient result falls compared to the distribution of results for the reference population, which is often (but not always) a representative sample of the entire population that the laboratory has tested for each biomarker. After rank ordering each individual test result from lowest to highest, the

reference population is divided into 5 equal groups, or **quintiles**, where each group represents 20% of the total count of individual results in the reference population.

The following example shows a patient's result (black diamond) that lies in the middle of the 4th quintile. The diamond placement below indicates that approximately 70% of all patients in the reference population for this biomarker had results that are lower than this patient's result.



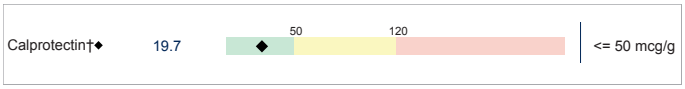
The quintile reporting bar is denominated in percentile units and the color thresholds on the bar approximate standard deviation (SD) thresholds, assuming a normal distribution. In general, for a 2-tailed test as illustrated above, the green region includes plus or minus 1 SD from the population mean, or 68.2% of all results. The yellow areas include plus or minus 2 SD from the population mean, and encompass 95.4% of the distribution, and the red area represents the remainder of the population that falls outside of 2 SD in either direction.

By examining the quintile reporting bar for each biomarker in the context of the numerical result and reference range, the clinician can quickly identify results calling for closer clinical consideration. In conjunction with the patient's history and physical findings, biomarker results trending towards or outside of 2 SD may require additional evaluation.

Quintile reporting may also be useful when serial testing is used, to assess movement in either direction, and as a monitoring tool for effectiveness of interventions.

Some biomarkers have established threshold values associated with specific clinical conditions, histopathological findings, or recommended clinical interventions. These biomarkers are not reported using the quintile system but instead are reported on a colorimetric graphical bar denominated in the same laboratory measurement units as the biomarker result. The color-coded thresholds are defined by specific **Medical Decision Points,** or MDPs. For example, fecal calprotectin, PE1, and eosinophil protein X all have reference ranges based on a clinically characterized healthy reference population (i.e. not a symptomatic tested population) and cutoff points indicating a normal result, a borderline or weakly positive result, and an abnormal or strongly positive result.

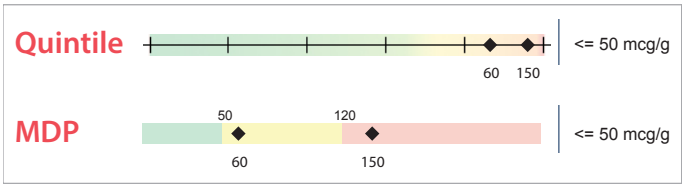
An example MDP reporting bar for a normal calprotectin result is shown here:



This fecal calprotectin result of <= 50 mcg/g stool in a patient meeting Rome criteria for Irritable Bowel Syndrome (IBS) virtually excludes the likelihood of Inflammatory Bowel Disease; however, a fecal calprotectin result above 120 would be a strong indication for additional evaluation (perhaps including colonoscopy) to determine the specific etiology of the inflammatory process.

Since 88% of patients with GI symptoms have a fecal calprotectin result < 50, a quintile graphical representation would restrict all clinically significant values (those above 50) to the right hand side of the reporting bar on the far side of the 5th quintile.

For the sake of illustration, consider how results reporting for a patient with an initial calprotectin value of 150 mcg/g – and subsequent value of 60 mcg/g – would appear in quintile and MDP representations.



Thus, the MDP colorimetric reporting bar facilitates visualization of a clinically significant improvement in fecal calprotectin levels (e.g. results from 150 mcg/g to 60 mcg/g) better than a quintile reporting bar (e.g. from 95th percentile to 90th percentile).[5]

Digestion and Absorption

For proper nutrition and gastrointestinal (GI) function, ingested nutrients must first be broken down (digested, in a biochemical sense), and the products of digestion must then be absorbed through a variety of physical and biochemical processes.

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
- **Fecal Fat**, a marker of fat breakdown and absorption

In good health, digestion is accomplished in several steps. First by chewing and other physical processes, and then by the actions of stomach acid and a host of enzymes produced in the pancreas and small intestine, breaking down the three major components of food: complex carbohydrates (starches), proteins, and fats.

Absorption of the resulting products of digestion then occurs by several distinct processes. Damage to, or impairment of, any of the processes involved in digestion or absorption results in two main problems: inadequate net absorption of nutrients, producing absolute or relative nutrient deficiencies, and/or delivery of intact nutrients to the colon, where gut microbes may inappropriately digest or ferment nutrients. Such fermentation results in byproducts leading to excessive osmotic loads and gasses, leading to abdominal discomfort, diarrhea, flatulence, and other common symptoms.[6]

Maldigestion is defined as impaired breakdown of nutrients, and is often the result of inadequate or impaired digestive enzymes (or gastric acid production), while malabsorption refers to impairments in absorption of the normal end products of digestion.[7]

When faced with a patient experiencing sub-acute or chronic GI symptoms, it is the task of the clinician to discern which, if any, of these processes is occurring, and then, if possible, to identify one or more underlying, primary causes. Finally, in many cases, once a primary cause has been identified, a rational and usually simple course of therapy may be prescribed, with the goal of repairing the underlying pathological processes.

In many cases, fecal biomarker testing is useful in discerning whether maldigestion, malabsorption, or both, are present. Such testing is also helpful in identifying the underlying causes, for which treatment may be available.

Pancreatic Elastase-1 (PE1)

The Biomarker

- The exocrine portion of the pancreas (the cells and structures not related to endocrine functions, such as insulin production) secretes numerous digestive enzymes, among them PE1
- PE1 is a robust proteolytic enzyme that reaches the colon without itself being digested, is not greatly affected by increases or decreases in intestinal transit times, and is not affected by pancreatic enzyme replacement therapy
- The PE1 reference range was adopted from an FDA-approved kit

Biomarker Key Points

- Noninvasive biomarker of pancreatic exocrine (i.e., digestive) function
- Is not affected by supplemental pancreatic enzymes
- Reflects true pancreatic exocrine function[8]

Fecal PE1 testing can be used for initial determination of pancreatic exocrine insufficiency in suspected patients, as well as the monitoring of pancreatic exocrine function in patients under treatment.

Patient Populations of Interest

Patients in whom PE1 testing may be useful include those with:

- Unexplained diarrhea
- Weight loss
- Other symptoms of maldigestion
- Abdominal pain
    - Including symptoms meeting clinical criteria for irritable bowel syndrome (IBS)
- Low bone density

In addition, pancreatic exocrine insufficiency may occur secondary to:

- Chronic pancreatitis
- Diabetes
- Celiac disease
- Cystic fibrosis
- Inflammatory bowel disease (IBD)
- Excessive alcohol consumption
- Gallstones

Fecal PE1 testing can be used for ***initial determination*** of pancreatic exocrine insufficiency in suspected patients

There is also evidence that aging populations may exhibit a progressive loss of PE1, since pancreatic exocrine function may decrease with age.[9-14]

Comparator/Gold Standard Tests

PE1 has a strong correlation with the gold-standard test for pancreatic insufficiency, the secretin-caerulein test.[8,15] (Caerulein is a synthetic analog for pancreozymin and stimulates pancreatic activity in a similar manner.)

Interpretation

Fecal PE1 Value (µ/g)	Interpretation[16,17]
> 200	Normal exocrine pancreatic function
100 to 200	Mild-to-moderate exocrine pancreatic insufficiency
< 100	Severe pancreatic insufficiency

Results are based on MDPs and are not represented by quintile values.

Notes on Interpretation

- Fecal PE1 testing may have reduced sensitivity for detecting mild pancreatic exocrine insufficiency in children.[18]
- Consumption of vegetarian or vegan diets, or other diets involving decreased meat intake, have been associated with reductions in fecal PE1.[19,20]
- Pancreatic exocrine insufficiency occurs in about 50% of type 1 diabetics, and in about 33% of type 2 diabetics.[21]
- Chronic pancreatitis patients may have compromised antioxidant systems.[6]

Outcomes and General Therapeutic Considerations

Patients with PE1 results suggestive of exocrine pancreatic insufficiency should undergo further investigation to determine the underlying causes of their dysfunction, as shown in the following table:

Additional Testing	Rationale
Evaluation of fecal fats[22]	Excess fecal fat may be due to: • Lack of bile acids (due to liver damage, hypolipidemic drugs, or impaired gallbladder function) • Celiac disease • Small bowel bacterial overgrowth • Other conditions and medications (e.g., Orlistat)
Full nutritional assessment	Defective exocrine pancreatic function may be associated with: • Abnormal blood lipids • Low levels of minerals (magnesium, zinc, selenium, and calcium) • Low levels of fat soluble vitamins (A, D, E, and K)[23]

Supporting the Patient with Evidence of Pancreatic Exocrine Insufficiency

Certain lifestyle, medication, and supplement interventions may be appropriate for patients with abnormal fecal PE1 results suggestive of pancreatic exocrine insufficiency.

Lifestyle Support[23]

- Small, frequent meals (better absorbed)
- Reduce alcohol consumption
- Smoking cessation

Medication/Supplement Support[22,24-26]

- Support patients with pancreatic exocrine insufficiency by pancreatic enzyme replacement therapy (PERT) at doses appropriate for degree of insufficiency and based on symptom improvement; in some conditions PE1 levels normalize as underlying disorders improve[27] (improved PE1 levels reflect functional improvements, not supplementary enzymes)

Products of Protein Breakdown

The Biomarker

When proteins or their digestion products (oligopeptides and amino acids) reach the distal colon, they are fermented by colonic organisms (proteolytic fermentation) into a group of compounds including the characteristic short-chain fatty acids (SCFAs), isovalerate, isobutyrate, and valerate.

Biomarker Key Points

- Normal protein digestion and absorption is relatively complete in stomach and small intestine
- Healthy colonic contents therefore include only small amounts of protein-derived SCFAs
- Protein fermentation can yield a diversity of end products, including SCFAs, amines, phenols, indoles, thiols, sulfur compounds and branched-chain fatty acids[28]
    - Though many have shown toxic properties in vitro and in animal models, the relationship between gut health and protein fermentation in humans has not been thoroughly investigated[29-31]
- Primary colonic SCFAs from protein breakdown are valerate, isovalerate, and isobutyrate[16,17]
- The result on the *GI Effects* report reflects a combined total of valerate, isovalerate, and isobutyrate measurements

Patient Populations of Interest

Patients with protein maldigestion, or those with abnormally large amounts of protein presented to the distal colon, may demonstrate increased products of colonic protein breakdown in the stool.

