



GI Microbial Assay Plus

Quantitative PCR Stool Technology for the Integrative and Functional Medicine Practitioner



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The GI Microbial Assay Plus (GI-MAP)

Quantitative PCR Stool Technology for the Integrative and Functional Medicine Practitioner

Microbiology and DNA Analysis

In the last few decades, DNA analysis has transformed the field of microbiology. The National Institutes of Health have followed suit with initiatives such as the Human Microbiome Project, which characterized the microbiome from over 15 habitats of the body in more than 200 healthy human subjects using DNA analysis.³ More than ever before, we are keenly aware of the health benefits or disease risks brought about by the microorganisms that inhabit the human body. Culture techniques, previously the standard, left up to 50% of bacterial species virtually invisible.⁴ When next-generation methods revolutionized this field, it allowed the identification of tremendous numbers of previously unknown organisms. Anaerobic bacteria make up a large part of the human microbiome and can be opportunistic and cause illness. Therefore, inability to cultivate these organisms left a large blind spot for clinicians when trying to diagnose the source of infection.

The **Gastrointestinal Microbial Assay Plus (GI-MAP™)** was designed to assess a patient's microbiome from a single stool sample, with particular attention to microbes that may be disturbing normal microbial balance and may contribute to perturbations in the gastrointestinal (GI) microbiota or illness. The panel is a comprehensive collection of microbial targets as well as immune and digestive markers. It screens for pathogenic bacteria, commensal bacteria, opportunistic pathogens, fungi, viruses, and parasites. It primarily uses automated DNA analysis to give integrative and functional medicine practitioners a better view into the gastrointestinal microbiome.

The GI-MAP measures pathogenic organisms that can cause hospital-acquired infections (HAI) such as *C. difficile* or norovirus, foodborne illness such as *E.coli* or *Salmonella*, and common causes of diarrhea such as *Campylobacter* or *Shigella*.⁵ This panel measures viral causes of gastroenteritis, unavailable by other common stool tests. It measures parasites such as *Cryptosporidium*, *Giardia*, and *Entamoeba histolytica*. The GI-MAP analyzes *Helicobacter pylori* and its virulence factors. It can detect opportunistic pathogens such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, associated with autoimmune molecular mimicry. It includes a panel of single-celled, amoebic parasites such as *Blastocystis hominis*, *Dientamoeba fragilis*, and *Entamoeba coli*. Worms such as *Necatur americanus* and *Trichuris trichuria* are reported on the GI-MAP as well as cytomegalovirus and Epstein-Barr virus. Fungal organisms include *Candida*, *Geotrichum*, *Microsporidia* and more.

Finally, the GI-MAP measures standard markers of immunity, inflammation and digestion including calprotectin, secretory immunoglobulin A (SIgA), anti-gliadin antibody, and pancreatic elastase-1.



Disruption of the Gastrointestinal Microbiome Can Cause:

Gastrointestinal Symptoms

- Abdominal pain⁶
- Bloating⁷
- Constipation⁷
- Crohn's disease^{8,9}
- Diarrhea^{6,7,10}
- Food poisoning¹¹
- Gastric cancer¹²
- Gastritis¹²
- Gastroenteritis^{13,14}
- Gastroesophageal reflux^{15,16}
- Irritable Bowel Syndrome^{13,17}
- Small Intestinal Bacterial Overgrowth (SIBO)¹⁸
- Ulcer¹²
- Ulcerative colitis¹⁹
- Vomiting²⁰

Autoimmune Conditions

- Ankylosing spondylitis²¹
- Reactive arthritis²¹⁻²³
- Rheumatoid arthritis²⁴

Allergic Disease

- Asthma²⁵
- Eczema²⁶⁻²⁸

Molecular Methods Revolutionize Diagnostic Testing

Diagnostic Solutions Laboratory (DSL) is using a novel DNA technique to detect a comprehensive list of stool bacteria, viruses, fungi, and parasites. Real-time polymerase chain reaction (RT-PCR) or quantitative PCR (qPCR) combines amplification and detection into one step. qPCR *“is one of the most powerful and sensitive gene analysis techniques available.”* It is used to quantify gene expression, analyze single nucleotide polymorphisms (SNPs), determine genotypes, detect pathogens, validate drug targets, and measure RNA interference.²⁹

In 1885, the first microbe was cultured. In 1969, breakthroughs in anaerobic microbial culture helped further define the GI ecosystem. But in 2000, molecular techniques, or DNA analysis, sparked what was initially described as a Renaissance and later called, the “molecular revolution.”³⁰ These molecular methods, also used in the landmark Human Microbiome Project, made it possible to see 50 percent more microbes than had ever been seen before.^{4,31,32}

qPCR “is one of the most powerful and sensitive gene analysis techniques available.”



qPCR is quickly becoming the standard for diagnostics due to the increased specificity, sensitivity, and reproducibility of PCR techniques. qPCR panels are able to rapidly detect viruses, parasites, and anaerobic bacteria, which can be missed by traditional methods.^{33,34}

Government and private institutions around the world are incorporating it into patient care. The FDA has cleared multiple molecular-based gastroenteritis testing panels.³⁴ While there are many PCR diagnostic tests for gastroenteritis, the GI-MAP stands alone as the largest panel that quantifies each pathogen and non-pathogen, making it the best tool to assess both acute and chronic GI complaints.

Polymerase chain reaction (PCR) methodologies (such as qPCR and standard PCR) are targeted approaches for rapidly detecting, identifying, differentiating, and quantitating specific microbes and genes of clinical relevance. These DNA replication methods make numerous copies of a target sequence of DNA in the presence of primers (short, single-stranded sequences of nucleic acids) and DNA polymerase (the DNA-replicating enzyme). Also known as amplification and hybridization, these two steps are required for accurate measurement of microbial targets. Amplification is the process of making many copies of the target gene. Hybridization matches the target gene to a complementary genetic sequence in a lock-and-key manner.

Table 1. Comparison of Microbial Detection Methods

Microbial Detection Method	Fully Quantitative	Highly Sensitive Detection <i>Measures Very Low Levels of Organisms</i>	Each Analyte Individually Validated	Provides only Clinically Relevant Organisms	Rapid Turnaround Time <i>Within Days</i>	Identifies Bacteria, Parasites, Fungi, and Viruses Down to the Strain Level	Identifies Genes Involved in Microbial Function
GI-MAP™ DNA Stool Testing by qPCR*	+++	+++	+++	++	+++	+++	++
Standard PCR	-	++	++	++	+++	++	++
Shotgun Metagenomic Sequencing	-	+	-	-	-	++	+++
Metatranscriptomic Sequencing	-	+	-	-	-	++	+++
16S Sequencing	-	+	-	-	-	-	-
Culture + MALDI-TOF MS	-	-	+	+	+	-	-
Microscopy	-	-	+	++	+	-	-

Performance Ranking of Each Microbial Detection Method

+++ Strong Performance ++ Moderate Performance + Poor Performance — Method Fails This Indicator

* Only GI-MAP is Fully Quantitative

In qPCR, segments of DNA that are highly specific for selected targets are amplified (replicated many times). Colorimetrically labeled DNA probes (single-strand nucleic acid sequences designed to bind the amplified target gene) make it possible to quantitate the amplification process as it occurs, in real time, yielding a truly quantitative DNA result. Diagnostic Solutions’ qPCR method uses highly-specific primers targeting each microbe (species, genus, or virulence gene) based on ribosomal RNA regions. The target specificity is further confirmed based on extensive verification testing using validated reference genomes.





Standard PCR is similar to qPCR, except that it is not regarded as truly quantitative, because the amplified DNA is quantified only at the final stage of the PCR process, making it impossible to determine the true quantity of target DNA. It may instead be estimated by comparing it to a standard curve or by analyzing the quality and yield of PCR-products with gel electrophoresis.²⁹

Setting the Bar for Microbial Detection and Quantitation

Diagnostic Solutions Laboratory uses qPCR because it is truly quantitative and more accurate than all other microbial detection methods available in the commercial setting (see Table 1). Knowing exactly how much DNA is present gives the practitioner important information for better clinical decision-making.

qPCR exceeds other DNA analysis techniques using multiplex polymerase chain reaction²⁹ and standard PCR for its ability to quantitate. In qPCR, all of the organisms are run separately, and in duplicate, which makes it possible to measure each organism accurately. There is no competition for chemical reagents in the same well that could lead to variation in the results. The GI-MAP has a larger analytical range, which means it measures higher and lower amounts of genomic DNA than other DNA analysis techniques. All results are quantitative (giving a precise measurement) instead of qualitative (positive or negative).

The GI-MAP qPCR method is superior to shotgun metagenomic sequencing, metatranscriptomic sequencing, and 16S sequencing for its ability to quantitate extremely low levels of organisms. Turnaround-time with qPCR may be as little as five days at Diagnostic Solutions Laboratory. Other stool testing options on the market take weeks or even months to provide results.

DNA or RNA Sequencing methods such as metagenomic and metatranscriptomic sequencing and 16S sequencing are increasingly available for stool microbe testing. In

A “Primer” on Amplification and Hybridization

Accurate measurement of DNA targets relies on two molecular methods: making copies of target genes (amplification) and matching single-stranded DNA from the targets to the probes in a lock-and-key manner (hybridization). After receiving stool specimens, nucleic acids are extracted and purified. The DNA is separated into single strands and each strand is duplicated using a primer. This process is repeated multiple times, which “amplifies” the gene targets. Amplification can generate thousands to millions of copies of a single target DNA sequence, amplicon. This makes it possible to measure even tiny amounts of DNA found in a stool specimen.

After amplifying the DNA targets found in the stool specimen, the specimen undergoes hybridization. It is treated with DNA probes. A probe is a segment of DNA that seeks to join with its complementary sequence and is colorimetrically labelled for measurement.

Hybridization is the binding (like a lock and key) of one single-stranded DNA segment to another complementary piece of DNA. This step is important for accurate identification of a microbe based on its DNA signature. Each probe has a unique DNA signature that will bind to the amplified target gene, *if* it is present in the stool specimen. If the probe does not perfectly match the target gene, then it falls away. This allows for accurate and sensitive detection of a target organism.



this methodology, the instrument sequences amplified DNA or RNA, and determines the order of nucleotides of the genes in the specimen. The gene sequences are compared to a gene library to identify the organisms in the stool. Sequencing DNA is very slow and has extended turnaround-times.

Sequencing approaches can provide approximate, relative levels of organisms, genes or transcripts, but are not considered sufficiently accurate for true quantitation. For this reason, results are expressed as a percent of total, not as quantitative values. In commercial settings, these techniques use lower numbers of reads (the number of base pairs sequenced from a DNA fragment) to cut costs, which produce less accurate results.

16S sequencing focuses on the 16S ribosomal RNA gene. This is not as comprehensive or accurate of a method because the 16S gene is only found in bacteria and archaea and varies widely in copy numbers. Sequencing methods available in the commercial setting can give qualitative information about the microbes in stool but cannot be used to diagnose a pathogen.

qPCR stands above culture techniques for microbial detection as well as ova and parasitology methods. These methods were used historically for stool analysis. Culture and Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry, relies on bacterial culture of the stool specimen. The organisms that are cultured are then identified using the MALDI-TOF. A limitation of this method is the reliance on bacterial and fungal culture. Microbes in the stool specimen can grow or decay after collection and in transit. Therefore, they may not represent the sample at the time of collection. Additionally, organisms that do not grow under typical culture conditions cannot be identified. DNA analysis of stool microbes more closely represents the actual microbial populations of the patient's gastrointestinal tract at the time of collection.

The qPCR method used in the GI-MAP is high throughput and is fully automated. The automated nature of this method minimizes the chance for human error. Historically, DNA analysis was labor intensive and prone to human errors in extraction, hybridization, and amplification.

GI-MAP Analytes

The human gastrointestinal microbiome houses trillions of bacteria and research shows that these microorganisms are essential for human metabolism,³⁵ nutrition, immune function,³⁶ and resistance to infection.³⁷ Over 500 different species of microorganisms from 30 different genera have been identified from the human gut. But in any one person, there are 100 million – 1 trillion microorganisms per gram of fecal content.³⁸ Most microbes in the human gut are believed to be beneficial or commensal. There are microbes that colonize many people but only become pathogenic in certain situations (opportunistic pathogens). Finally, there are pathogens that are widely recognized to cause disease in the human host.





Although they are ubiquitous, pathogenic bacteria do not cause illness in all people. This is because commensal gastrointestinal microbiota can protect the host from infection. When gut microbiota protects the intestines from pathogens and harmful microorganisms it is called, “colonization resistance.”³⁷ Animal models show that when normal gut microbiota are lacking, the host is more susceptible to GI infections with *Salmonella*. Similarly, after antibiotic treatment there is increased risk of pathogenic infections.³⁷ On the other hand, commensal bacteria such as *Lactobacillus* and *Bifidobacterium* can prevent gastrointestinal infection. Colonization resistance explains why most pathogenic bacteria fail to cause disease in healthy subjects.³⁹

Commensal bacteria naturally inhabit the human gastrointestinal tract and do not cause disease. Many are beneficial; they produce enzymes,⁴⁰ vitamins,⁴¹ short chain fatty acids,⁴² and other metabolic products that keep the bowels and the body functioning well. The incredibly complex interaction between human health and the gastrointestinal microbiome is the subject of multiple cutting-edge research studies.⁴³ Given the metabolic, nutritional, and immune-enhancing roles of these organisms, the microbiome deserves close analysis when treating patients with chronic illness.