Increased fecal presence of products of protein breakdown may be present in patients with:

- Hypochlorhydria (diminished hydrochloric acid secretion in the stomach), which is associated with:[32]
    - Advanced age: in roughly 30% of elderly patients, gastric acid secretion is diminished
    - Use of acid-blocking medications or dietary supplements that produce too high a gastric pH to allow for complete protein digestion in the stomach[33]
    - Food reactions; elevated gastric pH (less acidic) has been associated with increased risk for food reactions, possibly from hindering protein breakdown[34-36]
- Pancreatic exocrine insufficiency or pancreatitis; insufficient pancreatic proteases leave improperly-digested protein fragments that reach the colon[37,38]
- Excessive protein intake[39]
- Gastrointestinal bleeding or irritation, mucosal desquamation, and bacterial overgrowth; these conditions result in excessive self-derived proteins in the intestinal lumen[40]

Comparator/Gold Standard Tests

Currently there is no gold standard assessment for fecal products of protein breakdown. Products of Protein Breakdown are utilized as a contributory diagnostic tool. Testing for fecal nitrogen may indicate the presence of protein malabsorption, but fecal nitrogen is difficult to measure and is not in widespread clinical use.[41]

Interpretation

The result for products of protein breakdown reflects the sum of fecal valerate, isovalerate, and isobutyrate. On the *GI Effects* test report, the value is shown against a background representing population quintiles, as described in the introduction.

Outcomes and General Therapeutic Considerations

Patients with elevated products of protein breakdown should be evaluated for common causes of insufficient protein digestion and/or excessive protein presenting to the colon.

Source of Elevated Colonic Products of Protein Breakdown	Possible Causes	Therapeutic Response
Insufficient Protein Digestion[33,34]	Hypochlorhydria	• Reduce acid-blocking medications • Add betaine HCl
	Pancreatic exocrine insufficiency	• Evaluate fecal PE1
Excessive delivery of protein to colon[42]	High-proein diet	Review protein/ carbohydrate intake
	GI irritation/ inflammation, bleeding, bacterial overgrowth	Additional testing, e.g., fecal calprotectin, fecal eosinophil protein X (EPX), fecal occult blood, stool culture for beneficial bacteria

Total Fecal Fats
The Biomarker

Under normal conditions, the bulk of dietary fat is digested and absorbed in the small intestine, leaving only small amounts for delivery to the colon and fecal stream. Fecal fat measurements determine the amount of fat in stool, and may therefore identify fat maldigestion, malabsorption, or steatorrhea.

Biomarker Key Points

- The test is a fecal fat extraction method that results in a quantitative value.
- Fecal fat extraction methods have been found to correlate with degree of fat malabsorption.[43]
- Total fecal fat is made up of long-chain fatty acids (LCFAs), cholesterol, triglycerides, and phospholipids.

Patient Populations of Interest

Fecal fats should be measured in any patient for whom steatorrhea (passage of pale, bulky, and malodorous stools) may be a symptom of underlying digestive or non-digestive disorders.

Symptoms suggesting evaluation of fecal fat as a means of detecting root causes include:

- Fatigue
- Unexplained anemia
- Nutrient deficiencies
- Unintended weight loss

Comparator/Gold Standard Tests

The 3-day stool collection with total fecal fat determination is the gold standard test for fecal fat. This test is unwieldy and unpleasant for patients and lab personnel. The total fecal fat extraction on a single specimen provides a quantitative value to identify patients that may benefit from the more in-depth 3-day test. Limited research has found extraction methods to correlate with the gold-standard.[43]

Interpretation

Total fecal fat is the sum of fecal triglycerides, long-chain fatty acids, cholesterol, and phospholipids. Fecal fats are reported using the quintile system.

Outcomes and General Therapeutic Considerations

Fecal fat may be elevated in situations of ***fat maldigestion***, such as:

- Pancreatic exocrine insufficiency (inadequate lipase production or delivery)
  - Causes include chronic pancreatitis and cystic fibrosis
- Bile salt insufficiency (inadequate solubilization of fats for digestion)
  - Causes include liver damage, hypolipidemic drugs, impaired gallbladder function
- Hypochlorhydria (inadequate stomach acid)
  - Causes include aging and gastric acid-lowering drugs
- Small intestinal bacterial overgrowth and resulting acidic small-intestinal pH (impairment of small intestinal digestive enzymes)[15,30]
- Use of medications designed to impair intestinal lipase activity (Orlistat, Xenical, Alli), or use of synthetic fat-like products indigestible by normal lipase (Olestra)[44-47]
- Elevated fecal fat may be associated with deficiencies in fat-soluble nutrients, so consider nutritional assessment of essential fatty acids, fat-soluble vitamins, and minerals.[48,49]

Fecal fat may also be elevated in situations of ***fat malabsorption***, such as:

- Intestinal dysbiosis
- Intestinal parasites
- Gastric bypass, ileal resection, or other surgeries that limit absorptive surface area

Finally, fecal fat may be elevated in patients with:

- Irritable bowel syndrome (often as a symptom of pancreatic exocrine insufficiency)
- Inflammatory bowel disease
- Food intolerances[50]
- Celiac disease
- Excessive alcohol intake
- Chronic use of non-steroidal anti-inflammatory drugs (NSAID)[51]

Supporting the Patient with Elevated Fecal Fat Levels

Depending on root causes, patients with elevated fecal fat levels can be supported as follows: [6,45-47,52-54]

Suspected Cause of Elevated Fecal Fat	Support Measures	Rationale
Pancreatic exocrine insufficiency	Supplementary plant or pancreatic digestive enzymes	Lipases increase fat digestion
Disorders of bile formation/ transport (cholestasis)	• Bile salts or cholagogues, taurine or glycine • Diet changes	Enhance intestinal fat solubilization
Lipase inhibitors (orlistat, Xenical, Alli) or synthetic fat consumption (Olestra)	Discontinue these products	Permit normal fat digestion

Inflammation and Immunology

Interactions between the immune system and the GI tract are being recognized as of growing importance, not only in GI physiology and pathophysiology, but also in their influences on systemic health and disease.

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**, a marker of eosinophil-driven inflammation and allergic response
- **Fecal Secretory IgA**, a marker of gut secretory immunity and barrier function

Calprotectin
The Biomarker

Calprotectin is a protein produced in abundance by neutrophils, the ubiquitous immune system "first responders." When neutrophils accumulate at sites of inflammation, they release increased amounts of calprotectin in a way that closely correlates with findings on endoscopy and histology, and are thus useful in quantifying the degree of intestinal inflammation.[55,56]

This property makes calprotectin useful for differentiating inflammatory from non-inflammatory disease processes, e.g., distinguishing irritable bowel syndrome (IBS) from inflammatory bowel disease (IBD).[57]

Biomarker Key Points

- Calprotectin is described in the literature as a useful non-invasive screening tool for identifying which patients may benefit from endoscopy for suspected IBD
- Calprotectin is used in diagnosing IBD (Crohn's disease and ulcerative colitis) and for quantifying degree of inflammation
  - › Thus calprotectin may be useful for monitoring treatment and assessing for relapse in patients with known IBD
- Calprotectin is FDA-cleared to differentiate IBS from IBD[57]

Patient Populations of Interest

Patients with symptoms consistent with IBS should have fecal calprotectin testing done as a means of ruling out significant inflammation; those with positive Rome criteria and normal calprotectin (<= 50 mcg/g) have virtually no chance of having IBD.[58,59]

Rome III Diagnostic Criteria for IBS [23,60*]

Recurrent abdominal pain or discomfort** at least 3 days/month in the last 3 months associated with two or more of the following:

1. Improvement with defecation
2. Onset associated with a change in frequency of stool
3. Onset associated with a change in form (appearance) of stool

Studies suggest that a person with positive Rome criteria and a normal calprotectin (<= 50 µg/g) has virtually no chance of having IBD

** Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis*

*** "Discomfort" means an uncomfortable sensation not described as pain*

Comparator/Gold Standard Tests

The gold-standard comparator test for determining presence and degree of intestinal inflammation is endoscopy with biopsy and histology; fecal calprotectin correlates closely with this approach.

Interpretation

The expected values for fecal calprotectin are shown here:

Calprotectin Concentration (micrograms/g stool)	Interpretation	Follow-up
<= 50	Normal (no active GI Inflammation)	None
> 50 to 120	Borderline, suggestive of low-grade inflammation	Re-evaluate in 4–6 weeks
> 120	Abnormal	Determine source of inflammation and repeat as clinically indicated
> 250	Associated with high risk of clinical relapse in IBD[61]	Adjust therapy accordingly

On the *GI Effects* report, calprotectin levels are represented as normal, borderline, or high, and are not represented by quintile values.

- False negatives for calprotectin may be seen in patients with severe immune compromise, who are not able to mobilize neutrophils sufficient to raise levels of calprotectin in the intestine
- Calprotectin elevations are seen in conditions other than IBD, e.g., malignancy, infection; therefore, a primary diagnosis of IBD cannot be established solely on the basis of a positive fecal calprotectin result
- Elevated calprotectin may also result from chronic NSAID use; evaluate such use in all patients with elevated calprotectin

Outcomes and General Therapeutic Considerations

Calprotectin is a simple, reliable, and non-invasive test that is useful in:

- Selecting patients with abdominal symptoms who may require further diagnostic procedures[62]
- Aiding in distinguishing between IBD and IBS[63]
- Selecting/screening patients for endoscopy, especially children in whom general anesthesia might be required for invasive study[55]
- Determining disease activity and risk of relapse in IBD[64]
  - Therapy can then be initiated before inflammation reaches critical intensity
- Monitoring IBD treatment response and determining when a full clinical remission has been achieved[65]
- Evaluating efficacy in trials of new treatments for IBD

Supporting the Patient with Elevated Fecal Calprotectin Levels

- Because inflammatory, infectious, or neoplastic processes may result in an elevated calprotectin level, the cause of a value > 120 mcg/g warrants further investigation – including endoscopy or radiography – based on clinical correlation
  - Assessments to uncover causes of bowel inflammation are found at the end of this section on Inflammation and Immunology

Eosinophil Protein X (EPX)
The Biomarker

Many inflammatory and neoplastic processes in the gut involve increased activity of eosinophils, white blood cells that normally reside in the lamina propria (connective tissue layer) of the intestinal wall.

When the lamina propria is damaged, eosinophils migrate into the gut lumen, where they degranulate to release a variety of proteins with cytotoxic properties, which contribute to ongoing inflammation and tissue destruction.[66] One such protein is eosinophil protein X (EPX), which can be measured in fecal matter.[67]

Biomarker Key Points

- Fecal EPX offers the practitioner a noninvasive alternative to the invasive gold standard, allowing for better differential diagnosis
- EPX is considered the superior cationic protein for assessment of eosinophil function, because it most accurately reflects the degree of mucosal damage[68]
- Baseline EPX levels offer a way to determine and monitor GI inflammation associated with food allergy
  - Significant reduction in EPX after 3 months on an elimination diet has been demonstrated[69]
  - Return of EPX to normal levels (< 2.0 mcg/g) can be used to indicate clinical efficacy of elimination diets[69] or clinical remission of IBD[68]
- Systemic corticosteroids can reduce circulating levels of EPX[70]

Patient Populations of Interest

Clinically, elevations in EPX indicate the presence of an IgE-mediated inflammatory process. Patients at risk for having such processes, and in whom fecal EPX may be helpful in making diagnosis and treatment plans, include those with:

- Complaints of GI symptoms related to food intake (possible food allergy)
  - › Also for monitoring results of elimination diets or other interventions in patients with known food allergy
- Concerns about possible parasitic/worm infections (recent travelers with GI symptoms)
- IBD in need of non-invasive monitoring for disease activity and treatment monitoring (consider calprotectin as well)

Because food allergy may present with symptoms similar to those seen in IBS, also consider fecal EPX testing in patients with symptoms consistent with IBS.