Pathogens

The GI-MAP measures bacterial pathogens such as *Campylobacter*, *Escherichia coli* (*E. coli*) O157, *Enterotoxigenic E. coli*, Shiga-like toxin-producing *E. coli*, *Clostridium difficile*, *Salmonella*, *Shigella*, and *Vibrio cholerae*. The GI-MAP qPCR technique was developed, verified, and validated by Diagnostic Solutions Laboratory (DSL) and has been correlated with the previous FDA cleared assay. In addition, the DSL qPCR assay has been validated against known positive samples for all organisms and is capable of detecting as low as 0.1 cell per gram of stool. Diseased samples were used to construct reference ranges and cutoff values to correctly distinguish disease-causing amounts of pathogenic and opportunistic bacteria.

The pathogenic targets have been selected based on their clinical utility and analytical validity as DNA targets. For example, *Clostridium difficile* is positive when genes encoding for toxins A and B have been detected while other organisms are detected based on their unique DNA signatures. In one comprehensive review of rapid molecular technologies compared to conventional culture techniques, the authors concluded that there was sufficient evidence to recommend testing with PCR for *Campylobacter*, *E. coli* O157, and *Salmonella* and that it may yield better results than culture techniques.⁴⁴ Multiplex PCR was preferred over conventional microbiological techniques in 347 patients with gastroenteritis. Authors concluded that DNA analysis was faster for pathogen identification and provided clinicians with a larger panel of pathogens, helping to contain nosocomial outbreaks before they spread.⁴⁵

Bacterial pathogens are often spread due to contamination of food and water with fecal material containing these pathogens. Consult your Physician's Desk Reference for standard treatments for these pathogens. Antibiotic therapy is not always recommended because antibiotic resistance can worsen the infection. Hydration,



probiotics, and supportive therapies for the gut-immune system can help to remove the pathogen from the GI tract.

The presence of a pathogen does not, by itself, indicate disease.⁵ Results from laboratory tests must be interpreted together with clinical symptoms and history by a qualified health practitioner. With increased awareness of the complexity of the GI environment, a pathogen is likely to cause disease if there are vulnerabilities in the host's defenses. For example, imbalanced microbiota, poor immune defenses, poor diet, toxic exposures, antibiotics, or chronic GI symptoms could make a person more susceptible to harm from a pathogen. Whereas another person may carry a fecal pathogen but is in good health. In healthy patients, treating pathogens may not be necessary. However, continuing to support a beneficial and diverse microbiota and a strong gut-immune system will further protect the host from infection.^{36,46}

Despite what type of stool test is used, the transient nature of the microbiota must be acknowledged. Populations of microorganisms can change dramatically in short periods of time, especially under stress, with the use of antimicrobial medications, or changes in the diet, etc. The transient nature of gastrointestinal microorganisms makes it even more important to use the lab results together with signs and symptoms to determine if a particular lab finding is indicative of a clinical condition that requires treatment. Clinical monitoring and follow-up testing and confirmation by other testing methods helps to analyze the changes to the microbiome over time and verify clinically relevant findings.⁵ Similarly, a pathogenic organism finding on a test result does not necessarily indicate treatment, even when there are symptoms of disease. Healthy, immune-competent people can naturally eradicate a pathogen with basic healthcare practices and the passage of a few weeks, making treatment unnecessary.

***Clostridioides difficile* (previously *Clostridium difficile*, *C. difficile*, or *C. diff*)** is a well-known pathogen that can cause colitis and *Clostridium difficile*-associated diarrhea or CDAD. It commonly presents with mild to moderate diarrhea and occasionally abdominal cramping. *C. diff* is able to colonize the GI tract after a disturbance of the microbiota, generally after antibiotic therapy. *C. diff* releases toxins that cause inflammation and damage to the GI lining. It infects nearly 20% of hospitalized patients, making it the most common nosocomial infection.⁴⁷

Toxins A and B are the major virulence factors believed to be responsible for *C. diff* infection symptoms. They are proinflammatory and cytotoxic. They damage the cytoskeleton of intestinal epithelial cells, permitting fluid influx, they open tight junctions in the GI lining, and thereby damage the GI lining.

Toxins A and B have even shown systemic effects in animal models, suggesting that their bioactivity may not be localized to the GI tract. Toxins A and B are encoded by the *tcdA* and *tcdB* genes and are therefore detectable using DNA analysis.⁴⁸ Real-time polymerase chain reaction is considered a gold standard diagnostic methodology for *C. diff*.⁴⁷

***Escherichia coli* (*E. coli*)** is a large and varied species of bacteria that includes many strains. They colonize humans and animals and are spread through contaminated water, food, or contact with infected humans or animals.⁴⁹ *E. coli* can



cause infections outside of the GI tract such as urinary tract infections, meningitis, and intra-abdominal abscess.⁵⁰

While there are many harmless, and even beneficial *E.coli* strains, there are six strains that are notorious for their pathogenicity, and are measured on the GI-MAP:

- *Enterohemorrhagic E. coli*
- *E. coli* O157
- *Enteroinvasive E. coli/ Shigella*
- *Enterotoxigenic E. coli* LT/ST
- Shiga-like Toxin *E. coli* stx1 and stx2

Enteroinvasive E. coli and *Enterohemorrhagic E. coli* colonize the colon while the others colonize the small intestines and subsequently initiate diarrhea.⁵⁰

***Enterohemorrhagic E. coli* (EHEC)** is a moderately invasive bacteria known to cause hemorrhagic colitis, causing bloody diarrhea in infected individuals and may progress to hemolytic uremic syndrome (anemia and kidney failure). Infections are often from food or water borne sources including undercooked beef, raw milk, water, and unpasteurized juice.⁵¹ The shiga toxins produced by EHEC are often the source of illness in infected individuals, with symptoms lasting up to a week. Symptomatic individuals may experience fever, abdominal cramping, fatigue, nausea, and diarrhea. PCR methodology is noted to be an effective method for detection of EHEC in infected individuals.⁵²⁻⁵⁴

The ***E. coli* serotype O157:H7** has been implicated in many outbreaks and cases of bloody diarrhea and hemolytic uremic syndrome⁵⁰ and has a high prevalence worldwide.⁵⁴

***Enteroinvasive E. coli/Shigella* (EIEC)** is a pathogenic bacteria known to cause symptoms after ingestion of contaminated food. A highly invasive bacteria, EIEC may cause damage to the intestinal wall. EIEC can lead to dysentery similar to that caused by *Shigella*. Infected individuals may experience symptoms 12 to 72 hours after ingestion of contaminated food. Symptoms include: diarrhea (with blood and/or mucus), vomiting, fever, chills, fatigue, and abdominal cramping. Symptoms are generally self-limiting with no known complications.

Recent research suggests that EIEC and *Shigella* may be the same organism.⁵⁵ These two organisms were grouped together in older enzyme immunoassay tests because it was believed that EIEC and *Shigella* shared an identical toxin or cellular antigenicity. Infections may present with similar symptoms. Diagnostics Solutions Laboratory recognizes this organism as EIEC but has included the *Shigella* name to help provide continuity despite changes to the taxonomy. PCR methodology can distinguish between EIEC and *Shigella*.^{56,57}

Enterotoxigenic E. coli can cause traveler's diarrhea. Heat-labile toxin (LT) and heat-stable toxin (ST) are the enterotoxins responsible for diarrheal disease in humans. ST-producing *E.coli* is widely known to cause diarrhea but the mechanism is still unknown. LT acts similarly to the cholera toxin by activating adenylate cyclase, leading to diarrhea.⁵⁸



Shiga-like Toxin *E.coli* (STEC) has been involved in foodborne illness outbreaks.⁴⁹ It causes various GI illnesses, including bloody and non-bloody diarrhea. Stx1 and Stx2 are generally considered to be the virulent factors responsible for serious illness caused by STEC. Stx1 and stx 2 are genetic targets that help accurately detect the presence of Shiga-like toxin producing *E.coli* in stool samples.⁵⁹

Salmonella is the most common cause of foodborne illness, affecting 1.2 million Americans each year. 19,000 people are hospitalized and 400 people die from *Salmonella* each year in the U.S.⁶¹ It is the largest health burden of all the bacterial pathogens.⁶² *Salmonella enterica* and *Salmonella bongori* make up this genus. There are six subspecies of *S. enterica*. *Salmonella* species are subdivided into serotypes based on surface molecules: O-antigen is present in lipopolysaccharide and H-antigen is the protein found in the flagellar complex.⁶¹

Salmonella species typically cause gastroenteritis with fever, vomiting, and severe diarrhea. It usually resolves within one week. Systemic infections may occur and require antibiotic interventions. A few serotypes, such as *S. Typhi*, cause enteric fever which is characterized by a high fever, abdominal pain, and malaise, without diarrhea or vomiting.⁶¹

Salmonellosis often follows consumption of contaminated food or water. The number of *Salmonella* cells needed to produce disease varies widely, suggesting that even small amounts can initiate illness. As little as 10 cells (in contaminated food) can trigger illness, all the way up to 10⁵ to 10⁶ cells (based on clinical studies).⁶¹

Yersinia enterocolitica is a bacterium belonging to the family Enterobacteriaceae and is known to cause infection in humans, as well as pigs, cattle, and birds.⁶³ Common sources of exposure are contaminated water or undercooked pork, meats, dairy products.⁶⁴ Symptoms usually develop four to seven days after exposure and may include watery or bloody diarrhea and fever, vomiting, and abdominal pain often resembling appendicitis.^{65,66} *Y. enterocolitica* can mimic inflammatory bowel disease, especially Crohn's disease.⁶⁷

Y. enterocolitica are iron-loving bacteria. Therefore, individuals with hereditary hemochromatosis are usually more susceptible to infection with *Yersinia*. Genito-urinary infection with *Y. enterocolitica* have been associated with inflammatory diseases such as reactive arthritis, most likely due to an immune-mediated mechanism.^{22,23} There is some evidence that *Y. enterocolitica* is associated with autoimmune thyroid disorders including Graves's disease and Hashimoto's thyroiditis in genetically susceptible individuals. Higher antibodies to *Y. enterocolitica* are often found in these patients.^{68,69}

Infection is usually self-limiting and does not require treatment. However, for severe infections, pharmaceutical treatment with doxycycline in combination with an

Table 2. Food Sources of *Salmonella*

Poultry and Poultry Products
Meat
Dairy
Raw, Fresh, Ready-to-Eat Produce Such as: <ul style="list-style-type: none"> • Tomatoes • Leafy greens • Sprouts • Berries • Melons



aminoglycoside may be warranted. Additionally, trimethoprim-sulfamethoxazole, chloramphenicol, and rifaximin may also be useful treatments.⁷⁰

Parasitic Pathogens

A parasite is an organism that lives and feeds on a host organism at the expense of the host. Some parasites can cause infectious disease in humans, but others do not. Parasites can live inside the gut, removing vital nutrients, and damaging the gut lining. Some parasitic infections are easily treated, and others are not, with symptoms ranging from mild discomfort to severe problems, including death. It is commonly thought that parasitic infections occur mostly in underdeveloped countries, but these infections also affect people in developed countries including the United States. In fact, such pathogens can survive in their hosts and cause health problems that may be hard to identify. Parasitic pathogens that infect the gastrointestinal tract typically cause a wide variety of symptoms such as diarrhea, constipation, abdominal cramping, bloating, gas, nausea, and vomiting. In immunosuppressed patients, symptoms may involve the central nervous system.

Contaminated food and drinking water present the highest risk for parasite transmission, but lakes, swimming pools, and sexual contact are also ways a person can contract these pathogens. The fecal-oral route is a common way that parasitic pathogens are spread. Therefore, poor hygiene or any conceivable contact with fecal material could result in parasitic infection. Treatments should be specific and based on the type of parasite identified. Efforts should be made to interrupt the parasite's life cycle to prevent reinfection (handwashing, washing fruits and vegetables, avoiding a contaminated water source, treating household members if warranted, etc.). Once symptoms are gone, it is important to retest to make sure the parasite has been eradicated.

Cryptosporidium is notorious for being spread by swimming pools. A number of *Cryptosporidium* outbreaks have occurred after contamination of public swimming facilities. *Cryptosporidium* can cause gas, bloating, diarrhea, and abdominal pain. In a healthy, immune-competent person, this is a self-limiting infection and can be cleared within 2–3 weeks.

Entamoeba histolytica (E. histolytica) is a disease-causing parasite that can affect anyone, although it is more common in those who lived or travelled in tropical areas with poor sanitary conditions. Diagnosis can be difficult since, under a microscope, it looks similar to other parasites such as *Entamoeba dispar* and *Entamoeba hartmanni*. The latter two parasites generally do not cause illness. *E. histolytica* is transmitted via the oral-fecal route or from contaminated food or surfaces. Infected people do not always become sick and symptoms are often mild including stomach cramps and loose stools.

This parasite can infect the liver or spread to other parts of the body including the lungs and brain, although this is not as common. Research has shown that in a small percentage of patients with amebic liver abscess, the infection can cause brain abscess



with the patient presenting with central nervous system symptoms.⁷¹ Treatment for infection with *E. histolytica* includes antiparasitic drug therapy and may include a combination based on the severity of infection.

***Giardia intestinalis* (also known as *Giardia lamblia* or *Giardia duodenalis*)** is the most commonly identified intestinal parasite in the United States and the most commonly isolated protozoan worldwide.⁷² It may be asymptomatic or it can cause chronic diarrhea. It is found in outside water sources such as lakes, streams, and ponds, and it can also get past filtration systems. It is possible for as little as 10 cysts to cause infection. Animals carry *Giardia* and it is common in daycare workers and institutionalized patients. *Giardia* can cause significant symptoms in people with malnutrition, immunosuppression, or cystic fibrosis. Travelers, immunocompromised patients, and certain sexually active homosexual men have high risk for developing giardiasis.

***Giardia* Can Cause:⁷²**

- Diarrhea (90%)
- Fatigue
- Abdominal distention and cramps (70–75%)
- Gas
- Nausea and vomiting
- Foul-smelling, greasy stools
- Steatorrhea
- Anorexia
- Weight loss (66%)
- Neurologic symptoms such as irritability, sleep disorder, depression, neurasthenia
- Urticaria
- Malnutrition
- Growth retardation in children

Giardiasis can mimic celiac disease,⁷³ gall bladder, or peptic ulcer disease. Metronidazole, tinidazole, and nitazoxanide are approved pharmaceutical treatments for giardiasis. Stool ova and parasitology (x3) is the traditional method for diagnosis of *Giardia* infection. PCR can detect *Giardia* in stool samples at levels of 10 parasites per 100 microliters of stool and is able to identify both mild and asymptomatic infections. In one study, a stool PCR test for *Giardia* showed excellent sensitivity and specificity (>98%).



Viral Pathogens

Adenovirus and norovirus are viral causes of gastroenteritis that are normally self-limiting in healthy individuals. When a clinician is looking for a microbial cause of gastroenteritis, they would be remiss to overlook these viruses as possible causes of diarrhea, abdominal pain, and vomiting. In a study of 4,627 patients with gastroenteritis, PCR stool technology detected norovirus in 36% and rotavirus A in 31% of samples.⁷⁴ Another study of over 300 people with acute diarrhea over the course of a year showed 36.0% were positive for norovirus and 17.3% were positive for rotavirus, while 5.4% were positive for adenovirus. In total, viruses accounted for 58.7% of cases of acute gastroenteritis,⁷⁵ pointing to the value of viral detection in stool specimens.

Previous tests with the GI-MAP (unpublished) showed high incidence of viral pathogens and evidence of chronic carriers. This may be related to the persistence and pervasiveness of viruses. Norovirus was detectable for over three years in groundwater and infectious for at least 61 days.⁷⁶ There are no standard treatments for viral gastroenteritis in healthy hosts. Antivirals are not recommended.⁷⁷ Supportive care for the gastric mucosa, hydration, and immune-boosting agents may be warranted.

Adenoviruses 40 and 41 cause gastroenteritis. They are a common cause of diarrhea in infants and children but can also affect adults. These pathogens can replicate readily in the intestine. They are the only adenovirus types that are shown to be causative agents of gastrointestinal disease. However, other adenoviruses may cause gastroenteritis. Fever and watery diarrhea are usually limited to 1–2 weeks. Adenoviruses 40 and 41 may also be present in the stool of asymptomatic carriers and may not require treatment.⁷⁷

Adenoviruses 40 and 41 belong to the larger group of adenoviruses, including 52 different serotypes, known to cause a variety of illnesses from respiratory tract infections (common cold, sore throat, bronchitis, pneumonia) to bladder infection and cystitis. They are hardy viruses that are transmitted through close contact such as touching an infected person or surface, then shaking hands or touching your eyes, nose or mouth. Other routes of transmission include blood, air particles (coughing or sneezing) and the oral-fecal route. Adenoviruses rarely cause severe illness, but infants and those with weakened immune systems have a higher risk of developing a more serious illness from the infection.

Norovirus GI & GII, or Norwalk virus, is the most common cause of non-bacterial gastroenteritis in the world. It is widely known for causing the stomach flu on cruise ships.⁷⁸ Three genotypes of this diverse virus, GI, GII, and GIV, can infect humans. Genotype group II, genotype 4 (GII.4) is the most common and accounts for the majority of outbreaks around the world.⁷⁹ Norovirus, which can have a sudden or gradual onset, typically develops 24–48 hours after contact with an infected person or ingestion of contaminated food or water. Symptoms include nausea and vomiting, diarrhea, abdominal cramps, low-grade fever, muscle aches, fatigue, and headache. Norovirus is generally short-lived, lasting about 24–72 hours but it is highly contagious due to its stability in the environment and resistance to heat, cold, and



disinfectant solutions. It can survive on hard surfaces for weeks and up to 12 days on contaminated fabrics.⁸⁰ Infection affects the microvilli of the small intestine, not the colon. Those infected can shed the virus for up to two weeks after recovery, continuing to spread the virus.

Noroviruses are the most common cause of sporadic diarrhea in community settings and cause up to half of all outbreaks of gastroenteritis.⁸¹ Treatments for norovirus include hydration and electrolytes primarily, and in some cases antiemetics for nausea and vomiting, and analgesics for pain and headache. Intravenous fluid and electrolytes may be needed in extreme cases. PCR is a highly sensitive and specific method for detection of norovirus.⁸²

Helicobacter pylori

H. pylori and eight virulence genes are included on the GI-MAP. *Helicobacter pylori* has been evolving with human beings for well over 50,000 years, since they migrated out of Africa.¹² *H. pylori* colonization has been implicated in a variety of gastroduodenal diseases including gastritis, gastric cancer, and duodenal and peptic ulcer.⁸³ *H. pylori* has also been detected by stool PCR in cases of dyspepsia, abdominal pain, and chronic gastrointestinal symptoms.⁸⁴⁻⁸⁶ It is infamous for its causal link to ulcers and gastric cancer, which resulted in a Nobel prize awarded to Robin Warren and Barry Marshall in 2005. However, some sources are suggesting its role, at least in part, as a commensal organism. *H. pylori* may protect its host from certain atopic disorders,¹⁶ as well as other diseases such as esophageal cancer⁸⁷ reflux, and obesity.¹⁶

Numerous papers suggest the clinical utility of PCR testing for *H. pylori*. Detection of *H. pylori* in biopsy specimens by PCR has proven superior to other methods.^{85,86,88} It has shown sensitivity and specificity reaching that of the diagnostic “gold standard,” which is endoscopy with biopsy and urease test.⁸⁴⁻⁸⁶ *H. pylori* genotyping may be useful for resistant *H. pylori* infections that have failed to respond to triple antibiotic therapy.⁸⁵ In one study of RT-PCR, authors stated it was a “highly accurate noninvasive method to detect *H. pylori* infection in stool and at the same time allows for culture-independent clarithromycin susceptibility testing.”⁸⁵

Population data shows that *H. pylori* virulence varies geographically. It is associated with high rates of cancer in certain regions, but not in others. The difference may lie in *H. pylori*'s genetics.¹² Host immune status and acid secretion seem to be other important factors contributing to *H. pylori*'s colonization and pathogenesis.⁸³ The *H. pylori* virulence factors that are most well recognized are *vacA* and *cagA*.

Fifty percent of the world's population is believed to be infected with *H. pylori* but only 2% of those develop gastric cancer.⁸⁹ *H. pylori* may be asymptomatic and require no treatment or only supportive care to improve the intestinal mucosa and gastrointestinal lining.

Acute *H. pylori* infection results in hypochlorhydria, whereas chronic infection results in either hypo- or hyperchlorhydria, depending upon the anatomic site of infection (within the stomach or duodenum). Most patients chronically infected with *H. pylori*



manifest a pangastritis with reduced acid secretion due to bacterial virulence factors, inflammatory cytokines, and various degrees of gastric atrophy.⁹⁰

Virulence Factors

Positive virulence genes represent the potential for an *H. pylori* strain to create pathology (Table 3). Information about the potential for virulence may help the clinician determine if *H. pylori* treatment is necessary.

BabA (Blood group antigen binding adhesin) is an outer membrane adhesin protein that facilitates binding of *H. pylori* to the gastric mucosa. BabA is thought to play a significant role in inducing inflammation in the gastric mucosa and in promoting long-term infection. Higher expression levels of BabA are associated with severity of inflammation and the development of clinical disease.¹⁰¹

CagA (Cytotoxin-associated protein A) presence in *H. pylori* strains has been significantly associated with gastric cancer and peptic ulcer.¹⁰² The gene codes for a type IV secretion system which allows the bacterium to inject the cagA protein into the host cell. Once inside the host's gastric epithelial cells, cagA can disrupt cell signaling, leading to abnormal proliferation, motility, and changes in the cytoskeleton.¹⁰² These changes to normal cell signaling can initiate cancer.

DupA (Duodenal ulcer-promoting gene A) is strongly linked to an increased risk for developing duodenal ulcers, but not gastric cancer. DupA is thought to be involved in inducing the inflammatory cytokine IL-8, as well as secretion of urease and inhibition of mitochondria-mediated apoptosis. However, the function of the DupA protein has not yet been well-established.¹⁰³

IceA (Induced by contact with epithelium A) has been linked to increased expression of the inflammatory cytokine IL-8, and the development of gastric inflammation, peptic ulcer disease, and gastric cancer, in some studies. However, the function of the IceA protein has not yet been established.^{104,105}

OipA (Outer inflammatory protein A) is an adhesin protein found in the outer cell membrane of *H. pylori*, and functions in adherence of *H. pylori* to gastrointestinal mucosa. OipA contributes to the activity of the CagA virulence factor, and to *H. pylori*'s ability to induce inflammation via IL-8. It is associated with gastric cancer and peptic ulcers.¹⁰⁶

Vacuolating toxin A (vacA) has been associated with gastric cancer, peptic ulcer, and duodenal ulcer.¹⁰² The vacA gene is present in all strains of *H. pylori* but is polymorphic, which leads to different levels of vacuolating toxin. VacA toxins interact with certain receptors on host cells, setting off a chain of events including mitochondrial damage, inhibition of T-lymphocytes, and interference of antigen presentation.¹⁰²

VirB and VirD of the Cag Pathogenicity Island (Cag PAI) This "island" includes two genes: virB and virD. Cag PAI is a section of the *H. pylori* genome that encodes CagA and a Type IV Secretion System, a multiprotein complex that mediates the transfer of



H. pylori virulence factors — including CagA — into gastric epithelial cells. The presence of Cag PAI is associated with highly virulent strains of *H. pylori*.¹⁰⁷

Table 3. *Helicobacter pylori* Virulence Genes, Mechanisms, Interactions, and Disease Associations⁹¹⁻¹⁰⁰

Gene Acronym and Name	Genetic Characteristics	Associations with Disease
BabA Blood group antigen binding adhesin	<ul style="list-style-type: none"> Promotes DNA breakage in host cell Improves <i>H. pylori</i> adherence (“stickiness”) to epithelial cells May promote other virulence factors, especially CagA 	May promote carcinogenesis
CagA Cytotoxin-associated protein A	<ul style="list-style-type: none"> Promotes <i>H. pylori</i> adhesion and colonization Affects barrier function of gastric epithelial tight junctions Promotes loss of cell polarity Antagonizes VacA Evades the immune system and affects the activity of dendritic cells and B-cells Considered part of the “pathogenicity island” which includes VirB and VirD virulence factors. This is a closely-associated group of genes that work synergistically and often transfer as a unit. 	Promotes carcinogenesis, strong association. Also associated with peptic ulcer disease
DupA Duodenal Ulcer-Promoting gene A	<ul style="list-style-type: none"> Promotes Inflammation 	Associated with duodenal ulcers, specifically
IceA Induced by contact with epithelium A	<ul style="list-style-type: none"> Transcription of this gene is only initiated after adhesion to the gastric epithelium Promotes inflammation and associated with elevated IL-8 	Associated with dyspepsia and gastric & duodenal ulcers NOT associated with gastric cancer
OipA Outer inflammatory protein A	<ul style="list-style-type: none"> Promotes Inflammation Drives IL-8 production 	Associated with carcinogenesis and peptic ulcer disease
VacA Vacuolating toxin A	<ul style="list-style-type: none"> Enters the host cell by endocytosis Affects mitochondrial function Disrupts tight junctions Causes a programmed necrosis by inducing the production of large vacuoles inside the host cells; inducing cellular swelling; disrupting cell barrier thus causing nutrient leakage Facilitates nutrient acquisition (iron, minerals, amino acids, etc.) Inhibits antigen presentation in vitro Antagonizes CagA 	Associated with gastric inflammation, peptic ulcer disease, and gastric cancers
VirB & VirD of the Cag pathogenicity island (Cag PAI)	<ul style="list-style-type: none"> Part of the CagA “pathogenicity island” Both genes can potentiate CagA virulence factor by aiding in its transmission to host epithelial cells In the absence of CagA, these virulence factors are unlikely to change clinical outcome of <i>H. pylori</i> infections. 	Evaluate in combination with CagA virulence factors. VirB & VirD, if positive, can potentiate CagA virulence and clinical associations



Commensal/Keystone Bacteria

Trillions of microorganisms inhabit the human intestine to make up a complex ecosystem that plays an important role in human health. The gut microbiota is diverse, varies among individuals, and can change over time, especially during developmental stages and with disease. The predominant phyla of bacteria in the gut are *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*. The fungi that are part of the gut microbiota include *Candida*, *Saccharomyces*, *Aspergillus*, and *Penicillium*.