Comparator/Gold Standard Tests

The gold standard test for quantifying eosinophils in the gut is whole gut lavage. This is an invasive procedure requiring endoscopy, and has limited utility in the office setting. Clinical research indicates significant correlation between whole gut lavage fluids and eosinophil mediators in stool, such as fecal EPX.[71]

Interpretation

The normal reference range for EPX is < 7.0 mcg/g of stool. Levels above 7.0 suggest increased eosinophil activity in the gut lumen, suggestive of allergic or inflammatory reactions.

- Baseline EPX levels may be used to determine inflammation associated with food allergy
  - › Subsequent testing can be used to monitor results of dietary changes
- Elevations of EPX correlate with disease activity in ulcerative colitis and Crohn's disease[68]
- Serial testing of EPX offers a non-invasive means of evaluating disease activity and for predicting relapses in patients with IBD[72]

Outcomes and General Therapeutic Considerations

EPX levels above 2.0 mcg/g of stool are suggestive of elevated eosinophil activity, which may be associated with the following:[68,71,73]

- IgE-mediated food allergy
- Intestinal parasitic infection
- IBD

Less commonly, fecal EPX may be elevated in:[66,71,72,74,75]

- Atopic dermatitis
- Gastroesophageal reflux disease (GERD)
- Collagenous colitis
- Allergic colitis
- Excessive alcohol intake
- Chronic diarrhea
- Protein-sensitive enteropathy
- Gastrointestinal cancer
- Eosinophilic gastroenteritis (rare)

When evaluating a differential diagnosis that includes bowel cancer, calprotectin may be useful as an adjunctive marker along with radiologic and/or endoscopic evaluation

Conversely, in patients with known food allergy or IBD, acquisition of a normal EPX (< 2.0 mcg/g stool) can indicate the efficacy of treatment, such as elimination diet or remission of IBD.

Supporting the Patient with Elevated Fecal EPX

To further delineate the root cause(s) of an elevated fecal EPX result, consider additional testing to uncover causes of bowel inflammation, which are found at the end of this section on Inflammation and Immunology.

Fecal Secretory IgA (sIgA)

The Biomarker

Secretory IgA, or sIgA, is a class of antibodies produced by and secreted from mucosal surfaces, especially the GI and respiratory tracts. In the gastrointestinal epithelium, sIgA is the first line of defense against the entry of enteric toxins and pathogenic organisms from the colon. Colonic sIgA is closely involved in maintenance of the gut epithelial barrier, and in the development of immune tolerance of normal, beneficial commensal gut organisms, as well as of common molecular epitopes found in foods.[76,77]

Measurement of sIgA in fecal material, or fecal sIgA, therefore, may provide longitudinal information about the status of the intestinal epithelial barrier and its function in both immune exclusion (prevention of pathological material and organisms from gaining entry into the general circulation) and in immune inclusion (delivery of commensal bacteria and their products to the gut and systemic immune system for recognition and the development of tolerance, which spares these beneficial organisms from destruction by the immune system).[78,79] To date, there is little support from scientific data for the use of sIgA as a stand-alone diagnostic study; rather, measurement of sIgA has been most valuable in determining the outcomes of selected interventions that modulate GI barrier functions, including both disturbances of immune exclusion (in which excessive foreign matter is allowed to enter the organism, resulting in colonization by pathogens) and immune inclusion (in which immune tolerance fails or is weakened, resulting in allergic manifestations).[79]

Biomarker Key Points

- Fecal sIgA in the gut:
  - Regulates the balance of commensal (beneficial) bacteria[80,81]
  - Prevents colonization by pathogens[82]
  - Promotes tolerance of commensal organisms[76,77]
  - Maintains GI barrier function[78]
  - Promotes formation of normal biofilm containing beneficial organisms in the gut[83]

Patient Populations of Interest

Patients with known or suspected disruptions of the GI epithelial barrier may suffer from manifestations of "leaky gut" such as excessive bacterial translocation (failure of immune exclusion) or food allergies (failures of immune inclusion and tolerance development).[83-90]

Management of such patients may involve treatment with probiotics for restoration of a healthier colonic flora, or with exclusion diets in attempts to identify and eliminate sources of food allergies/intolerances. Measurement of sIgA is a common means of monitoring response to therapy in studies of such interventions, and clinicians may find it useful to determine sIgA levels prior to and during the course of treatment.[91-94]

The sIgA study is not recommended for use as a primary diagnostic test in patients suspected of having congenital immunoglobulin deficiencies such as selective IgA deficiency; such immune deficiencies should be evaluated by serological tests based on symptoms and family history.[95]

Comparator/Gold Standard Tests

There are no standard comparator tests for secretory IgA production in the gut.

Interpretation

Fecal sIgA results are reported on the *GI Effects Comprehensive Profile* using the quintile reporting system.

- There are no validated correlations between sIgA levels and specific disease states
- Fecal sIgA is most commonly used as an outcomes measure in clinical trials
- Determination of a pre-intervention sIgA level, followed by serial post-intervention levels, may clarify responses to food elimination diets, pre- and probiotic supplementation regimens, and similar therapeutic maneuvers
- Increased sIgA levels may indicate a normal, transient, immunological response to intestinal viral or bacterial pathogens[96]
  - Monitoring the level may aid in identifying resolution of issue

Outcomes and General Therapeutic Considerations

Observation of the sIgA levels following therapeutic interventions may help to determine whether and how much a patient is responding to such therapies. Fecal sIgA levels should not be used in isolation to make diagnostic or therapeutic decisions, but rather should form part of the picture of gut barrier function in the context of additional clinical data.

Lactoferrin (Add-on)

The Biomarker

Lactoferrin is an iron-binding glycoprotein secreted by most mucosal membranes; it is a major granular component of neutrophils (white blood cells). Liberated from the neutrophils in response to inflammation, lactoferrin binds to iron, impeding microbial growth and facilitating generation of hydroxyl radicals.[97]

Biomarker Key Points[98]

- Expressed by surface epithelial cells and found in most exocrine secretions, including breast milk, tears, nasal secretions, saliva, intestinal mucus, and genital secretions
- Lactoferrin is a multifunctional protein with antibacterial and immune modulatory activities and is a component of the first line of host defense
- Its expression is upregulated in response to inflammatory stimuli
- In the gastrointestinal tract, lactoferrin serves as a non-specific marker of inflammation

Patient Populations of Interest

Potential indications for testing are patients exhibiting gastrointestinal symptoms with suspected inflammation. Although clinical cut-offs have as yet to be determined definitively, lactoferrin may be useful in assisting the clinician in (1) identifying which patients may need further evaluation for inflammatory bowel disease[99-101] and (2) differentiating between IBD and non-inflammatory irritable bowel syndrome (IBS).[102]

Comparator/Gold Standard Tests

The gold-standard comparator test for determining presence and degree of intestinal inflammation is endoscopy with biopsy and histology. Fecal lactoferrin has been correlated to histological findings.[103]

Interpretation

A positive lactoferrin test generally indicates inflammation of the intestinal mucosa.

Outcomes and General Therapeutic Considerations

Lactoferrin is a non-invasive screening test and can assist the physician in stratifying symptomatic patients who require further evaluation; subsequent calprotectin testing can provide additional useful diagnostic information and assist in triage for endoscopic referral.

Supporting the Patient with Abnormal Lactoferrin Results

To further delineate the root cause(s) of an abnormal lactoferrin result, consider additional testing to uncover causes of bowel inflammation, which are found immediately below.

Supporting the Patient with Gastrointestinal Inflammation and Immune Reactions

Regardless of the specific findings, the support for patients with evidence of GI inflammation and/or immune reactions involves the following steps:

- Eradicate known pathogens or other infectious agents[104]
- Consider supporting commensal bacteria with probiotic supplements and dietary changes[92,105-107]
- Consider intestinal mucosal and anti-inflammatory support: appropriate nutrients, dietary changes, and botanicals[108-115]
- Rule out food sensitivities or allergies; consider elimination diet and/or IgG and IgE food sensitivity testing[116-118]
- Support immune status with supplemental whey protein or increased fiber

Consider further evaluation of underlying causes as shown in the following table:

Tests for Discerning Underlying Causes of Bowel Inflammation

Intestinal Permeability (IP) Assessment	IP is a noninvasive assessment of impaired permeability or leaky gut, which can affect barrier and immune function. Chronic irritation in the gut lining can lead to maldigestion and malabsorption.
Food Antibody Assessment	Assessment of immune-mediated responses to specific foods, both IgE and IgG classes.
Celiac Panel	Celiac disease is an autoimmune response to the gluten protein, gliadin, and to proteins with similar structure; damage occurs to the villa in the small intestine. The panel includes highly sensitive markers for identifying celiac disease.
ImmunoGenomic™ Profile	Evaluates genetic variations that modulate immune and inflammatory activity; these polymorphisms can result in stimulation of mechanisms that lead to chronic, overactive inflammatory responses.

Gastrointestinal Microbiome

The gastrointestinal microbiome is emerging as an exciting and powerful area for managing not only GI health, but that of the entire organism. It is estimated that the GI microbiome contains at least 10 times as many individual cells as are found in the somatic cells of human beings, with at least 1000 times the amount of genetic information.

Biomarkers of the GI Microbiome provide information about the health, function, and diversity 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.

The biomarkers are:

- **Metabolic indicators**, which demonstrate specific and vital metabolic functions performed by the microbiota
- **Commensal Bacteria**, which demonstrate the composition, diversity, and relative abundance of gut organisms, all of which are being linked to general health
- **Bacterial and mycological culture**, which demonstrate presence of specific beneficial and pathological organisms
- **Parasitology**, which demonstrates presence of parasites

Clinically significant imbalances in the GI microbiome (dysbiosis) have been associated with:[119-121]

- Irritable bowel syndrome (IBS)
- Inflammation
- Inflammatory bowel disease (IBD)
- Immune Modulation
- Metabolic Disorders
- Body weight & fat distribution
- Insulin sensitivity/type 2 diabetes
- Autism
- Other acute and chronic disorders[23,61,122-126]

There are multiple ways of assessing the GI microbiome and its impact on human health.

Humans have a conserved set of gut microbiota that is generally shared by most individuals, though each person has their own distinct and highly variable microbiota. The commensal gut microbiota interacts extensively with the host, influencing multiple metabolic and physiological functions.

Metabolomic methods involve measuring and interpreting the metabolic products of bacterial activity in the colon; these include determinations of fecal short-chain fatty acids and of enzymes elaborated by gut organisms, such as beta-glucuronidase.

Taxonomic methods are rapidly maturing, and include traditional bacterial and fungal culture and microscopy, as well as newer methods based on the microbial genomes of individual bacterial groups, such as polymerase chain reaction (PCR).

Metabolic Products: Short-Chain Fatty Acids

The Biomarker

Commensal gut bacteria anaerobically ferment resistant starch and dietary fiber (including prebiotics) to produce the beneficial short-chain fatty acids (SCFAs) acetate, propionate, and butyrate.[127] In particular, n-butyrate is the obligate fuel source for colonocytes, and inadequate levels are associated with disordered colonic health.[128,129]

Studies show distinct differences between healthy populations and those with GI disorders in terms of gut microbiota composition and SCFA production and distribution.

In the gut, short-chain fatty acids:

- Maintain intestinal barrier function
- Provide fuel for colonocytes (n-butyrate)
- Regulate colonic absorption of water and electrolytes
- Salvage unabsorbed carbohydrates
- Support commensal bacteria

SCFA levels are influenced by many factors, including:

- Diet composition
- Fecal ammonia content (indicative of excessive undigested protein)
- Obesity
- Environment

Biomarker Key Points

- Fecal SCFA concentrations are a metabolomic indicator of the health of the GI microbiome
  - › Low concentrations suggest either dysbiosis (abnormal levels or function of gut bacteria) or inadequate substrate for commensal organisms to ferment
- While optimal levels have not yet been identified, higher fecal concentrations of SCFAs have been associated with decreased GI disease in epidemiologic studies

Patient Populations of Interest

Studies have demonstrated distinct differences between healthy populations and those with gastrointestinal disorders when comparing the composition of gut microbiota and SCFA distribution. Lower levels of SCFAs are associated with colorectal diseases in population studies. Altered SCFA production has been seen in IBD and IBS. Fecal SCFA testing is a non-invasive contributory diagnostic tool in evaluating such patients.

Comparator/Gold Standard Tests

There is currently no gold-standard test for assessing SCFA production. SCFAs can also be measured in the blood. All three major SCFAs [acetate, butyrate, and propionate] are present in portal blood at concentrations several times greater than peripheral venous blood indicating the gut as a major source of these fatty acids.[130] Because SCFAs are rapidly utilized in the body, it is problematic to correlate blood levels with fecal SCFAs.

Interpretation

Optimal levels have not yet been determined for fecal SCFAs, however, in general, higher levels are considered beneficial.

The relative concentrations of n-butyrate, acetate, and propionate are also reported as percentages of total SCFAs. All results are reported and graphically displayed using the quintile reporting system.

- Low SCFA production is associated with:
  - Decreased carbohydrate or fiber intake[127]
  - Low levels of fecal anaerobic or commensal bacteria (dysbiosis)[62,131,132]
  - Dysbiosis has been associated in research with inflammatory processes (e.g., IBD),[133] and functional bowel disorders (e.g., IBS)[134-136]

Outcomes and General Therapeutic Considerations

Low fecal SCFA levels typically indicate disordered metabolic processes in the colonic commensal community, e.g., inadequate amounts of beneficial bacteria or inadequate substrate for those bacteria to produce their beneficial metabolic products, SCFAs.

Diets high in fiber and resistant starch, and relatively low in protein content, may increase SCFA production with resulting reduced risk of colorectal diseases and lowering of cholesterol and blood sugar.[137-145]

Supporting the Patient with Low Fecal SCFA Concentrations

Support of patients with low fecal SFCAs is centered on increasing metabolic substrates for beneficial SCFA-producing organisms:

- Prebiotic supplementation
- Increased dietary carbohydrate and fiber intake
- Increased consumption of resistant starch, which is known to increase levels of fecal butyrate[146]
- Evaluate and treat abnormalities of commensal gut bacteria

Beta-glucuronidase
The Biomarker

Beta-glucuronidase is an enzyme that breaks down complex carbohydrates. Additionally, it acts to deconjugate glucuronide molecules from a variety of toxins, carcinogens, hormones, and drugs, which are naturally glucuronidated in the liver to facilitate biliary excretion. Deconjugation of these molecules in the gut permits their reabsorption via enterohepatic recirculation, producing higher than desired blood levels of potentially harmful compounds.

Beta-glucuronidase activity must be sufficient to permit deconjugation and absorption of desirable molecules, while remaining low enough to prevent widespread deconjugation and subsequent reabsorption of toxins and other undesirable molecules.

Additionally, many beneficial nutrients are ingested as the glucuronide conjugate of the active molecule, which must be deconjugated in order for the beneficial molecule (the "aglycone") to be absorbed. Such nutrients include lignans, flavonoids, ceramides, and glycyrrhetinic acid.

Thus, a proper balance of glucuronidase in the gut lumen is essential. Beta-glucuronidase is inducible in colonocytes, but it is also produced by anaerobic gut bacteria (particularly *E. coli,* but also *Peptostreptococcus, Bacteroides, and Clostridium*).

Biomarker Key Points

- Limited research suggests an association between elevated fecal beta-glucuronidase and colon cancer risk[64,147,148]
- Low fecal beta-glucuronidase may also represent a problem, because the enzyme is needed to release the active aglycone forms of many dietary phytonutrients

Patient Populations of Interest

Evaluating beta-glucuronidase may be of interest to clinicians interested in evaluating substances that require deconjugation of glucuronide molecules, such as hormones, vitamin D, toxins, and phytonutrients.

Comparator/Gold Standard Tests

There is currently no gold-standard test.

Interpretation

Results are reported using the quintile reporting system.

Abnormally high levels of this biomarker warrant further investigation; abnormally low levels may diminish the bioavailability of many phytonutrients.

Outcomes and General Therapeutic Considerations

Further evaluation of patients with elevated fecal beta-glucuronidase includes consideration of exposure to and intake of toxins, hormones, and drugs.

For patients with persistently low fecal beta-glucuronidase, consider genomic testing and detoxification profiles to assess genetic markers and functioning of the glucuronidation pathway.

Supporting the Patient with Elevated fecal beta-glucuronidase:

For patients with ***elevated*** fecal beta-glucuronidase:

- The following supplements may be helpful:
  - Calcium-D-glucarate
  - Milk thistle
  - Probiotics (*Lactobacilli* and *Bifidobacteria*)
- The following dietary management may be helpful:
  - Increased consumption of vegetables and insoluble fiber

Commensal Bacteria

The Biomarkers

Commensal bacteria populate the human GI tract, especially the colon.

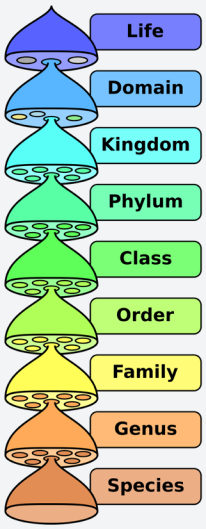
More than 95% of commensal gut organisms are anaerobic. Because these organisms are therefore difficult to recover by traditional (aerobic) culture techniques, there has been a major shift in research towards molecular DNA techniques, which are now considered the standard for anaerobic bacteria assessment in research. These techniques permit identification and quantification of multiple organisms with a single specimen.

PCR TECHNOLOGY

The *GI Effects* tests utilize DNA by Polymerase Chain Reaction (PCR) technology to identify commensal bacteria.

PCR utilizes probes that target specific, highly conserved segments of DNA, which allows identification of unique chosen sequences.

These probes may be created at any level of the rank of organisms: Phylum, Class, Genus, or Species.



The Polymerase Chain Reaction (PCR) methodology is capable of identifying and quantifying organisms. PCR probes can identify bacterial populations at any level of taxonomy, as broadly as phylum and as narrowly as species. This ability permits analysis of the gut microbiome at any desired degree of complexity.

Such analyses are of growing importance in human health and disease. The commensal gut microbiome has a diverse set of direct interactions with the human host. These interactions influence multiple metabolic and physiological functions, including:

- Producing short-chain fatty acids that nourish colonocytes and modulate gut physiology
- Modulating the systemic and intestinal immune systems
- Modulating GI hormone production
- Maintaining gut barrier function and motility
- Modulating oxidative responses

- Producing vitamins (e.g., biotin, vitamin K)
- Metabolizing xenobiotics and phytochemicals
- Preventing colonization by potential pathogens

In addition, the normal gut microbiome as a whole is important in preventing colonization by pathogens, a concept known as "colonization resistance."[149,150]

Clinically significant disruption or impairment of the healthy microbiome is referred to as "dysbiosis," although there is as yet no standard definition of this term, nor is there an identified "ideal" or "normal" microbial community.[151-163] Some researchers suggest that dysbiosis may occur in three primary types: transient, disrupting, or persistent.[133]

Biomarker Key Points

Assessment of an individual's commensal bacterial population may provide clinical utility by permitting:

- Evaluation of 24 key bacterial groups/species that are associated with GI and systemic health
- Detection and characterization of states of imbalance between these groups
- Monitoring of changes following clinical interventions (e.g., antibiotic, probiotic, or dietary therapies)
- Tailoring of treatments to individual needs
- Identification of possible origins of metabolic disturbances that lie in alterations of the gut microbiome
- Detection of dysbiosis
  - Commensal bacteria should be diverse and balanced in terms of population levels
  - A low or imbalanced population of commensal bacteria may be an indicator of dysbiosis[133]

Patient Populations of Interest

Because of the large numbers of conditions that are associated with imbalances in the composition of the gut microbiome, many patient populations may benefit from evaluation of GI commensal organisms. These include patients suffering from:

- Irritable bowel syndrome (IBS)
- Inflammation
- Inflammatory bowel disease (IBD)
- Immune Modulation
- Metabolic Disorders
- Body weight & fat distribution
- Insulin sensitivity/type 2 diabetes
- Autism
- Other acute and chronic disorders[23,61,122-126]
- Conditions associated with "leaky gut" or impaired intestinal barrier function

Comparator/Gold Standard Tests

No gold standard exists for evaluation of the commensal bacterial community. DNA-based techniques such as PCR are now considered the standard for anaerobic bacteria assessment in research and such techniques are now being applied in the clinical realm. These techniques allow for vastly increased detection and quantitation of large numbers of organisms across the entire phylogenetic spectrum.

Interpretation

The commensal bacteria PCR testing panel produces results for 24 important bacterial groups/species known to be associated with both GI and systemic health.

- Organisms are reported according to phylum, and sub-categorized by specific genus and, in most cases, by species
- The abbreviation "spp." is used to indicate multiple species within a genus
- Commensal bacteria PCR results are reported as the number of colony-forming units (CFU) per gram of stool, expressed in a computer version of scientific notation, where the capital letter "E" indicates the exponent value
- Thus, in the example below, the result for Bacteroides–Prevotella group is 4.3E7 (or 4.3 x 10^7) CFU/g stool, representing 43 million individual organisms per gram of stool

Recall that there is a great deal of variability in terms of specific species present in any one individual gut microbiome, so we should not necessarily expect that a reference population of 100 healthy individuals would have the exactly the same composition of bacteria present. As noted in published research, not all commensal bacteria are present in every individual – or if present, may be present in such small amounts relative to other gut microbial species that the level of sensitivity of the molecular assay cannot detect their presence. For some of the commensal targets in *GI Effects*, a portion of the quintile bar is shaded in grey to represent the proportion of the healthy reference population for whom levels of the specific commensal target were below detection limits. For instance, *Oxalobacter formigenes*, one of the 24 targets on *GI Effects*, has a unique ability to metabolize

oxalates in the gut and therefore colonization with this organism may reduce the risk of calcium oxalate kidney stones with healthy levels associated with a 70% reduced risk of being a recurrent calcium oxalate stone-former. However, we know from the literature that *Oxalobacter* is not normally present in 23% to 54% of healthy adults, which is consistent with our finding that 20% of our healthy cohort had a <detection limit (<DL) result for this bug.