These commensal (friendly) bacteria coexist with their human host and perform many important functions. They extract nutrients and energy from our diets, maintain gut barrier function, produce vitamins (biotin and vitamin K), and protect against colonization by potential pathogens.³⁷ Research has demonstrated the microbiota's capacity to interact with the immune system as an important health benefit.¹⁰⁸ The microbiota also has anti-inflammatory and antioxidant activity.¹⁰⁹ It is essential that commensal bacteria are diverse and balanced since disruption to the normal balance (or dysbiosis) has been associated with obesity, malnutrition, inflammatory bowel and other autoimmune diseases, neurological disorders, and cancer.¹¹⁰ A limited list of commensal microbiota is included in the GI-MAP test as a general screen for levels of normal, protective microbiota or to monitor probiotic supplementation.

Bacteria within the *Clostridia* class, such as *Faecalibacterium prausnitzii* and *Roseburia* spp., serve important roles in the microbial community of the large intestine.¹¹¹ They are major producers of butyrate and other short-chain fatty acids (SCFA) from non-digestible carbohydrates.^{112,113} SCFA are important regulators of mucosal integrity, immune balance, and pathogen resistance.^{114,115} Butyrate is a health-promoting SCFA that not only serves as an important source of energy for colonic epithelial cells, but also promotes the production of anti-inflammatory regulatory T cells in the intestinal mucosa.

Bacteroides fragilis is a human commensal bacterium that colonizes the lower gastrointestinal tract in mammals. *Bacteroides* species are some of the first microorganisms to colonize the human gut and are present in high numbers. *B. fragilis* is a very common, important, Gram-negative anaerobe yet it accounts for only approximately 0.5% of the *Bacteroides* species found in the gut.¹¹⁶ In its usual role as a commensal gut bacterium, *B. fragilis* has beneficial, immunomodulatory activity. However, if *B. fragilis* enters the bloodstream, as a result of intestinal permeability, trauma or surgery, it can cause serious infections.¹¹⁶

B. fragilis has been the subject of rigorous investigation in recent years because it appears to have a protective effect against inflammation and possibly against autoimmune disorders. *B. fragilis* repairs defects in the gut barrier by influencing tight junction proteins and cytokine expression.⁴⁰ When autistic-like mice were given *Bacteroides fragilis*, it normalized intestinal permeability, restored microbial balance, and removed behavioral and cognitive symptoms.⁴⁰ *B. fragilis* has also been shown to correct gastrointestinal pathology in animal models of colitis⁶³ and inhibit neuroinflammation in mouse models of multiple sclerosis.⁶⁰ Its anti-inflammatory activity is attributed to a surface molecule called polysaccharide A



which promotes regulatory T cells and anti-inflammatory cytokines through toll-like receptor 2 (TLR2) signaling.¹¹⁷

Bifidobacteria spp. and ***Lactobacillus spp.*** are a natural part of the microbiota in the human body. They are often described as beneficial or commensal bacteria. They are given therapeutically as probiotics. These beneficial bacteria promote good digestion, regularity, boost the immune system,¹¹⁸ and help control intestinal pH.¹¹⁹ *Bifidobacteria* and *Lactobacillus* help prevent the overgrowth of *Candida albicans*, *E. coli*, and other pathogenic bacteria.^{39,120} *Bifidobacteria* and *Lactobacillus* species also help to indirectly support butyrate production by producing acetate and lactate, which a number of Clostridia species can use to produce butyrate.¹²¹

Enterococcus spp. are gram-positive, lactate producing, facultative anaerobes that are part of the *Firmicutes* phyla.¹²² *Enterococcus* colonize along the intestinal epithelium below the mucous layer. Residing in this location gives it the ability to increase intestinal mucosal barrier function.¹²³ *Enterococcus* play a key role in preventing foodborne infections with their production of bacteriocins providing inhibitory activity against the following organisms: *Shigella flexneri*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Bacillus subtilis*.¹²⁴ *Enterococcus* species also produce B12 in the intestinal tract.¹²⁵ High levels may be reflective of small intestinal dysbiosis¹²⁶ and achlorhydria (or the lack of stomach acid). *Enterococcus faecalis* can survive in pancreatic juice and/or bile,¹²⁷ be associated with antibiotic resistance,¹²⁸ virulence factors,¹²⁹ and has the potential to be associated with pancreatitis.¹²⁷ For these reasons, it is wise to reference the *Enterococcus faecalis* measurement if elevated commensal *Enterococcus* species are detected on the report.¹²⁷ *Enterococcus* species are considered mucosa-associated microbiota. Therefore, low levels may be indicative of decreased mucosal and/or epithelial health.¹³⁰

Escherichia spp. are gram-negative, facultative anaerobes that are part of the *Proteobacteria* phyla. *Escherichia* species as a group are often thought of as pathogens, however they are typically a regular part of the gut flora.¹³¹ Most strains of *Escherichia* species are non-pathogenic and inhibit pathogenic *E. coli* strains.¹³² *Escherichia* species are among the first bacterial species to colonize the intestine. During a vaginal delivery, the infant is exposed to maternal fecal matter and subsequent handling that contains facultative anaerobic species such as *Escherichia coli*, *Staphylococcus*, and *Streptococcus* that then take residence in the infant's gut. These initial organisms produce an anaerobic environment in the first few days of life that allow strict anaerobes like *Bacteroides* species and *Bifidobacterium* species to then be able to colonize and thrive.¹³³ Commensal *E. coli* can modulate inflammation in the following ways: inducing host immune defense against pathogens, strengthening the intestinal barrier, and directly inhibiting pathogenic *E. coli* strains. Specifically, *E. coli* strain Nissle 1917 (EcN) has supported ulcerative colitis patients in maintaining disease remission, inducing host immune defense against pathogens, strengthening the intestinal barrier, and directly inhibiting pathogenic *E. coli* strains.¹³² In addition, a vegetable-fat-based diet, while not ideal for *E. coli* growth compared to other diets, allows *E. coli* to produce more lactic acid that can inhibit *P. aeruginosa* growth.¹³² When *Escherichia* species are low, experimental animals are more vulnerable to pathogenic *E. coli* and *Pseudomonas* infections. When *Escherichia* species are elevated, this can be indicative



of increased intestinal inflammatory activity, as shown in animal models.^{132,134} Refer to the pathogenic *E. coli* section on page one of the report as well as Escherichia species in the commensal section.¹³⁴

Enterobacter spp. are gram-negative facultative anaerobes that are part of the *Proteobacteria* phyla. *Enterobacter spp.* are natural commensals of the human gut microbiota. There are certain subspecies/species that have been associated with hospital-acquired infections and outbreaks.¹³⁵ Elevated levels may indicate increased intestinal inflammatory activity due to these bacteria containing LPS capsules.¹³⁶

Akkermansia muciniphila is a mucus-degrading bacterium that plays an important role in supporting the gut microbial ecosystem.^{137,234} By breaking down mucus polysaccharides, *A. muciniphila* releases sugars and generates metabolic products that may be used by other community members, such as *Clostridia*, for their own energy needs.¹³⁷⁻¹³⁹ Thus, *A. muciniphila* helps to support the production of important gut microbial products such as butyrate. Reduced levels of *A. muciniphila* have been linked to metabolic dysfunction and obesity. Very high levels in certain cases have been associated with neurodegenerative diseases, such as Parkinson's disease and multiple sclerosis.^{140,141} Causation has not been established, however.

Faecalibacterium prausnitzii is a major butyrate-producing species, that is particularly abundant in the colon.¹³⁷ Reduced levels of *F. prausnitzii* have been found in several intestinal diseases, such as Crohn's disease, ulcerative colitis, and colorectal cancer, as well as in a number of other chronic diseases.^{137,142}

Roseburia spp. – A genus of Gram-positive anaerobic bacteria in the *Clostridia* class that inhabit the human colon. The *Roseburia* genus has five well-characterized species, all of which produce short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. *Roseburia* can also produce butyrate from acetate, promoting balance in energy homeostasis. The genus is widely recognized to influence colonic motility, support immunity, and suppress inflammation. Low levels are associated with several diseases, including irritable bowel syndrome, obesity, Type 2 diabetes, nervous system conditions and allergies.¹⁴³⁻¹⁴⁶

Phyla Microbiota

Gram-negative *Bacteroidetes* and gram-positive *Firmicutes* are bacterial phyla that dominate the entire human digestive tract, including the mouth, nose, throat, and colon.³ Other subdominant phyla are: *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia*.¹⁴⁷ Phyla are a high-level taxonomic rank, above the taxonomic classifications of species, genus, family, order, and class. Because they are heavily represented in the human GI tract, the amounts of *Bacteroidetes* and *Firmicutes* bacteria have been used by scientists to characterize gastrointestinal bacterial composition.

Research over the last twenty years shows that human gut microbiota are involved in energy harvest and storage,¹⁴⁸ lending them the nickname, "fat bugs." Initially, studies showed a characteristically high ratio of *Firmicutes* to *Bacteroidetes* (F/B ratio) in obese



subjects when compared to lean subjects.¹⁴⁹ And when obese subjects lost weight, there was a simultaneous change in the *Firmicutes* to *Bacteroidetes* ratio, favoring that of lean subjects.³⁵ Some authors have challenged those results, suggesting instead that obese subjects have lower microbial diversity.¹⁴⁸ Overall, it seems clear that there is GI microbial imbalance in people with obesity and this could be a modifiable factor for patients with metabolic disorders.

Diet is one of the most powerful modulators of the GI microbiome. A high fat diet is a driver of microbial changes and can increase the F/B ratio. It is difficult to determine if the characteristic obese microbial pattern is caused by obesity or a diet that promotes obesity. Recent findings suggest that it is the diet, and not obesity itself, that leads to imbalanced GI microbial patterns.^{147,148} Patients with a high F/B ratio may benefit from a lower fat diet and probiotics and prebiotics aimed to balance the *Firmicutes* and *Bacteroidetes* phyla. In one study, 30 grams of glutamine taken orally every day for two weeks lowered the F/B ratio.¹⁵⁰

Opportunistic and Overgrowth Microbes

The GI-MAP was designed to detect pathogenic and opportunistic organisms that may be causing symptoms or illness. Many bacteria measured on the GI-MAP are opportunistic pathogens, meaning that they only cause disease and illness in some individuals, particularly the immune-compromised. Many people come into contact with opportunistic pathogens and experience no symptoms, probably because opportunists are suppressed by the balance of commensal bacteria.³⁹ Overgrowth and excessive colonization by opportunistic bacteria may occur when the commensal bacteria are impaired by poor diet, antibiotic use, parasitic infection, or a weakened immune system. Opportunistic pathogens are not recognized by conventional medical authorities to commonly cause illness, and finding measurable quantities in the stool may be considered clinically insignificant.

Yet certain opportunistic pathogens are recognized in the integrative and functional medical field as creating imbalance in the gut microbiota or otherwise preventing proper healing of the GI mucosal barrier. Some of these organisms have been implicated in contributing to extra-intestinal disease. Therefore, testing and treating overgrowth of these organisms may be beneficial in managing some cases of chronic GI illness.

Pseudomonas spp. are gram-negative bacteria found widely in the environment. *Pseudomonas aeruginosa* is the most common species causing infection and can affect every portion of the intestine. In the gastrointestinal tract it can cause inflammation, epithelial barrier dysfunction, tight cell junction interruption, and intestinal permeability.¹⁵¹ This bacterium exhibits enhanced virulence with stress, trauma, surgery, and cancer.¹⁵¹ Symptoms of enteric infection include fever, dehydration, abdominal distention, diarrhea, and physical findings of Shanghai fever.¹⁵² The infection usually affects young children and adults with hematologic malignancies



and neutropenia. Outside the GI tract, it can cause urinary tract infections, dermatitis, bacteremia, bone and joint, respiratory, and systemic infections especially in immune-compromised individuals.

Desulfovibrio spp. is a genus of Gram-negative, sulfate-reducing, anaerobic bacteria containing more than 30 species. These bacteria produce hydrogen sulfide (H_2S), a metabolite which can influence cell signaling and reduce oxidative stress at low concentrations and pose toxicity at higher concentrations.^{153,154-158} *Desulfovibrio* species can be carried in the human gastrointestinal tract asymptotically or they can be opportunistic pathogens. They are found in a variety of sources including dogs, pigs, hamsters, sewage, and water sources.^{153,159}

Desulfovibrio species have been linked to bacterial overgrowth and inflammatory bowel disease, including ulcerative colitis and Crohn's disease.^{153,159-161} Clinically, high levels of H_2S have been associated with colon cancer, ulcerative colitis,^{153,162} and cellular damage to colonocytes. Additionally, it should be noted that *Desulfovibrio* is one of several genera including *Bacteroides*, *Pseudomonas*, *Clostridium*, and *Escherichia* that are known to form biofilms¹⁶² in the intestine.