The number of tails (1 tail or 2 tail) for the quintile reporting bar for a specific organism is determined empirically by the underlying distribution of the reference population data (i.e., does the data look like it has 1 tail or 2 tails) and by the clinical associations published in literature (i.e., is there published evidence suggesting that High and/or Low levels of this organism are associated with health or with disease). For example, *Bacteroides vulgatus* could not be detected in 5% of our healthy reference population and therefore the reference range value for this bug is <4.6E9, the 97.5% percentile value. We also know from published literature that high levels of *B. vulgatus* are found to be present in stools of severely autistic children when compared to controls. However, we also know that, compared to healthy controls, low levels of *B. vulgatus* are associated with obesity, T2D, and IBS. Therefore, the quintile reporting bar for *B. vulgatus* is 2-tailed even though we are only able to statistically determine the 97.5% percentile value on the right-hand tail.

Outcomes and General Therapeutic Considerations

Once the profile of the commensal bacteria community for an individual patient is known, interventions can be applied in some cases to modulate it favorably.

Supporting the Patient with Disrupted Commensal Bacteria (Dysbiosis)

Dietary changes:

- Increased intake of fiber and whole, complex carbohydrate and resistant starch[41]
  - Introduce high-fiber foods gradually to avoid exacerbation of GI symptoms[76]

Supplements:

- To promote and sustain beneficial commensals, consider addition of probiotics (live bacteria) and/or prebiotic supplements (products to support indigenous microbiota) to alter commensal bacteria; for example:[91]
- Probiotics
  - *Lactobacillus*
  - *Bifidobacteria*
- Prebiotics
  - Psyllium
  - Oat bran
  - Oligofructose
  - Xylooligosaccharide
  - Inulin
  - Beta-glucan
  - Arabinogalactan
- Botanical products may also be used to decrease or modulate gut bacteria

Note that:

- Alterations in the macronutrient composition of the diet, such as in vegan, high-protein, or gluten free diets, have shown changes in the composition of the gut microbiome.[164,165,166-170]
  - Short-term dietary changes have been shown to rapidly change the microbiome[171]
    - Animal-based diets were shown to increase the abundance of bile-tolerant organisms, such as *Bacteroides* (plus other Bacteroidetes genera), with a concomitant relative decrease in saccharolytic bacteria
    - A primarily plant-based diet exhibited higher populations of saccharolytic bacteria, such as *Roseburia* and *F. prausnitzii*, which metabolize dietary plant polysaccharides
- Unbalanced or low commensal bacteria levels have been associated with antibiotic or botanical intake and inappropriate probiotic or prebiotic supplementation

Individual Commensal Bacteria

***Bacteroides-Prevotella* Group** Bacteroidetes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	L	L/H	L	L/H		LD

- Abundance associated with lower bacterial gene richness in the gut[172]
- Reduced patterns of *Bacteroides* reported in IBS and ulcerative colitis;[173] conversely, other researchers found increased levels in IBD[174-177]
- When compared with fibromyalgia patients, early RA patients showed less *Bacteroides-Prevotella-Porphyromonas*[178]
- Higher levels associated with excessive weight gain in pregnancy[179] and in obesity[180,181]
- Other researchers reported lower *Bacteroides* (as part of *Bacteroides-Prevotella* group) in obese subjects compared to lean[182]
- Ratio of *Bacteroides-Prevotella* group to other gut bacteria correlated positively and significantly with plasma glucose;[183]
- In contrast, some have reported half the *Bacteroides* abundance in T2DM compared to those with normal glucose tolerance or those with pre-diabetes[184]

B. vulgatus Bacteroidetes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	L		L/H	L	H	LD

- *Bacteroides* spp. associated with lower bacterial gene richness in the gut[172]
- Lower levels of *B. vulgatus* have been seen in IBS patients in comparison to healthy controls[185]
- Low relative proportions of *B. vulgatus*, along with high concentrations of *Lactobacillus* spp. observed in the microbiota of obese children when compared to lean;[186] B. *vulgatus* also found under-represented in microbiota of type-2 diabetics[187]
- *B. vulgatus* found to be present in significantly higher numbers in stools of severely autistic children when compared to controls[188]
- While increased *B. vulgatus* prevalence was associated with the genotype of infants at high risk of celiac disease development,[189] another study found that *B. vulgatus* was more frequently detected in controls than in patients with treated celiac disease ($p<0.01$)[190]

***Barnesiella* spp.** Bacteroidetes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
			L			

- Currently identified as a common but low-abundance genus[191]
- Higher fecal *Barnesiella* (two logs difference) associated with protective effect against Vancomycin-Resistant Enterococcus in stem cell transplant patients[192]

***Odoribacter* spp.** Bacteroidetes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
		L				

- Lower concentrations in humans have been reported in ileal Crohn's and pancolonic ulcerative colitis[193]

***Prevotella* spp.** Bacteroidetes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
		H	H	H	L	LD

- Abundance associated with lower bacterial gene richness in the gut[172]
- Has been found higher in IBD,[177,194] in smokers with (and without) IBD (in association with higher *Bacteroides* and lower *F. prausnitzii*),[174] and in type-2 diabetes[184]
- *Prevotella* found over-represented in new-onset rheumatoid arthritis (RA), when compared to other groups (healthy controls, treated RA patients, and psoriatic arthritis patients, who had higher *Bacteroides*)[195]
- Lower levels found in autism[196] and in debilitated aging[197]
- Family Prevotellaceae exhibited a >6-fold increase in obese subjects when compared to the healthy group, with most of the Prevotellaceae sequences belonging to a single-genus, *Prevotella*.[198]

* *LD = Robust levels of this organism associated with Low Diversity of gut bacteria*
HD = Robust levels of this organism associated with High Diversity of gut bacteria

Individual Commensal Bacteria

Anaerotruncus colihominis Firmicutes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
				L		HD

- Abundance associated with higher bacterial gene richness in the gut[172]
- Inversely associated with BMI and triglycerides[172,199]

Butyrivibrio crossotus Firmicutes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
				L		HD

- Abundance associated with higher bacterial richness in the gut[200]
- Abundance may help protect against weight gain[200]

***Clostridium* spp.** Firmicutes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
		L/H			H	LD

- Many of its species are associated with lower bacterial gene richness[172]
- Higher *Clostridium* counts and increased number of *Clostridium* species reported in autism;[201-203] vancomycin (which targets *Clostridium*) improves symptoms in children with late-onset regressive autism[202-204]
- Both higher[193] and lower[205] abundance of *Clostridium* has been observed in IBD

Coprococcus eutactus Firmicutes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	L				L	HD

- Abundance associated with greater bacterial gene richness in the gut[172]
- *Coprococcus* may be less prevalent in autistic children compared to neurotypical children;[196] may be result of intestinal disaccharidase deficiencies common in autism[206]
- In IBS, reduced abundance reported (in association with elevated *Ruminococcus* spp.)[207]

Faecalibacterium prausnitzii Firmicutes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
		L/H		L		HD

- In a healthy gut, represents more than 5% of the total bacterial population[208] and is comprised of only one species
- Abundance associated with higher bacterial gene richness in the gut[172]
- Controls inflammation through inflammatory-cytokine inhibition;[209] lower counts reported in IBD:[174,193,210] Crohn's disease[211-215] and ulcerative colitis (UC),[216] although increases have been noted[215]
- Appears to protect against glucose intolerance and type 2 diabetes;[184] possibly due to anti-inflammatory effects[182] and/or positive effects on insulin resistance status[182,184]

***Lactobacillus* spp.** Firmicutes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	L/H			H		HD

- Abundance associated with higher bacterial gene richness in the gut[172]
- Studies have reported altered levels in IBS, with some finding higher concentrations[217-219] and others finding lower concentrations[220-222]
- Lower levels reported to correlate with symptom severity in IBS[207]
- Increased levels seen in obese patients compared to lean controls[223]

***Pseudoflavonifractor* spp.** Firmicutes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
						HD

- Abundance associated with higher bacterial gene richness in the gut[172]

Individual Commensal Bacteria

***Roseburia* spp.** Firmicutes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	L	L	L	L		HD

- Abundance associated with higher bacterial gene richness in the gut[199]
- Less abundant in individuals with IBS, particularly constipation-predominant IBS[224]
- Counts lower in type 2 diabetics;[183] trending inversely with plasma glucose[183]
- Lower in IBD[193,216] and early-onset rheumatoid arthritis[178] (as part of decreased *E. rectale-C. coccoides* group)

***Ruminococcus* spp.** Firmicutes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	L/H	H			H	LD

- Abundance associated with low bacterial gene richness in the gut[172]
- Human studies have reported that Ruminococcus spp. tend to be more abundant in IBD;[225] active UC,[176] active CD,[226] and ileal CD[227]
- Levels are variable in IBS, depending on IBS subtype, with some researchers reporting increased concentrations[228] and some finding decreased amounts[222]
- May be more prevalent in autism[202]

***Veillonella* spp.** Firmicutes phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	L/H				L	HD

- Abundance associated with higher bacterial gene richness in the gut[172]
- Imbalances noted in IBS, although findings are mixed: some studies reported higher concentrations in IBS,[218] in IBS-C,[221] IBS-D;[219] others have reported lower counts[217] or lower counts weakly correlating with greater symptom severity[207]
- Found less abundant in autistic children compared to neurotypical children[196]

***Bifidobacterium* spp.** Actinobacteria phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	L	L	L	L/H	L	HD

- Abundance associated with higher bacterial gene richness in the gut[172]
- Modulates local and systemic immune responses[229]
- Abundance lower in IBD[212,213]
- Abundance lower in IBS;[219,230,231] low levels also correlate with symptom severity in IBS[207]
- Lower levels seen in type 2 diabetes,[187,232,233] pediatric allergy,[234] and autism[235]
- Increased levels in obese subjects compared to lean/overweight;[236] infants with lower *Bifidobacterium* may have increased risk for weight gain in childhood[237]
- Abundance decreases after weight loss[238] and gastric-bypass surgery[182]

B. longum Actinobacteria phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
				H		HD

- Abundance associated with higher bacterial gene richness in the gut[172]
- Abundance decreases with weight loss[238]
- Found Increased in obese subjects compared to lean/overweight[236]

Collinsella aerofaciens Actinobacteria phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	L	H		H		

- Lower counts reported in IBS;[222,239] lower levels may correlate with greater severity of IBS symptoms[207]
- Higher concentrations reported in IBD; thought to be result of abnormal host responses to the bacteria[177]
- *Collinsella* spp. reported higher in type 2 diabetes;[184]

* *LD = Robust levels of this organism associated with Low Diversity of gut bacteria*
HD = Robust levels of this organism associated with High Diversity of gut bacteria

Individual Commensal Bacteria

Desulfovibrio piger Proteobacteria phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	H	H		L	H	

- Reported higher in IBD[205,240,241]
- Sulfate-reducing bacteria higher in constipation-predominant IBS[224] compared with healthy subjects
- *Desulfovibrio* spp. also found higher in autism[242]
- May be lower in obesity: preschoolers[243] and adults[244]