Low or insufficient levels of *Desulfovibrio* may also have an adverse effect on colon health. *Desulfovibrio* species in the gut have been associated with human health and beneficial microbial populations.¹⁶³ Normal levels of H_2S help inhibit pathogens and support overall antioxidant capacity by promoting healthy mucosa in the colon.

***Methanobacteriaceae* (family)** are methane-producing, bacteria-like microbes that play an important role in the gut ecosystem by facilitating carbohydrate fermentation and production of short-chain fatty acids by commensal bacteria.¹⁶⁴ Elevated levels of *Methanobacteriaceae*, have been linked to chronic constipation, irritable bowel syndrome and obesity, whereas reduced levels have been found in patients with inflammatory bowel disease.¹⁶⁴⁻¹⁶⁶ One study found a strong co-occurrence between *Blastocystis* and *Methanobrevibacter smithii*, the most common member of the *Methanobacteriaceae* family in the human gut microbiome.¹⁶⁷

Autoimmune-Related Gastrointestinal Bacteria

Opportunistic gastrointestinal pathogens are gaining attention for their ability to initiate autoimmune thyroiditis and inflammatory arthritis such as rheumatoid arthritis and ankylosing spondylitis. *Klebsiella spp.*, *Proteus mirabilis*, *Citrobacter spp.*, and *Yersinia* are bacteria that could contribute to inflammatory arthritis in susceptible individuals. *Yersinia enterocolitica* infection has been associated with Hashimoto's thyroiditis and Grave's disease⁶⁹ and higher antibodies to *Yersinia enterocolitica* have been found in these patients.⁶⁸ Enterovirus is also associated with immunogenic thyroiditis.¹⁶⁸ Analysis of gastrointestinal microbes is recommended in chronic autoimmune disorders that don't respond to the usual therapies.



In healthy individuals, opportunistic pathogens should not present a problem. A healthy gastrointestinal barrier, good levels of commensal microbiota, and strong immune defenses in the gut should eliminate the potential pathogen within a few weeks, causing little to no symptoms. However, when the intestinal barrier is breached, normally harmless opportunistic microbes can pass through the barrier, creating extraintestinal infection and illness. Intestinal permeability, or leaky gut, has been documented in a number of autoimmune diseases: ankylosing spondylitis, rheumatoid arthritis, celiac disease, inflammatory bowel disease, IgA nephropathy, nonalcoholic steatohepatitis, and multiple sclerosis.^{170,171} Patients with these conditions or documented intestinal permeability may be at risk if gut microbiota are imbalanced.

Some theories of microbial-initiated autoimmune disease are molecular mimicry, the bystander effect, and the hygiene hypothesis. Molecular mimicry is a common explanation for how a microbial infection can initiate autoimmune disease, presumably due to antibacterial and cross-reactive autoantibodies. It is believed that microbial antigens resemble self-antigens. These cross-reactions essentially “confuse” the immune system which mistakenly mounts an attack against self-tissues. The bystander effect theory proposes that microorganisms damage self-tissues, exposing self-antigens to immune attack. Finally, the hygiene hypothesis presumes that decreased exposure to microbes increases the Th1 response which can lead to autoimmunity.¹⁷⁰

Spondyloarthropathies are a family of chronic, multi-system, inflammatory diseases involving the sacroiliac joints and axial skeleton and they may have an infectious trigger.²¹ They include: ankylosing spondylitis, arthritis associated with ulcerative colitis or Crohn’s disease, psoriatic arthritis, and reactive arthritis. All of these share a genetic predisposition and all are characterized by enthesitis, or inflammation of the sites where ligaments and tendons insert into the bone.²¹ They are usually rheumatoid factor negative and they show an association with human leukocyte antigen B27 (HLA-B27). A prominent hypothesis is that HLA-B27 may resemble or act as a receptor for bacterial antigens, triggering the autoimmune attack on self.²¹

Reactive arthritis can be brought on by genito-urinary infections with *Proteus mirabilis*^{172,173} or gastrointestinal infections with bacterial agents such as *Chlamydia*, *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*,^{22,23} and *Clostridium difficile*. Parasites such as *Strongyloides stercoralis*, *Giardia lamblia*, *Ascaris lumbricoides*, and *Cryptosporidium* species can also result in reactive arthritis.^{174,175} Aggressive cases could evolve into ankylosing spondylitis.²³ Substantial data supports a causative role for *Proteus mirabilis* in rheumatoid arthritis while ankylosing spondylitis and Crohn’s disease have been related to *Klebsiella* microbial infections.²⁴ Evidence of *Salmonella* has been found in cases of ankylosing spondylitis.^{176,177}

Other data shows abnormal serum antibody responses to *Klebsiella* and *Proteus mirabilis* in the spondyloarthropathies,¹⁷⁴ high levels of IgG antibodies to *Klebsiella* in patients with ankylosing spondylitis, Crohn’s disease, and ulcerative colitis, and antibodies to *Proteus* in rheumatoid arthritis.¹⁷¹ While cultures of synovial fluid do not yield gastrointestinal microbes, there is evidence of bacterial antigen and immune responses in the synovium of the joint, suggesting that microbes do play a role in the pathology.¹⁷⁸



Fecal studies have not been used to provide firm evidence of the causative relationship of stool microbes with autoimmune syndromes. However, stool testing for opportunistic pathogens seems a reasonable avenue in chronic, intractable, and painful autoimmune conditions, especially if onset closely followed a gastrointestinal infection.

Systemic sclerosis is an autoimmune disorder that involves significant gastrointestinal pathology. The gut microbiota of patients with systemic sclerosis has been shown to harbor increased levels of opportunistic bacteria such as *Fusobacterium* spp., and *Prevotella* spp., along with decreased levels of beneficial bacteria, including *Faecalibacterium* and *Bacteroides*.¹⁷⁹

***Fusobacterium* spp.** are gram-negative bacteria that are most commonly found in the oral cavity and upper gastrointestinal tract, but may also be present in the lower GI tract, where they have been linked to several diseases related to chronic inflammation, including inflammatory bowel disease and colorectal cancer.¹⁸⁰⁻¹⁸³ *Fusobacterium* spp. are considered normal members of the oral microbiome, but *Fusobacterium nucleatum* is involved in oral diseases such as periodontitis. *Fusobacterium* spp. are thought to play important roles in the formation of multi-species biofilms under normal as well as in pathogenic conditions.¹⁸⁰

***Klebsiella* spp.** are gram-negative bacteria normally found in the intestinal tract that are associated with a wide range of small intestinal disorders including alterations of motility, diarrhea, gas, abdominal pain, and bloating. Its overgrowth in the small intestine can also cause histaminosis and gut inflammation through the release of histamine by the bacteria.¹⁸⁴ Those with a history of long-term antibiotic use are at risk.

***Proteus* spp.** are known to be human opportunistic pathogens. They may be isolated from urine, wounds, and other clinical specimens. The gastrointestinal tract may be a reservoir for *Proteus* species. *Proteus* species in soil or water is an indicator of fecal pollution. *Proteus* may be asymptomatic or cause diarrhea. *Proteus* can cause food poisoning when water or food contaminated with *Proteus* is consumed. Wild and domestic animals can carry *Proteus* bacteria.¹⁸⁵ *Proteus* may produce lipopolysaccharides and increase inflammation.

Proteus mirabilis is the most common cause of *Proteus* infection in humans. It is widely found in soil, water, and environmental habitats such as long-term facilities and hospitals and is often the source of hospital- and nursing home-acquired infections including septicemia, pneumonia, and wound infections.¹⁸⁵ In serious wound infections, *P. mirabilis* can enter the blood stream inducing an inflammatory response that can cause a systemic inflammatory response and sepsis.

As an opportunistic gastrointestinal pathogen, *P. mirabilis* may contribute to inflammatory arthritis in susceptible individuals⁶⁹ and there is substantial data supporting it as a causative role in rheumatoid arthritis.²⁴ Other data shows abnormal serum antibody responses to *P. mirabilis* in the spondyloarthropathies¹⁷⁴ and antibodies to *Proteus* in rheumatoid arthritis.¹⁷¹

As a urease-producing organism, *P. mirabilis* is known to be the cause of some genitourinary infections,¹⁸⁶ specifically impacting the kidneys, bladder, and urethra. Patients



with long-term catheterization are particularly susceptible to urinary tract infections caused by *P. mirabilis*.¹⁸⁷ The most common infection involving *P. mirabilis* occurs when the bacteria moves to the urethra and urinary bladder.¹⁸⁸ In individuals with kidney stones, *P. mirabilis* can cause reinfection in individuals that have been treated with antibiotics.^{186,188} *P. mirabilis* is generally susceptible to broad-spectrum penicillins or cephalosporins, except in severe cases.^{189,190}

Fungal Organisms

Fungal organisms are a part of the normal human digestive tract, but fungal overgrowth can cause illness in susceptible people. Common symptoms associated with fungal overgrowth are gas, bloating, constipation, diarrhea, eczema, and other signs of fungal infection such as athlete's foot, vaginal yeast infections, thrush, and jock itch. Stool testing, using GI-MAP, for fungi such as *Candida*, *Microsporidia*, and *Geotrichum* can often reveal a hidden source of continual fungal growth — the gut. Fungal overgrowth is usually controlled with a diet low in sugars and starches. In some cases, antifungal medications are necessary.

Candida spp. are part of the normal microbiota in the alimentary canal and on mucocutaneous membranes. *Candida* colonizes oropharyngeal sites in 30–55% of healthy young people and can be found in normal stool specimens.^{191,192} However, *Candida* is also the most pathogenic fungal threat to humans, especially in immunocompromised patients. Three-fourths of women will have vulvovaginal candidiasis at least once in their lives. It is the fourth most common pathogen found in blood cultures of patients with systemic infections. Some sources say *Candida* can cause diarrhea which resolves with nystatin treatment.¹⁹¹

Fungal dysbiosis or “*Candida sensitivity*” has been suggested to cause a cluster of symptoms for which no etiology has been established, including: gastrointestinal complaints, fatigue, lethargy, skin rashes, urinary frequency, muscle or joint pain, abdominal pain, diarrhea, constipation, flatulence, allergies, and vaginitis. High quality evidence to support this hypothesis is lacking. However, studies that identify patients at high risk of having fungal overgrowth (past history of antibiotic use), respond dramatically to a low-sugar, low-starch diet and antifungal medications.¹⁹³ Dr. Carol Jessop reported a dramatic improvement in 1,100 patients with chronic fatigue syndrome after giving oral nystatin and a special diet (low sugar, alcohol, fruit, fruit juice) for 3–12 months.^{193,194}

Conventional medicine does not recognize the existence of a subclinical *Candida* overgrowth condition that causes chronic symptoms. Endoscopy with or without biopsy is necessary to establish the diagnosis of gastrointestinal candidiasis.¹⁹¹ Blood culture is used to detect disseminated candidiasis but it can miss 40–50% of cases.¹⁹¹

Because the condition of *Candida* overgrowth is so poorly understood, there are no diagnostic tools for it. Stool *Candida* and urine D-arabinitol can be used together to investigate *Candida* overgrowth in the colon and the small intestine, respectively.



Candida antibodies may be used to determine abnormal immunological responses to *Candida*.

Microsporidium spp. were first identified as parasites of the silkworm but are now recognized as fungi. They are often difficult to diagnose but significant progress has been made with molecular diagnostics for detection of these organisms.¹⁹⁵ The GI-MAP specifically detects *Encephalitozoon intestinalis*, the microsporidia known to affect the gastrointestinal tract. These opportunistic pathogens often infect immunosuppressed individuals such as those with HIV infection, organ transplantation, or chemotherapy, but can also infect healthy people. Common symptoms include diarrhea and wasting due to enteric infection.¹⁹⁵ *E. intestinalis* can disseminate to ocular, genitourinary, and respiratory tracts. Treatment often includes antifungal medications along with diet and nutritional interventions to help with chronic diarrhea.

Rhodotorula spp. are fungi commonly found in the environment and in various sources including soil, plants, bathrooms, and liquids (milk, water, juice).¹⁹⁶ *Rhodotorula spp.* may be a commensal fungus in most individuals. *Rhodotorula spp.* is often found in patients who are immunosuppressed or are under treatment that requires the use of central venous catheters.¹⁹⁷

Viruses

Cytomegalovirus (CMV) is a herpes virus that has affected 60% of the US population.¹⁹⁸ Almost one in 3 children have CMV by 5 years old and half of all adults have been infected with CMV by 40 years of age.¹⁹⁹ It is transmitted by direct contact with infectious body fluids such as urine or saliva. It can be passed around among children in daycares and by childcare workers.

Primary CMV infection may cause no symptoms or mild flu-like symptoms, usually 9–60 days after infection. Enlarged lymph nodes and spleen may be detected. Extreme fatigue may persist after laboratory values are normal. Patients with clinical mononucleosis or fever of unknown origin should be tested for CMV. Immunoglobulin tests can help to diagnose a primary infection. In immunocompetent patients, CMV can cause severe community-acquired viral pneumonia, transaminitis, splenomegaly, colitis, encephalitis, cytopenias, and fever of unknown origin. CMV is more common in immunocompromised patients than in immunocompetent patients.²⁰⁰

The virus can remain dormant in the body and reactivate later in life. How and why and the time course for viral reactivation is unknown but it usually occurs when the patient has other infections or is under high stress. When a person is infected with CMV, its DNA can be detected by PCR in all different cell types and organ systems of the body.¹⁹⁸

A positive finding for CMV in stool on the GI-MAP indicates active CMV infection, not past infection. No treatment is recommended for asymptomatic CMV. In patients with compromised immune systems and life-threatening illnesses due to CMV, antiviral treatment may be indicated. Patients can prevent spreading CMV with regular handwashing, especially when in contact with young children.¹⁹⁹



CMV and Gastrointestinal Disease – RT-PCR detection of CMV in fecal specimens correlates with plasma CMV levels and can aid in the diagnosis of cytomegalovirus-related gastrointestinal disease.²⁰¹ High levels of CMV DNA were detected in inflammatory bowel disease (IBD) patients, in both those who were newly diagnosed as well as those who were already taking immunosuppressive medications. The prevalence of CMV in IBD patients suggests that it is not only a consequence of immunosuppressive therapy but that it may play more of a role in IBD pathophysiology than previously believed.²⁰² The frequency of CMV infection in IBD patients was 10–36% and may contribute to colitis symptomology.²⁰³ In a Japanese population, CMV infection (detected by stool PCR) was common in ulcerative colitis (UC) patients and even more so in UC patients who had active disease and were on immunosuppressive therapy. One study showed that 12.3% of IBD patients had CMV infection.²⁰⁰ CMV can cause colitis even in immunocompetent hosts. CMV colitis may be indistinguishable from *C. difficile* (abdominal pain and watery or bloody diarrhea), except it will be resistant to *C. difficile* treatment. CMV can coexist with *Clostridium difficile* infection and can be detected by stool qPCR.²⁰⁴

Intestinal nematodes infect one-fourth to one-third of the world's population.²

CMV and Autoimmunity – CMV has been implicated in the development of autoimmune diseases: systemic lupus erythematosus, systemic sclerosis, diabetes mellitus type 1, and rheumatoid arthritis. In some autoimmune conditions, such as lupus and systemic sclerosis, patients have far higher antibodies against CMV than healthy controls. The high prevalence of CMV throughout the world's population (40–99%) makes it difficult to definitively prove a link between CMV and autoimmune conditions.^{205,206} The GI-MAP stool test detects active Epstein-Barr Virus (EBV) infections, not past infections. EBV can cause infectious mononucleosis (mono) and it can affect the brain, spinal cord, and nerves. EBV can affect the blood and bone marrow, leading to lymphocytosis. Symptoms include:

- Fatigue
- Fever
- Swollen lymph nodes (neck)
- Inflamed throat
 - » Enlarged spleen
 - » Rash
- Swollen liver

EBV can be difficult to diagnose. EBV is commonly contracted in childhood, but symptoms are mild and may be indistinguishable from other typical childhood illnesses. Adolescents and adults who contract EBV may experience symptoms for two to four weeks. Some people may feel fatigued for weeks or months. After primary infection, EBV remains in the body in an inactive state. It can reactivate and produce symptoms in people with weakened immune systems. EBV is more common in 206 immunocompromised patients than in immunocompetent patients.²⁰⁰



If the virus reactivates, it is contagious and can be spread to others. EBV is spread through bodily fluids especially saliva. There is no cure for EBV. Treatments include rest, hydration, and treatments for the symptoms of fever and pain. It can also be treated with antiviral medications and supplements.

Cordyceps may help to suppress the virus²⁰⁷ and vitamin D may help prevent autoimmune sequelae of EBV.²⁰⁸ Prevent transmission of EBV by washing hands, and avoiding people who have EBV infection, especially avoiding contact with saliva such as kissing, sharing drinks or food or toothbrushes.²⁰⁶

EBV blood antibodies are used to diagnose an EBV infection.²⁰⁶ A primary EBV infection is often characterized by anti-viral capsid antigen (VCA) IgM, or high anti-VCA IgG antibodies, without antibodies to EBV nuclear antigen (EBNA). IgG antibodies to the EBV early diffuse antigen can also indicate current or recent infection.²⁰⁹ Past infections are usually characterized by antibodies to both VCA and EBNA and may be elevated years after the primary infection.²⁰⁶

EBV and Autoimmunity – Primary infection with EBV causes mononucleosis, Burkitt's lymphoma, gastric cancer, nasopharyngeal carcinoma, and autoimmune diseases.⁸⁹ EBV has a central role in the pathogenesis of systemic autoimmune diseases, specifically rheumatoid arthritis, systemic lupus erythematosus, and Sjogren's syndrome.²¹⁰ EBV has been suggested to increase the risk of developing multiple sclerosis, an autoimmune condition of the central nervous system that eventually destroys the myelin sheaths of neurons.²¹¹ Other researchers suggest that EBV is a contributory factor in autoimmune thyroid disorders.²¹²

EBV and Gastrointestinal Illness – EBV increases the risk of gastric cancer because the virus invades epithelial cells. EBV coinfection with *H. pylori* may contribute to inflammation and the development of gastric cancer.⁸⁹ The frequency of EBV infection in IBD patients ranges from 30–64%.^{200,203} EBV may cause colitis in addition to the preexisting IBD. Areas of more severe mucosal damage in IBD patients corresponded with higher viral loads. Authors recommended diagnosing EBV and CMV in patients with IBD through qPCR analysis of mucosal biopsies.²⁰³



Parasites (Non-Pathogens)

Non-pathogenic parasites are present in the gastrointestinal tract and generally are self-limiting and do not cause illness. However, some research shows an association between non-pathogenic parasites and gastrointestinal symptoms.²¹³ Therefore, testing of these microorganisms may be useful in some cases. Recent research shows certain parasites, such as *Blastocystis hominis*, as an emerging potential pathogen.²¹⁴

Blastocystis hominis is found throughout the world in both people with and without symptoms. Common signs of infection with *Blastocystis* include diarrhea or watery stools, abdominal pain, anal itching, constipation, excess gas, and dermatologic issues. Some research recommends treatment for people with gastrointestinal and dermatologic symptoms but no treatment for those who are asymptomatic.²¹⁵ There may also be an association between *Blastocystis* and chronic digestive disorders, such as irritable bowel syndrome.²¹⁶

Chilomastix mesnelli is considered non-pathogenic and may not require treatment. However, there have been several cases associating it to diarrhea. In stool, it is a sign of exposure to fecal material. Chronic infection could have an effect on the immune response. Long term, it could create a dysbiotic environment, allowing for a secondary infection, with possibly a more opportunistic protozoa. Treatment may be considered for that reason in certain cases.

Cyclospora cayetanensis is a parasitic protozoan commonly associated with water- and food-borne outbreaks, often causing traveler's diarrhea in infected hosts via oral-fecal transmission of sporulated oocyst in its infectious stage. Travel to tropical regions and imported fresh produce²¹⁷ from tropical regions contaminated with feces have been known to be sources for outbreaks of cyclosporiasis.²¹⁷ The thick bilayered wall of *Cyclospora* oocyst allows the organism to survive in harsh environments such as the acidic conditions of the stomach and water treatment^{218,219} such as chlorination. Cyclosporiasis is characterized by symptoms of prolonged watery diarrhea, intestinal distress, abdominal cramping, loss of appetite, weight loss, nausea, and vomiting.^{220,221} Individuals may also experience flu-like symptoms such as headaches and a low fever. Infection is usually self-limiting, with symptoms typically lasting approximately seven days.^{220,222} In more persistent cases lasting more than seven days treatment with an antibiotic combination of trimethoprim and sulfamethoxazole,²²³ may be necessary.

Dientamoeba fragilis (D. fragilis) is found in the gastrointestinal tract of humans and pigs and is frequently associated with traveler's diarrhea. Found worldwide with high prevalence in developed countries, fecal-oral transmission via food and water is the most common source of exposure to *D. fragilis*. While generally considered non-pathogenic, in the presence of other parasites, and in the immunocompromised, symptoms may be more prevalent in infected individuals.²²⁴ *D. fragilis* infection may present with abdominal pain, diarrhea, irritable bowel syndrome, or eosinophilic colitis.²²⁵ *D. fragilis* and *Enterobius vermicularis* (pinworm) are often detected together²²⁶ in stool samples and may cause symptoms of diarrhea, abdominal pain, and fatigue. Treatments for *D. fragilis* may include paromomycin or metronidazole; studies have found the former to be more effective against *D. fragilis* alone.²²⁵ Co-infection with



pinworm may warrant a longer, or different, course of treatment.²²⁶ Ensuring proper sanitation practices, such as hand washing before food preparation and after using the restroom, help to minimize exposure to *D. fragilis*.

Entamoeba coli is an amoeba found in the large intestine. Generally, it is not considered pathogenic. However, when it is found in stool samples it can indicate the presence of other potentially pathogenic organisms.

Pentatrichomonas hominis (formerly Trichomonas hominis) is a protozoan found in the cecum²²⁷ of the large intestine and generally considered to be non-pathogenic with most cases being asymptomatic. *P. hominis* is found in less than 2% of the population and is transmitted by fecal-oral contamination. *P. hominis* can be a sign of other dysbiosis, pathogenic infections, or weak digestion.²²⁸ It is more common in children than adults, likely due to poor hygiene.²²⁹ It also colonizes the gastrointestinal tract of many mammalian hosts and therefore it is possible to have zoonotic transmission, particularly from dogs or cats.²²⁹

It generally does not cause symptoms. However, in rare cases, it can cause abdominal pain and diarrhea and is a potential cause of pediatric diarrhea.²²⁹ Since it is a non-pathogen, treatment may not be needed. When present, address other imbalances found on the GI-MAP. Treatment with antiparasitic herbs may be considered, especially in cases where this is the only dysbiotic organism and symptoms are present. There are cases in the literature that report using metronidazole for *P. hominis*.



Worms

Worms tested on the GI-MAP will be labeled, “Detected,” or “Not Detected.” A “Detected” finding means that a significant quantity of DNA was present and consistent with a worm infection. It may include a multicellular adult worm (a few billion cells) or a large volume of eggs (likely generated by an adult worm). A “Not Detected” result means that no DNA was found, or it was below the threshold. Very low levels of worm DNA (in the form of worm eggs) can be present in the GI tract due to background levels in food or water but would not be considered clinically relevant.

Ancylostoma duodenale* and *Necatur americanus are roundworms commonly known to cause hookworm infection by penetrating the skin.²³⁰ Human infection with *A. duodenale* or *N. americanus* is believed to affect 439 million people around the world. *A. duodenale* is prevalent in southern Europe, northern Africa, India, Asia, the Caribbean islands, South America, and small areas of United States.²³¹ Hookworm infected 12–15% of schoolchildren in the southeastern U.S. in the 1970s. *N. americanus* may still be found in pockets of the southeastern U.S.²³² Hookworm infection is associated with poverty, poor sanitation, inadequate housing construction, and lack of access to medications.²³²

Hookworm infection may cause no symptoms. Early symptoms of hookworm infection are itching and a localized rash where the larvae penetrated the skin. Heavy infections may present with abdominal pain, diarrhea, fatigue, weight loss, anemia, and loss of appetite.^{233,234} Hookworm infection may affect physical and cognitive growth of children.²³² Hookworm is contracted via skin contact with soil that has been contaminated with larvae. Walking barefoot on soil or ingesting soil that may be contaminated with human feces could introduce hookworm into the human body. *A. duodenale* also lives in the small intestine of hosts such as cats and dogs;²³⁵ therefore pets may also be a source of exposure. In cases of heavy hookworm infection, symptomatic individuals can be treated with albendazole or mebendazole.²³⁶ Individuals presenting with anemia may benefit from iron supplements.²³⁷

Ascaris lumbricoides is one of the most common intestinal roundworms. Hosts may be asymptomatic or they may present with pulmonary or even severe GI symptoms. Four million people in the United States are thought to be infected with *Ascaris*. International travelers and recent immigrants (especially from Latin America and Asia) are at high risk of acquiring *Ascaris*. It is indigenous to the rural southeastern United States. *Ascariasis* can cause intestinal and biliary tract obstruction and may lead to abdominal surgical emergencies. Symptoms of ascariasis relate to larvae migrating through the lungs: fever, cough, wheezing, and dyspnea. In the later phase of infection, *Ascaris* causes gastrointestinal symptoms such as diffuse or epigastric abdominal pain, nausea, vomiting, frequent throat clearing, dry cough, “tingling throat,” appendicitis, pancreatitis, and obstruction.² In the early phase, eosinophils may be high in blood, but stool ova and parasitology will likely be negative. PCR tests are available to identify helminth infections. Albendazole and mebendazole are commonly used to treat symptomatic and asymptomatic infections.²



***Trichuris trichiura* (whipworm)** is known to cause mild to moderate symptoms in individuals via fecal-oral transmission of contaminated produce or person-to-person contact.²³⁸ *T. trichiura* is prevalent in Asia, Africa, South America, and rural southeastern United States. Individuals exposed to *T. trichiura* are usually asymptomatic, however some individuals may experience painful diarrhea with mucus, and blood.²³⁸ In cases of heavy infections, symptomatic individuals can be treated with albendazole and mebendazole.²³⁶ Individuals presenting with anemia may benefit from iron supplements.²³⁹

Taenia spp. (tapeworm) may be found in stool after ingestion of contaminated or undercooked pork (*Taenia solium*) or beef (*Taenia saginata*).^{240,241} *T. solium* is found worldwide and is most prevalent in poorer communities where humans live in close contact with pigs and eat undercooked pork. *T. saginata* is prevalent in Africa, parts of Eastern Europe, the Philippines, and Latin America where raw beef is often eaten or where individuals live in close contact with cattle.^{240,242,243} Humans are the only definitive host for both *T. solium* and *T. saginata*.²⁴⁴ PCR methods are sensitive and specific for detecting *Taenia* species in stool.^{243,245}

Infection usually involves just a single tapeworm after ingestion of undercooked pork or beef from infected animals that have ingested eggs or tapeworm segments.²⁴⁵ Individuals with taeniasis are usually asymptomatic or have mild symptoms.²⁴⁶ Passage of pieces of tapeworm can cause discomfort.