Escherichia coli Proteobacteria phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	H	H		L/H		

- Increased counts reported in inflammatory bowel disease;[177,205,213,245-247]
- Increased levels found in diarrhea-predominant IBS[248]
- Higher in overweight pregnant women compared to normal weight pregnant women and in women with excessive weight gain during pregnancy[249]
- Reported to increase with weight loss after gastric bypass, correlating negatively with leptin levels[182]

Oxalobacter formigenes Proteobacteria phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
				L		

- Normally present in 46–77% of healthy adults[250]
- Unique ability to metabolize oxalates in the gut[250-252]
- Dietary oxalate consumption generally increases *O. formigenes* abundance in controls, but not stone formers[250]
- Colonization with this bacteria may reduce risk of oxalate stone formation,[250,252] with healthy levels associated with 70% reduced risk of being recurrent calcium-oxalate stone-former[250]

Methanobrevibacter smithii Euryachaeota phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
	L/H	L/H		L/H		HD

- Abundance associated with higher bacterial gene richness in the gut[172]
- Lower counts of *Methanobrevibacter* species reported in human obesity;[180,253] higher amounts reported in anorexia;[223] in contrast, one study confirmed a positive association with increased BMI and body fat in methanogen-colonized populations[254**]
- Higher levels linked to IBS-C; reduced levels linked with IBS-D[255**]
- Methanogens found higher in people with colon cancer, colonic polyposis, ulcerative colitis,[256**] and diverticular disease (sigmoidoscopy enema samples)[257]
- Some studies have reported lower counts in IBD;[[255,258**]] [259] conversely, other have reported Increased abundance;[256**]

** Breath-testing study, an accepted indirect measure of gut methanogens; although *M. smithii* is currently considered the dominant methanogenic archaeon in the gut,[260,261] other methanogenic bacteria may also be contributors to breath methane.[259]

***Fusobacterium* spp.** Fusobacteria phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
		H	H	H		

- Although part of normal human gut flora, species of *Fusobacterium* strongly associated with numerous diseases, including colorectal cancer (CRC).[262-265] appendicitis,[266] dental plaque/ periodontal disease,[267] hepatic cirrhosis,[268] and inflammatory bowel disease[176,269,270]
- *Fusobacterium* correlates positively with TNF-alpha, suggesting involvement of mucosal inflammation[264]
- Obese, older subjects with metabolic syndrome demonstrated increased Fusobacterium as compared to younger subjects[199]

* *LD = Robust levels of this organism associated with Low Diversity of gut bacteria*
HD = Robust levels of this organism associated with High Diversity of gut bacteria

Individual Commensal Bacteria

Akkermansia muciniphila Verrucomicrobia phylum	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity* Association
		L		L	L	HD

- Dominant mucus-layer species;[271] may represent 3-5% of microbial community in healthy adults[272]
- Abundance associated with higher bacterial gene richness in the gut[172]
- Plays role glucose homeostasis[184]
- Abundance inversely correlated with IBD (both Crohn's and UC)[194,225,273] and appendicitis[266]
- Abundance inversely correlates with body weight in pregnant women[249] and children[243]
- Some have reported decreased *A. muciniphila* in pre-diabetes and decreased Verrocomicrobiae abundance in T2D and pre-diabetes[184]
- Lower in autism[196,235]

Firmicutes/Bacteroidetes Ratio

The Biomarker

The two largest phyla making up the gut microbiome in humans are Firmicutes and Bacteroidetes. The relationship of these two large groups, expressed as the Firmicutes/Bacteroidetes ratio, has been associated with a number of pathological conditions:[274]

- Obesity has been specifically associated with a greater abundance of Firmicutes and/or a drop in Bacteroidetes (i.e., an increase in the ratio); some research, however, has shown no change or even an increase in Bacteroidetes in obesity[253,274,275]
- Disruptions of metabolic homeostasis[276]
  - e.g., type 2 diabetes and non-alcoholic fatty liver disease[277,278]
- Elevated markers of inflammation such as IL-6[279]

Biomarker Key Points

The Firmicutes/Bacteroidetes Ratio captures the following members of each class:

Total Firmicutes	Total Bacteroidetes
Anaerotruncus colihominis	*Bacteroides-Prevotella* Group
Butyrivibrio crossotus	*B. vulgatus*
Clostridium spp.	*Prevotella* spp.
Coprococcus eutactus	*Barnesiella* spp.
Faecalibacterium prausnitzii	*Odoribacter* spp.
Lactobacillus spp.	
Pseudoflavonifractor spp.	
Roseburia spp.	
Ruminococcus spp.	
Veillonella spp.	

Comparator/Gold Standard Tests

Because of the relative newness of the Firmicutes/Bacteroidetes Ratio as determined by PCR, there are no existing comparator tests.

Interpretation

- The quintile reporting system is used for all commensal bacteria results, including the F/B ratio, based on a clinically characterized healthy reference population.
- The differences observed in the gut microbiota composition of obese and lean subjects in some clinical trials has led to multiple preliminary studies on the use of (beneficial) probiotic bacteria to alter the obese phenotype. These studies have largely shown that probiotic intervention has a beneficial effect, and may lead to novel interventions for overweight or obese human patients.[280]

Outcomes and General Therapeutic Considerations

Although no specific therapeutic interventions are yet known for a markedly elevated or depressed Firmicutes/Bacteroidetes Ratio, general intervention strategies directed against some of the commensals may impact this ratio and ultimately gut health.

Supporting the Patient with Disrupted F/B Ratio

For imbalances in the F/B Ratio it is best to start by correlating with microbial diversity and abundance, and base treatments on those analytes as noted out-of-range in the four functional pillars.

Bacteriology (Culture)

Traditional bacterial culture complements DNA-based tests such as PCR to provide a more complete survey of a patient's gut microbiota beyond the specific organisms targeted by PCR. Culture methods have established clinical utility and defined parameters that have been long recognized as "gold standard" in traditional clinical diagnostics.

Beneficial Bacteria (Culture)

Cultivable gut bacteria include beneficial bacteria such as *Bifidobacteria*, *Lactobacilli*, and *E. coli*, all of which are thought to exert positive local and systemic effects through their anti-inflammatory and immune-modulating properties. Conversely, impaired commensal bacterial populations have been associated with intestinal and chronic diseases. *Bifidobacteria*, *Lactobacilli*, and *E. coli* are also targeted by PCR to provide more specific quantitation of their levels.

Biomarker Key Points

- *Lactobacillus* species and *E. coli* are facultative anaerobes, capable of surviving in an environment with limited oxygen.
- *Bifidobacteria* are obligate anaerobes, and must be grown in anaerobic chambers.

Culture is required for determining therapeutic interventions such as sensitivities to pharmaceutical or botanical antibiotics.

Patient Populations of Interest

Bacterial culture remains of great utility in evaluating patients with symptoms of possible gastrointestinal infection or dysbiosis.

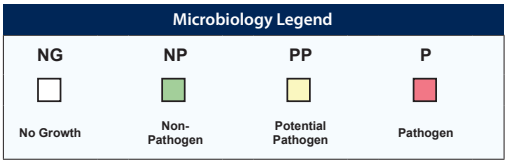
Comparator/Gold Standard Tests

Bacterial culture is the gold standard for identification of populations of cultivable organisms.

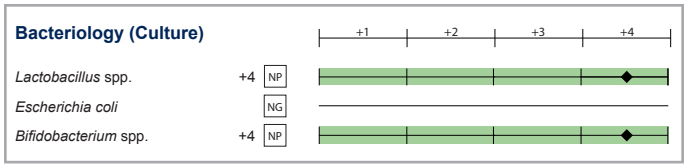
Interpretation

Results of bacteriology culture are reported as "No Growth," or growth in one of three categories of bacteria, using a color-coding system:

- No growth (white)
- Non-pathogen (green)
- Potential pathogens (yellow)
- Known pathogens (red)



The quintile reporting system is not used for this test; instead, growth is reported as present at one of four semi-quantitative levels of abundance.



Outcomes and General Therapeutic Considerations

Patients with low growth of beneficial bacteria may benefit from dietary manipulations and supplements similar to those discussed in the section on commensal bacteria, including pre- and probiotics and elimination of antibiotics when feasible.

Additional Bacteria (Culture)

Bacterial culture of fecal material may also yield additional organisms of interest in the assessment of the gut microbiome. Many such organisms constitute part of the normal aerobic or commensal flora, and are not recognized as major pathogens. They are typically readily cultured for identification when they occur at clinically significant levels.

Additional bacteria also include organisms that may be potential pathogens (PP on the report form), or "opportunistic" pathogens. Not usually pathogenic, these organisms may overgrow during periods of perturbation in the gut environment and their presence on culture may be an indicator of imbalance or dysbiosis. The significance of these organisms must be interpreted in the context of a specific patient's clinical presentation (e.g. symptoms, immunosuppression, etc.).

Biomarker Key Points

Additional or opportunistic pathogens are typically restrained and controlled by balanced levels of commensal organisms, but their overgrowth may occur when commensal bacterial populations are impaired by:

- Infection with overt pathogens or parasites
- Poor diet
- Antibiotic use
- Lowered gut immunity

Patient Populations of Interest

Patients whose management might benefit from testing for Additional Bacteria include those with symptoms of unexplained or persistent diarrhea, especially those with known or suspected imbalance of the normal gut flora (dysbiosis), and those who are immunosuppressed.

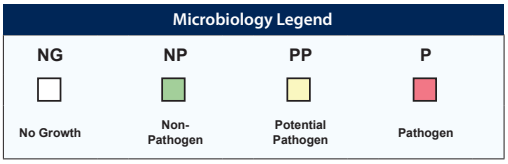
Comparator/Gold Standard Tests

This test uses bacterial culture. Identification of cultured bacteria is via Vitek-MS using Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF). The MALDI-TOF mass spectrometry platform utilized for the rapid identification of bacteria and yeast from pure cultures on the *GI Effects Comprehensive Profile* report relies on the most extensive FDA-cleared library of microbial targets available on the market, which can accurately identify approximately 200 different additional bacterial species.

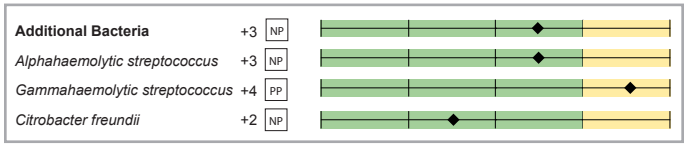
Interpretation

Additional bacteria are reported using the same conventions as for Bacteriology Culture, above.

- No growth (white)
- Non-pathogen (green)
- Potential pathogens (yellow)
- Known pathogens (red)



The quintile reporting system is not used for this test; instead, growth of any additional bacteria is reported as present at one of four semi-quantitative levels of abundance, as shown in the following example:



The presence of Potentially Pathogenic organisms (PP) at higher levels, or of Pathogenic bacteria (P) at any level, should trigger increased concern. Such levels may be of clinical relevance in patients with bacterial gastroenteritis including:

- Travelers' diarrhea[281-285]
- Food poisoning[286]
- IBD[287]
- IBS[288]

Presence of opportunistic or potentially pathogenic organisms may also indicate intestinal microflora imbalance, or dysbiosis, poor diet, antibiotic use, or lowered gut immunity.