Taeniasis symptoms include: abdominal pain, nausea, weakness, increased appetite, loss of appetite, headache, constipation, dizziness, diarrhea, pruritus ani, hyperexcitability, and anemia.^{234,246} Adult worms can be eliminated with albendazole or praziquantel.^{247,248}

Intestinal Health

Beyond the microbial communities of the GI tract, the GI-MAP looks at the foundational health of the GI, including immune function, inflammation, digestion, gliadin (gluten) reactivity, intestinal permeability, and metabolic activity of the gastrointestinal biome. Diagnostic Solutions Laboratory uses clinically validated ELISA assays to accurately quantify these markers in human stool samples: pancreatic elastase-1, secretory IgA (sIgA), anti-gliadin sIgA, eosinophil activation protein (EDN), calprotectin, and zonulin-1.

Enzyme-linked immunosorbent assays (ELISAs) are commonly used in clinical laboratories to detect and quantify a wide range of biomarkers in human specimens. There are three basic components to the ELISA “sandwich” assay: the capture molecule, the target molecule (from the human specimen), and a detector molecule. The capture molecule specifically binds to the target molecule and holds it in the reaction while all other molecules present in the sample are removed by washing. The capture molecule and target molecule are then bound to the detector molecule, creating the “sandwich” that holds the target molecule in the center. The detector molecule is tagged with a substrate that, when exposed to a specific enzyme, emits



a signal that can be measured quantitatively. The signal may be a color precipitate, fluorescence, or luminescence (light) and it is directly proportional to the amount of target molecule present in the clinical sample. For example, the more light emitted, the more of the target substance is present in the human sample.

Diagnostic Solutions Laboratory uses chemiluminescent ELISAs, which means that the enzyme attached to the detector molecule produces a light-emitting signal. There are several advantages of chemiluminescent ELISAs compared to other ELISA assays. Chemiluminescent ELISAs have an increased analytic range, which allows DSL to accurately quantify analytes present in a clinical sample at both higher and lower concentrations compared to non-chemiluminescent assays. In addition, chemiluminescent ELISAs can also more specifically detect the target molecule with less variation compared to other ELISA assays.

Steatocrit has been used widely since 1981 to detect steatorrhea in patients with pancreatic insufficiency and small intestinal malabsorption.²⁴⁹ It is a simple test that uses centrifugation to separate the solid, aqueous, and lipid layers of the stool. The lipid layer is measured in the steatocrit and this makes up the total fecal fat.²⁴⁹ The acid steatocrit method has been shown to correlate well with 24-hour and 72-hour fecal fats.^{250,251}

Fecal Pancreatic Elastase-1 is an accurate functional screening marker for pancreatic exocrine insufficiency. Pancreatic elastase is an enzyme produced by the pancreas to help break down proteins. Pancreatic insufficiency occurs when the pancreas is not working well and becomes inflamed (pancreatitis). Pancreatic insufficiency presents with symptoms of steatorrhea, abdominal pain, and malabsorption because it interferes with the body's ability to absorb nutrients from food, including fat-soluble vitamins.^{252,253}

This test also accurately predicts a patient's response to pancreatic enzyme supplementation, especially in patients with unexplained diarrhea and suspected pancreatic insufficiency.²⁵⁴ In patients with pancreatic insufficiency, 80% responded favorably to supplementation with pancreatic enzyme therapy; with an average dose of 120,000 units of lipase.²⁵⁵

Table 4. Staging of Pancreatic Insufficiency Based on Fecal Elastase-1

Fecal Elastase-1 Result	Clinical Significance
> 200 µg/g	• Normal pancreatic function
100–200 µg/g	• Mild pancreatic insufficiency
< 100 µg/g	• Moderate to severe pancreatic insufficiency

Low elastase-1 may suggest an underlying hypochlorhydria or it may be seen in small intestinal bacterial overgrowth (SIBO). Vegetarians have lower fecal elastase-1 than meat-eaters.²⁵⁶ Stress or vagal nerve dysfunction may interfere with healthy pancreatic function. Liquid stools can dilute elastase-1 levels.²⁵⁷ Consider elastase-1 levels in patients with symptomatic *H. pylori* infection or *Giardia intestinalis*. The fecal pancreatic



elastase-1 test may also be useful for monitoring diabetics because both insulin and non-insulin-dependent diabetes can impair pancreatic function.²⁵⁸

Causes of Exocrine Pancreatic Insufficiency²⁵⁴

Pancreatic Disorders

- Chronic pancreatitis (alcoholism)
- Acute pancreatitis
- Autoimmune pancreatitis
- Pancreatic cancer
- Benign pancreatic neoplasms
- Cystic fibrosis
- Shwachman-Diamond syndrome

Extrapancreatic Disorders

- Type I diabetes
- Type II diabetes
- Inflammatory bowel disease
- Celiac disease
- Pediatric intestinal transplantation
- HIV syndrome
- Gastrointestinal surgery
- Sjogren's syndrome
- Aging
- Tobacco usage
- Somatostatin analogs therapy

Beta-glucuronidase (β -glucuronidase) is an enzyme produced by cells in the liver, kidney, intestinal epithelium, endocrine, and reproductive organs.²⁵⁹ However, the major producers of beta-glucuronidase are these bacteria: *Bacteroides fragilis*, *Bacteroides vulgatus*, *Bacteroides uniformis*, *Clostridium paraputrificum*, *Clostridium clostridioforme*, *Clostridium perfringens*, *Escherichia coli*, *Eubacterium*, *Peptostreptococcus*, *Ruminococcus*, and *Staphylococcus*. It is found in 97% of *E.coli* strains.²⁶⁰ The enzyme hydrolyzes B-glucuronide to make glucuronic acid and an aglycone, such as imine, thiol, or alcohol. Glucuronidation by way of beta-glucuronidase is a major route of detoxification in the human body.²⁶⁰ However, this enzyme can also convert pro-carcinogens to carcinogenic compounds.²⁵⁹

High levels of fecal beta-glucuronidase can indicate unfavorable changes in the colon. When the enzyme is elevated in plasma, there is an increased risk of hormone-sensitive cancers, such as those of the breast or prostate.²⁵⁹ Evidence of increased enzymatic activity of intestinal microorganisms may suggest increased risk of digestive tract cancer.²⁶¹ Toxins stimulate beta-glucuronidase activity and dietary red meat and protein increases the enzyme. Antibiotics increase beta-glucuronidase levels. A low-calorie, vegetarian diet can reduce fecal beta-glucuronidase levels.²⁵⁹

Occult Blood – FIT (Fecal Immunochemical Test) is a newer occult blood screening method for colorectal cancer. It has replaced the fecal occult blood tests (FOBT) of the past due to increased specificity, sensitivity, and decreased costs.²⁶² FIT testing allows quantitative measurement of fecal hemoglobin concentration. It is more sensitive for detecting adenomas and colorectal cancer, especially at lower positivity thresholds. Most international guidelines call for colorectal cancer screening between ages 50 and 75 years old in average risk individuals. When used correctly, fecal occult blood tests can decrease morbidity and mortality from colorectal cancer. In one study of nearly



140,000 subjects, repeat FIT testing detected more colorectal cancer and advanced adenomas than sigmoidoscopy,²⁶³ in part due to better participation.

Secretory Immunoglobulin A (sIgA) is an antibody protein secreted into the gastrointestinal tract as a first line of immune defense against pathogenic microorganisms.²⁶⁴ This immunoglobulin influences the gut microbiome²⁶⁴ and helps to maintain barrier function²⁶⁵ by forming complexes with gut pathogens and allergens, preventing them from penetrating the intestinal barrier. Impairment of sIgA may increase the risk of infectious, allergic, and inflammatory diseases of the intestine.²⁶⁶ Chronic stress may also disrupt levels of sIgA. Elevated levels of sIgA may indicate an activated immune response to chronic infections or inflammatory reactions.

The presence of **fecal anti-gliadin antibodies** can indicate an immune response to gluten in the diet. Gliadin is a component of gluten, the protein found in wheat and other field grass grains such as barley, malt and rye. Because gliadin could stimulate intestinal immunity and increase levels of fecal anti-gliadin antibody long before serum concentrations of anti-gliadin antibody are detectable,^{267,268} it is sometimes used as an early indicator of gluten reactivity. High levels of fecal anti-gliadin antibodies can provide clinicians with an effective treatment strategy: a gluten-free diet.

Eosinophil Activation Protein – EDN, EPX (eosinophil protein X) is a protein released by activated eosinophils in the lumen of the intestines, which can be detected in feces. It has strong cytotoxic characteristics. The protein plays a significant role in a variety of inflammatory and mast-cell mediated pathologies. It helps to fight pathogens, particularly viral infections.

The accumulation of eosinophil activation protein in the intestine is associated with inflammation and tissue damage, and the level of eosinophil activation protein in the stool can serve as an objective measure for chronic inflammation in the GI tract. In the case of inflammatory bowel disease, the marker can be used to evaluate disease activity and predict relapse.²⁶⁹ Eosinophil activation protein can also be used to determine the effectiveness of a food elimination diet to control symptoms or disease progression.

Possible Causes of Elevated Eosinophil Activation Protein:²⁷⁰⁻²⁷²

- Respiratory allergies
- Asthma
- Food allergies and sensitivities
- Inflammatory Bowel Disease (IBD)
- Irritable Bowel Syndrome (IBS)
- Eosinophilic esophagitis (EE)
- Functional dyspepsia
- Acid reflux
- Intestinal barrier damage/dysfunction
- Anxiety (IBS-related anxiety)
- Intestinal parasites

Fecal calprotectin is the most studied marker of gastrointestinal inflammation²⁷³ and the gold standard marker for the diagnosis and monitoring of inflammatory bowel disease (IBD).²⁷⁴ It is used to discriminate IBD from irritable bowel syndrome (IBS).^{273,274} Calprotectin is a calcium-binding protein that is found at high concentration in





neutrophils. Calprotectin is also found in monocytes, macrophages, and gut epithelial cells.²⁷⁵ In IBD, there is a migration of inflammatory cells such as neutrophils to the inflamed intestinal mucosa. Because leukocytes are shed into the intestinal lumen, pro-inflammatory proteins such as calprotectin can be identified and measured in stool specimens.²⁷⁶ Fecal calprotectin levels are proportional to the level of neutrophil infiltration and inflammation in the gut.²⁷⁵

Calprotectin has been shown to correlate with histologic and endoscopic measures of inflammatory bowel disease severity.²⁷⁶ It is non-invasive, stable,²⁷⁴ and shows a considerable sensitivity and specificity of 93% and 96%, respectively, when used to screen for IBD activity.²⁷⁷ High calprotectin can also be detected in colorectal cancers, diverticular disease, and infectious gastroenteritis.²⁷³

Fecal calprotectin can elevate with enteropathy caused by excessive non-steroidal anti-inflammatory medication use.²⁷⁵ For this reason, it may be beneficial to temporarily discontinue NSAIDs, when possible in select patients, prior to measuring fecal calprotectin.²⁷⁵

Calprotectin Reference Ranges – While the utility of calprotectin as a standalone fecal biomarker for the diagnosis of IBD is well established, the specific concentration of fecal calprotectin that is diagnostic of IBD has not been widely agreed upon. This ongoing debate is complicated by the variety of tests commercially available. In addition, the utility of fecal calprotectin to differentiate healthy patients from non-IBD patients has not been well established.

Commercially available fecal calprotectin ELISA kits historically suggest a 50 µg/g cut-off value for differentiation of IBD from non-IBD patients. Unfortunately, utilization of this “standard” value is not exceptionally helpful as it does not efficiently diagnose IBD for all patients and not across all available ELISA kits. While healthy individuals usually maintain fecal calprotectin levels less than 50 µg/g, a meta-analysis indicated that a 100 µg/g cut-off value was a better predictive value compared to the 50 µg/g cut-off value.²⁷⁸ Other studies have suggested using even higher calprotectin ranges of 400 to 1000 µg/g for monitoring active IBD, while calprotectin levels >600 µg/g are strongly associated with IBD.²⁷⁹⁻²⁸²

While clinically relevant results (normal or elevated) should be comparable across different laboratories, it would be ideal to test and repeat test with the same laboratory, using the same testing platform, when monitoring calprotectin levels over time. A cross comparison of calprotectin assays indicated acceptable diagnostic accuracy of each testing platform but lacked absolute value agreement between platform results. This lack of absolute value consistency can be due to a variety of factors, including use of monoclonal vs. polyclonal antibodies, use of different substrates, concentration of capture and detection molecules used, variation in the calibrators used, as well as pre-analytic processing protocols (sample homogenization and calprotectin extraction).²⁷⁸ Given the variation in testing platforms, lack of standardization, and diverse patient populations, laboratories must utilize both laboratory-specific validation data and the available scientific literature to establish a clinically relevant reference range for their fecal calprotectin assay.