Outcomes and General Therapeutic Considerations

Once identified, pathogenic and potentially pathogenic/opportunistic bacteria may be decreased through pharmacological and botanical treatments, and by restoring a healthy intestinal microflora balance.

Pharmacological treatments are best utilized subsequent to standard methods of diagnosis, including sensitivity profiles. When opportunistic bacteria are found, they are automatically cultured and the sensitivity of pharmaceuticals and active botanical ingredients are assessed. Agents marked Sensitive or "S" have been shown to be effective treatments in culture/sensitivity studies and may be used as indicated.

Mycology (Culture)
The Biomarker

The fungal kingdom includes yeasts and molds. Yeasts are single-celled organisms in the fungal kingdom; 1500 species have been described to date. All humans house fungal colonies in their colons, typically without detriment. Yeasts are likely to be normal members of the human microflora and certain strains (e.g., *Saccharomyces boulardii*) have been shown to:

- Reduce symptoms of diarrhea in children
- Prevent reinfection with *C. difficile*
- Reduce bowel movements in patients with diarrhea-predominant IBS
- Reduce the incidence of antibiotic-, travelers'- or HIV-associated diarrhea

Biomarker Key Points

- Fungi are detected in fecal samples by standard culture techniques
- Pathogenic and potentially pathogenic (opportunistic) fungi are associated with gastrointestinal symptoms, especially in immune-compromised people
- Fungal infections can produce imbalances of GI microorganisms (dysbiosis)

Patient Populations of Interest

Patients at increased risk of significant fungal infections (mycosis) include people with:

- Compromised immunity
  - Those on corticosteroids
  - Those with diabetes
  - The very young or very old
  - Yeast overgrowth syndrome (clinical presentation does not have the severity described in conventionally recognized fungal infections)

Comparator/Gold Standard Tests

This test uses fungal culture, which is the gold standard. Identification of cultured yeast/fungi is via Vitek-MS (MALDI-TOF). The MALDI-TOF mass spectrometry platform utilized for the rapid identification of bacteria and yeast from pure cultures on the *GI Effects Comprehensive Profile* report relies on the most extensive FDA-cleared library of microbial targets available on the market.

Interpretation

Fungal culture is reported as Mycology (Culture) on the test report, using the same conventions as for Bacteriology Culture, above.

- No growth (white)
- Non-pathogen (green)
- Potential pathogens (yellow)
- Known pathogens (red)

The quintile reporting system is not used for this test; instead, growth is reported as present at one of four semi-quantitative levels of abundance. The example shows growth of a potential pathogen (PP) in the second quadrant of growth.

When fungi are found, they are automatically cultured and the sensitivities of pharmaceuticals and active botanical ingredients are assessed. Agents marked Sensitive or "S" have been shown to be effective treatments in culture/sensitivity studies and may be used as indicated.

Outcomes and General Therapeutic Considerations

Once identified, treatment for pathological yeast species and yeast overgrowth should be directed at eliminating the offending species and restoring microbial balance. Treatments may be pharmaceutical or botanical. Pharmaceutical treatments should be used following standard methods of diagnosis.

Supporting the Patient with Abnormal Fungal Culture

In supporting patients with yeast problems, consider the degree of infection, the patient's overall immune status, diet (carbohydrate/sugar intake), and offer support for a potentially impaired immune system.

Parasitology
The Biomarker

Though prevalence data for intestinal parasitic infection in the US is limited, one survey found one-third of 5792 fecal specimens to be positive for parasites, with positivity peaking seasonally between July and October.[289] According to the American Association for Clinical Chemistry (AACC), the most common parasites in the United States include: *Cryptosporidium*, *Entamoeba histolytica*, and *Giardia*.

A single fecal specimen tested for parasites by O&P may detect approximately 90% of GI parasite infections.[89]

The parasitology test uses two complementary methodologies:

1. Microscopic examination of fecal specimens for ova and parasites (O&P), the gold standard of diagnosis for many parasites
2. Enzyme immunoassay (EIA) for the identification of *Cryptosporidium, Entamoeba histolytica*, and *Giardia lamblia*

Biomarker Key Points

- EIA is a biochemistry-based test that detects immunogenic macromolecules such as toxins or organism-specific antigens
- EIA is widely recognized for its diagnostic utility for detection of pathogenic antigens

Patient Populations of Interest

Screening for parasites is appropriate in patients presenting with:

- Excessive or persistent diarrhea
- Stools containing blood or mucous
- Severe abdominal pain (chronic or subacute)
- Nausea and vomiting

Because of the diversity of presentations in patients with GI parasites, it may be useful to test for parasites in patients with such symptoms who:

- Have unexplained persistent headache and fatigue
- Have been exposed to a parasitic outbreak at daycare or school
- Have traveled outside of the U.S.
- Have consumed untreated water

Comparator/Gold Standard Tests

Microscopic examination of fecal samples for ova and parasites is the gold standard test for such examinations, and continues to have the highest proven diagnostic and clinical utility for parasite detection.

Although the examination of at least three samples on at least 3 separate days remains the recommendation of the Centers for Disease Control, some literature suggests that approximately 90% of enteric parasite infections may be detected in a single stool sample collected for O&P examination, with small increases in sensitivity and negative predictive values for additional samples.[89] For patients where parasitic infection is suspected or needs to be excluded, at least three samples on three separate days should be submitted for evaluation.

Interpretation

The normal result for O&P on microscopic exam is "negative" or "no organisms seen." Parasitology EIA tests are reported as "Negative" (in range) or "Positive" (out of range) on the report, with each EIA-evaluated organism listed separately (*Cryptosporidium, E. histolytica*, and *G. lamblia*).

Outcomes and General Therapeutic Considerations

Because parasitic infections typically require pharmaceutical treatment with anti-parasite medications, an accurate and timely diagnosis is essential. In a patient with a high degree of clinical suspicion for parasites, a positive test warrants appropriate interventions.

The Table below lists common fecal parasitic pathogens along with symptoms and therapeutic considerations.

Common Parasitic Protozoans

Parasitic Protozoans	Symptoms	Therapeutic Considerations
Blastocystis hominis Transmission: Unknown; consider contaminated food or water, or exposure to infected animals suspected.	Watery or loose stools, diarrhea, abdominal pain, anal itching, weight loss, constipation, fatigue, and excess flatulence have been reported in persons with *Blastocystis* infection. Many people are asymptomatic.	The clinical significance of *Blastocystis* spp. is controversial, although there is increasing evidence that it may be a pathogen in some individuals with symptoms meeting criteria for Irritable Bowel Syndrome.90 For additional information, see **www.dpd.cdc.gov/dpdx/HTML/Blastocystis.htm**
***Cryptosporidium* spp.** Transmission: Fecal contamination of food or water, including swimming pools and municipal water supplies.	Patients will be symptomatic or present with diarrhea varying from mild to severe, abdominal cramping, weight loss, anorexia, nausea, vomiting, flatulence, malaise, and mild fever.	Most people who have healthy immune systems will recover without treatment. Diarrhea can be managed by drinking plenty of fluids to prevent dehydration. Immunosuppression increases infection severity. See **www.dpd.cdc.gov/dpdx/HTML/Cryptosporidiosis.htm**
Dientamoeba fragilis Transmission: Unknown; often associated with pinworm infection or fecal contamination.	Patients will be asymptomatic or present with diarrhea, nausea, and vomiting; abdominal tenderness is possible.	There is no consensus as to best clinical practice; goal is eradication of parasite. See **www.dpd.cdc.gov/dpdx/HTML/Dientamoeba.htm**
Entamoeba coli, Entamoeba histolytica* and *Entamoeba dispar Transmission: Contaminated food or water, pets, sexual contact. More common in people who live in tropical areas with poor sanitary conditions. It can be a pathogenic amoeba.	Several protozoan species in the genus *Entamoeba* colonize humans, but not all of them are associated with disease. *Entamoeba histolytica* is well recognized as a pathogenic amoeba, associated with intestinal and extra-intestinal infections. Only about 10% to 20% of people who are infected with *E. histolytica* become sick. A severe form of *E. histolytica* is associated with stomach pain, bloody stools, and fever (may resemble ulcerative colitis). *E. dispar* is non-pathogenic.	Only one antibiotic is used in non-symptomatic *E. histolytica* infection; two antibiotics if patients are symptomatic. See **www.dpd.cdc.gov/dpdx/HTML/IntestinalAmebae.htm** and **www.dpd.cdc.gov/dpdx/HTML/Amebiasis.htm**
Giardia lamblia Transmission: Contaminated water, food, or fecal-oral transmission. *G. lamblia* is the leading cause of intestinal parasitic infection in the US. See **www.dpd.cdc.gov/dpdx/HTML/Giardiasis.htm**	Patients can be asymptomatic. If symptomatic, will present with acute to chronic diarrhea with bloating, intestinal malabsorption, and steatorrhea. Giardiasis has been associated with agammaglobulinemia, chronic pancreatitis, achlorhydria, and cystic fibrosis.	Several prescription drugs are available to treat giardiasis. See **emedicine.medscape.com/article/176400-clinical**

Bacteria Sensitivity

When bacterial culture yields pathogenic or potentially pathogenic organisms, the Bacteria Sensitivity section reports the results of in vitro testing for susceptibility.

- **Prescriptive Agents** lists detected organisms along with their relative sensitivity to prescription antibiotics or antimicrobials. Conventional definitions of "sensitive," "intermediate," and "resistant" organisms are used.
- **Natural Agents** lists detected organisms along with their relative degree of growth inhibition by herbal and other natural substances. "High" inhibition indicates a greater ability of the substance to limit microbial growth, while "Low" inhibition suggests less ability to limit growth.

Mycology Sensitivity

When fungal culture of stool yields fungal organisms, the Mycology Sensitivity section reports the results of in vitro testing for susceptibility. Prescriptive and Natural Agents are listed and categorized similarly to those reported for Bacteria Sensitivity.

- **Prescriptive Agents** lists detected organisms along with their relative sensitivity to prescription antibiotics or antimicrobials. Conventional definitions of "sensitive," "intermediate," and "resistant" organisms are used.
- **Natural Agents** lists detected organisms along with their relative degree of growth inhibition by herbal and other natural substances. "High" inhibition indicates a greater ability of the substance to limit microbial growth, while "Low" inhibition suggests less ability to limit growth.

Additional Tests

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

Color

Stool color is primarily associated with diet and medication use, though it may also be an indicator of various GI health conditions.

Consistency

Stool consistency may vary from significantly hard to watery. The technical ability to recover diagnostic biomarkers from stool may be influenced by extremes of consistency. Consistency is self-reported by the patient.

Occult Blood

The term "occult" in this context simply means blood not evident to the naked eye, that is, blood present in microscopic quantities only. Normally, stools should be entirely free of blood.

The Hemosure diagnostic kit uses fecal immunochemical testing (FIT); it has higher specificity than the common guaiac test 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. Further investigation is warranted in the presence of a positive test.

There are no drug or dietary restrictions prior to collecting the sample in relationship to FIT; vitamins or foods do not affect the FIT.