Add-On Tests

Optional Add-On Tests That Can Be Ordered With the GI-MAP

Zonulin

Zonulin is a protein secreted by intestinal cells that regulates intercellular tight junctions.^{1,283} Tight junctions are the connections between epithelial cells that make up the gastrointestinal lining. Zonulin increases intestinal permeability in the jejunum and ileum²⁸⁴ and is considered a biomarker for barrier permeability.^{1,283} Tight junctions can be opened or closed, depending on the physiological need. Zonulin's role is to open tight junctions in the gut. In the case of enteric infections, high zonulin can "open the floodgates" and flush out bacteria and toxins.¹ Certain gut bacteria and gliadin (the main staple protein from wheat) can activate the zonulin system.^{283,285}

The intestinal barrier is a critical interface between the lumen of the gut and the internal milieu. Dysfunction of this barrier is believed to initiate immune dysfunction because it allows macromolecules from the gut lumen to pass into the bloodstream.²⁸⁶ Intestinal permeability, also known as "leaky gut," has been associated with inflammatory bowel disease, celiac disease, food allergy, irritable bowel syndrome, critical illness, autoimmune diseases,²⁸⁷ and obesity and metabolic disease.²⁸⁸ In many cases, permeability precedes disease.¹

Zonulin regulates barrier permeability. Serum zonulin correlates with intestinal permeability and lactulose/mannitol tests for intestinal permeability.^{284,289} High serum zonulin has been associated with celiac disease, type 1 diabetes,²⁸⁹ insulin resistance and type 2 diabetes,²⁸⁴ cancers, neurological conditions, and autoimmune diseases.¹

Fecal zonulin is available for investigational use but has not been correlated with circulating (serum) levels as of this writing. Serum zonulin may constitute zonulin secretion not only from intestinal cells, but also from extraintestinal tissues such as the liver, heart and brain.²⁹⁰ Stool may therefore present an appropriate specimen for analyzing only intestinal production of zonulin. Fecal zonulin has been used in human studies as a marker of intestinal permeability. In athletes, fecal zonulin levels improved (decreased) after 14 weeks of probiotic supplementation.²⁸³ Treatment with zeolite lowered stool levels of zonulin in athletes and presumably improved intestinal barrier function.²⁹¹

Serum Zonulin is High in a Number of Immune-Mediated Conditions¹

Autoimmune Diseases

- Celiac disease
- Ankylosing spondylitis
- Inflammatory bowel disease
- Type 1 diabetes



■ Gluten Peptide

Gluten Peptide is an immunogenic fragment of gliadin that serves as a stool marker of gluten consumption. Gluten-containing grains, such as wheat, rye, and barley, are typically digested into their gliadin and glutenin subunits. One specific section of the alpha-gliadin peptide, known as the 33-mer peptide, is resistant to digestion in the GI tract and is a primary immune trigger in those with celiac disease.^{292,293} Even in non-celiac gluten sensitivity, the 33-mer protein can be antigenic, inducing food sensitivities and intestinal permeability also known as “leaky gut.”²⁹⁴ In the presence of intestinal permeability, this antigenic peptide can drive extraintestinal symptoms including skin manifestations, headaches, behavioral symptoms, and more.²⁹⁵



The fecal gluten peptide test is a non-invasive, direct quantification of the 33-mer gliadin peptide present in stool samples. Because this peptide is highly resistant to human digestion, it correlates to the amount of gluten recently consumed. The fecal gluten peptide can be accurately detected in a person’s stool two to four days after consumption of gluten.

Currently, the only approach to managing celiac disease is to maintain a strict gluten-free (GF) diet for life. Additionally, as non-celiac, gluten-related disorders are emerging more and more in literature and clinical practice, many people choose to follow a GF diet to reduce gastrointestinal inflammation and associated symptoms, and to prevent or heal intestinal permeability.

Although conceptually straightforward, there are inherent challenges to following a GF diet. Estimated compliance rates vary considerably (anywhere from 17–80%)^{296,297} and in 2024 the Celiac Disease Foundation reported that up to 50% of celiac patients still report symptoms while following a GF diet. There are abundant opportunities for accidental ingestion of gluten from hidden food sources and even medications. Food labeling and label reading is an added complication. A 2019 study found that up to 32% of restaurant-labeled GF foods can be cross-contaminated.²⁹⁸

Patients who continue to show the clinical signs of celiac disease even when following a GF diet are said to have non-responsive celiac disease. Although a subset of these patients may have refractory celiac disease (failure to respond to a GF diet even after 6–12 months), some are simply reacting to gluten cross-contamination. A Gluten Contamination Elimination Diet, which removes hidden traces of gluten can resolve the symptoms of celiac disease in these patients.²⁹⁹

The importance of monitoring a GF diet is obvious, however there are currently no clear guidelines for monitoring adherence or assessing the outcome. Recent studies suggest that patients cannot rely on dietary best practices, or even symptoms, to understand if they have been exposed to gluten.^{296,300} For this reason, measuring fecal gluten peptide is ideal to monitor occasional exposures (known and unknown) or exposures that may be routine in a person’s lifestyle.

Fecal gluten monitoring is useful to:

- Quantitatively evaluate the amount of gluten peptide in stool based on the last two to four days of exposure
- Monitor adherence to a gluten-free diet
- Monitor unintentional consumption of gluten for both celiac and non-celiac, gluten-sensitive patients
- Assist in the treatment of non-responsive celiac disease

■ Universal Antibiotic Resistance Genes

Antibiotic Resistance Genes are genes carried by bacteria that confer a special resistance or protection from certain antibiotics. This test detects the presence of 55 genetic elements associated with resistance to 10 of the most popular classes of antibiotics. The presence of these genes in a bacterial population have been associated with moderate to high levels of antibiotic resistance in human gastrointestinal infections.



Information about a patient's antibiotic-resistant microbes can be clinically useful for those who have been hospitalized, treated with antibiotics, or who have stubborn, chronic infections. Antibiotic therapy can be better tailored to each unique patient when the antibiotic resistance profile for a patient's microbiome is known. Multiple antibiotic resistance genes could explain gastrointestinal infections that do not respond to, or are made worse by, antibiotic treatment.

Universal Antibiotic Resistance Genes Panel

– Identifies Microbial Resistance to These Classes of Antibiotics

- | | |
|--------------------|---|
| • β -lactams | • 5-Nitroimidazoles
(<i>non-Helicobacter pylori</i>) |
| • Fluoroquinolones | • Trimethoprim |
| • Vancomycin | • Sulfonamides |
| • Macrolides | • Methicillin |
| • Ciprofloxacin | • Chloramphenicol |

Tailoring a patient's pharmacotherapy using the Universal Antibiotic Resistance Genes³⁰¹ helps to: combat the rising public health threat of multidrug resistance, treat infections more efficiently, protect patients from unnecessary antibiotics, and reduce healthcare costs.^{302,303}

Both Gram-negative and Gram-positive bacteria can evade antibiotic therapy by carrying specialized mobile genetic elements on plasmids (or circular DNA molecules).



These antibiotic-resistant genetic elements can be transmitted through horizontal genetic exchange, a process where bacteria essentially swap useful genes to promote their survival. Horizontal genetic exchange helps them acquire and spread antibiotic resistance among bacterial populations and even between different species.³⁰³

Antibiotic resistance genes in the GI-MAP are “universal,” meaning that these genetic elements are not specific to a single bacterial species. When an antibiotic resistance gene is “present,” it indicates that population(s) of bacteria in the patient’s microbiome may have resistance to that entire class of antibiotics. It is, therefore, not advisable to use this class of antibiotics as the first choice in an antibiotic protocol. In addition, as different species of bacteria can rapidly share DNA via horizontal gene transfer, the presence of antibiotic resistance in any bacterial population is reason enough to avoid use of that drug class. An antibiotic-resistance gene is a threat to the entire microbiome.

Herbal Antimicrobial Agents

The GI-MAP does not culture fecal microbes for sensitivity testing to botanical agents. This technique is not validated or proven to correlate with patient dosages and clinical outcomes. Because of the antiseptic properties of many natural compounds, true sensitivity is difficult to decipher from simple antiseptic activity. For these reasons, Diagnostic Solutions recommends using a broad-spectrum, multiple-ingredient formula when treating dysbiosis.

Table 5. Herbal Antimicrobial Agents Commonly Used by Integrative and Functional Medicine Practitioners to Correct Dysbiosis

Antimicrobial Agent	Description and Clinical Use
Berberine	Berberine has shown effectiveness against ETEC-associated diarrhea and has been studied extensively for its antibacterial effect. ²⁹² It shows antimicrobial activity against fungi, protozoans, helminths, viruses, and chlamydia. ²⁹³
Garlic	Garlic has shown activity against bacteria, protozoa, helminths, viruses, and fungi. ^{294,295} It strongly suppressed gram-negative diarrheagenic pathogens (<i>Shigella</i> , <i>Salmonella</i> , <i>Proteus mirabilis</i> , and <i>E. coli</i>) isolated from stool samples. ²⁹⁶ Aqueous garlic extract inhibited <i>E. coli</i> O157:H7 and <i>E. coli</i> LF82, and enhanced the growth of <i>Lactobacillus reuteri</i> in vitro. ²⁹⁷ This suggests that antimicrobial herbs may spare beneficial microbiota.
Olive leaf	Olive leaf has antibacterial, antifungal, ^{298,299} and antiviral properties. ³⁰⁰⁻³⁰²
Caprylic acid	Caprylic acid reduces <i>Campylobacter</i> and <i>Salmonella</i> in the gastrointestinal tract and stool of poultry when added to the feed or water. ³⁰³⁻³⁰⁵ Caprylic acid has antiviral and antifungal properties. ^{306,307}
Wormwood	<i>Artemisia annua</i> (wormwood) demonstrates significant antimicrobial effects and has been used in the treatment of malaria and parasitic gastrointestinal infections.
Black walnut	<i>Juglans nigra</i> (Black Walnut) has a long history of use as an intestinal antiparasitic (i.e. vermifuge, anthelmintic), antibacterial, and antifungal.
Uva ursi	<i>Arctostaphylos uva-ursi</i> leaves have been used worldwide as a diuretic, astringent, antiseptic, and treatment for urinary tract and gastrointestinal infections.
Tribulus	<i>Tribulus terrestris</i> contains X steroidal saponins that show antibacterial and antiviral effects.
Citrus seed extract	Grapefruit and other citrus seed extracts have long been used as antiseptics and are used clinically to reduce fungal overgrowth by such common organisms as <i>Candida</i> and <i>Geotrichum</i> . Citrus seed extract also has demonstrated antibacterial action, most notoriously with hemolytic coliform bacteria.

Botanical and volatile oil extracts have a long history of traditional use as natural antimicrobials. Natural agents such as berberine, garlic, olive leaf, caprylic acid, wormwood, black walnut, uva ursi, citrus seed extract, and *Tribulus terrestris* provide a broad spectrum of activity against the most common pathogens that cause gastrointestinal illness and dysbiosis. Antimicrobial herbs do not pose the same risk for microbial resistance,³⁰⁴⁻³⁰⁶ as compared to antibiotics, because multiple active ingredients from the whole plant work together in synchrony. Their long historical use suggests low risk of adverse effects.



Conclusions

Diagnostic Solutions' qPCR stool testing methodology for the GI-MAP continues to be the most sensitive and specific detection method on the market. Compared to PCR detection of fecal microbes, qPCR can detect as low as 0.1 cell per gram of stool.

The GI-MAP quantifies a substantial list of pathogenic bacteria, fungi, and opportunistic pathogens, including novel targets such as viruses, microsporidia, and pathogenic virulence factors. Chronic gastrointestinal symptoms, intestinal permeability, hormonal imbalance, and food sensitivities may trace their origins back to imbalanced gut microbes. Further, chronic inflammatory arthritis could have a microbial component that may warrant investigation by stool studies.

This stool test offers integrative and functional medicine practitioners superior sensitivity and specificity to help resolve persistent and complex illnesses. Since the immune system, the intestinal barrier, and microbial diversity are intimately interwoven, thorough understanding of our gut microbiome holds promise for new approaches to treat and prevent disease.³²³





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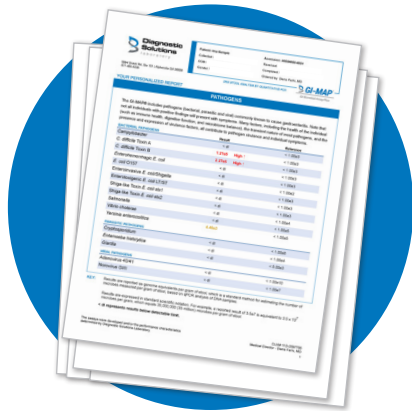
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