Pathogenic Bacteria (Add-on)

The Biomarker

Pathogenic bacteria are those organisms known to cause distinct human disease processes, and in that way they differ from normal or opportunistic organisms in the human GI microbiome. Pathogenic bacteria are detected on this test by enzyme immunoassay (EIA), which is the standard approach to diagnosis of pathogens.

Biomarker Key Points

Organisms for which EIA testing is done are:

- *Helicobacter pylori* (*H. pylori*)
- *Campylobacter* species
- Shiga Toxin-producing *E. coli*
- *Clostridium difficile (C. difficile)*

Patient Populations of Interest

Pathogenic Bacteria EIA testing will achieve its optimum utility when used in the context of an appropriate differential diagnosis that considers patient symptoms and produces a high index of suspicion for at least one clinically known syndrome or symptom complex. Testing of non-symptomatic patients is not recommended.

For each organism, symptomatology is as follows:

- *H. pylori*
  - Although prevalence of the organism is 35 to 40% among US adults, most patients remain asymptomatic[290] ("colonized" and not "infected") and therefore do not require testing to document the organism's presence
  - Indications for testing include upper GI symptoms or pathology such as:
    - Gastritis
    - Duodenal and peptic ulcer disease
    - Gastric lymphoma
    - Gastric cancer (in the patient or a relative)
- *Campylobacter* species
  - Asymptomatic carriage is common; testing of asymptomatic patients may be indicated during outbreaks
  - Indications for testing include:
    - Diarrhea (often bloody)
    - Abdominal cramps
    - Fever
  - Clinically apparent illness typically lasts about one week, but may be serious in immune-compromised hosts
  - An earlier culture-independent detection method using a DNA probe was less specific, detecting any *Campylobacter* species, not necessarily pathogenic ones
- Shiga toxin-producing *E. coli*
  - Asymptomatic carriage can occur
  - When present, symptoms are variable and may include:
    - Abdominal cramping
    - Water or bloody diarrhea
    - Vomiting
  - EIA confirms the presence of the pathogenic shiga toxin
  - Instead of routine screening, recommendations for testing now concentrate on clinically relevant populations; specifically, patients with significant diarrhea
- *C. difficile*
  - Asymptomatic colonization can occur; true infection is defined as presence of the organism and/or its toxin in the context of a symptomatic patient
  - When present, symptoms include:
    - Cramping
    - Lower abdominal pain/tenderness
    - Fever
    - Watery diarrhea
    - Nausea
    - Loss of appetite
- *C. difficile* is a well-known cause of antibiotic-associated diarrhea

Comparator/Gold Standard Tests

There is substantial peer reviewed literature indicating the diagnostic utility of the detection of a pathogenic antigen by EIA.

- EIA provides actionable clinical information

Interpretation

The positive finding for any pathogen on the report is considered significant.

Outcomes and General Therapeutic Considerations

Therapeutic intervention is warranted in any patient in whom the practitioner has a high clinical index of suspicion and in whom a diagnosis of any of these four pathogens is made.

The table below summarizes the organisms, their typical symptoms, and appropriate therapeutic considerations.

Pathogenic Bacteria by EIA

Pathogen	Symptoms	Therapeutic Considerations
***Campylobacter* spp.** Transmission: Consumption of contaminated food (particularly poultry), water, or contact with infected animals (particularly cats and puppies).	Symptoms include: diarrhea (often bloody), abdominal cramps, and fever. Illness typically lasts one week and may be serious in immunocompromised patients. *Campylobacter jejuni* infection has been associated with the onset of Guillain-Barré syndrome (GBS).	Patients generally recover without any specific treatment. They should drink extra fluids throughout the duration of diarrheal episodes. CDC review is at **www.cdc.gov/nczved/divisions/dfbmd/diseases/campylobacter/**
Clostridium difficile Transmission: Shed in feces.	Symptoms include: cramping, lower abdominal pain/tenderness, fever, watery diarrhea, loss of appetite, and nausea. It is a common cause of antibiotic-associated diarrhea (AAD).	In about 20% of patients, *C. diff* infection will resolve within 2 to 3 days of discontinuing the antibiotic to which the patient was previously exposed. CDC review is at **www.cdc.gov/HAI/organisms/cdiff/Cdiff_faqs_HCP.html#a9**
Shiga toxin *E. coli* Transmission: Consumption of contaminated food, unpasteurized (raw) milk, water that has not been disinfected, contact with cattle or the feces of infected people. STEC stands for shiga toxin-producing *Escherchia coli* (STEC).	The symptoms vary but may include abdominal cramping, watery or bloody diarrhea, and vomiting.	The infection can be self-limiting. Rehydrate, and consider pre-and probiotics to support infection resolution. Treatment is based on the site and severity of infection, and STEC status.CDC review at **www.cdc.gov/ecoli/index.html**. Also see **www.cdc.gov/ecoli/clinicians.html**
Helicobacter pylori Transmission: Is incompletely characterized. Person-to-person transmission is most commonly implicated with fecal/oral, oral/oral, or gastric/oral pathways.	Symptoms include: acute gastritis with abdominal pain, nausea, and vomiting. Non-ulcer dyspepsia is common. Development of severe *H. pylori* disease is partially determined by the virulence of the infecting strain.	Conventional recommendation is polypharmacy: antibiotics and proton-pump inhibitors (PPIs). See **www.acg.gi.org**. Botanical anti-*H. pylori* formulas may prove helpful. See **www.cdc.gov/ulcer/keytocure.htm**.

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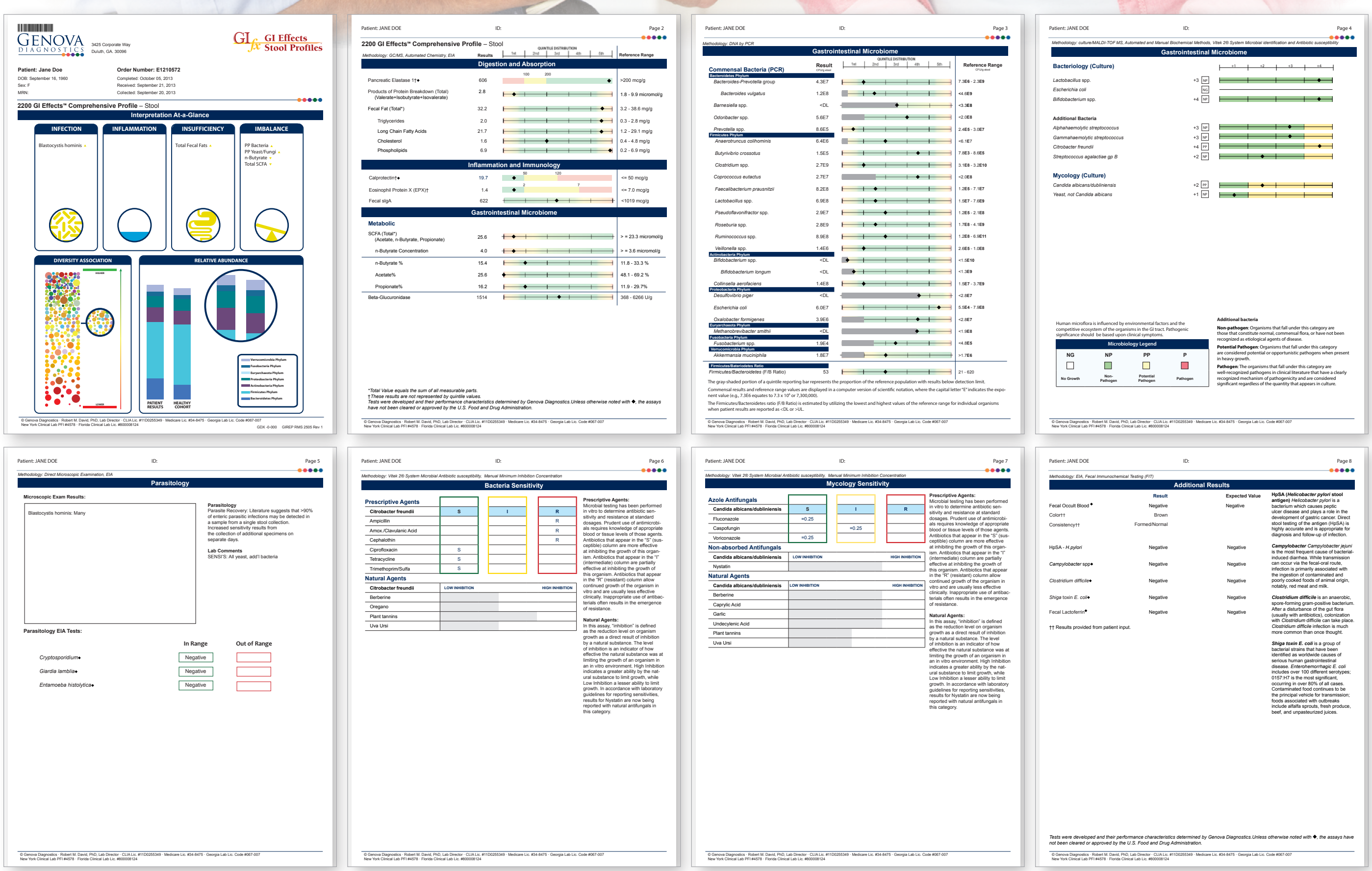
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Parasitology
Bacteria Sensitivity
Mycology Sensitivity
Additional Results

Gut Microbiota Clinical Association Summary*

	IBS	Inflammation	Immune Modulation	Metabolic Disorders	Autism	Diversity Association
Bacteroidetes Phylum						
Bacteroides-Prevotella Group	L	L/H	L	L/H		LD
B. vulgatus	L		L/H	L	H	LD
Barnesiella spp.			L			
Odoribacter spp.		L				
Prevotella spp.		H	H	H	L	LD
Firmicutes phylum						
Anaerotruncus colihominis				L		HD
Butyrivibrio crossotus				L		HD
Clostridium spp.		L/H			H	LD
Coprococcus eutactus	L				L	HD
Faecalibacterium prausnitzii		L/H		L		HD
Lactobacillus spp.	L/H			H		HD
Pseudoflavonifractor spp.						HD
Roseburia spp.	L	L	L	L		HD
Ruminococcus spp.	L/H	H			H	LD
Veillonella spp.	L/H				L	HD
Actinobacteria phylum						
Bifidobacterium spp.	L	L	L	L/H	L	HD
B. longum				H		HD
Collinsella aerofaciens	L	H		H		
Proteobacteria phylum						
Desulfovibrio piger	H	H		L	H	
Escherichia coli	H	H		L/H		
Oxalobacter formigenes				L		
Euryarchaeota phylum						
Methanobrevibacter smithii	L/H	L/H		L/H		HD
Fusobacteria phylum						
Fusobacterium spp.		H	H	H		
Verrucomicrobia phylum						
Akkermansia muciniphila		L		L	L	HD

** The literature-based clinical associations in this chart are not intended to indicate diagnostic patterns.*

Key

L = Low
H = High
LD = Robust levels of this organism associated with Low Diversity of gut bacteria
HD = Robust levels of this organism associated with High Diversity of gut bacteria

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