The GI Microbial Assay Plus

GI-MAP®

Quantitative PCR Stool Technology
for the 21st Century

Diagnostic Solutions laboratory




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The GI Microbial Assay Plus (GI-MAP)

Quantitative PCR: Innovative Stool Testing for the 21st Century

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 *Campylobacte*r 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 mirabilus*, 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 recent additions to 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. (*See the complete list of markers on the GI-MAP at the end of this article.)*

Disruption of the Gastrointestinal Microbiome Can Cause:

Gastrointestinal Symptoms

- Abdominal pain[6]
- Bloating[7]
- Constipation[7]
- Crohn's disease[8,9]
- Diarrhea[6,7,10]
- Food poisoning[11]
- Gastric cancer[12]
- Gastritis[12]
- Gastroenteritis[13,14]
- Gastroesophageal reflux[15,16]
- Irritable Bowel Syndrome[14,17]
- Small Intestinal Bacterial Overgrowth (SIBO)[18]
- Ulcer[12]
- Ulcerative colitis[19]
- Vomiting[20]

Autoimmune Conditions

- Ankylosing spondylitis[21]
- Reactive arthritis[21-23]
- Rheumatoid arthritis[24]

Allergic Disease

- Asthma[25]
- Eczema[26-28]

Methodology

Diagnostic Solutions Laboratory 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]

DSL has upgraded their technological platform to use qPCR because it is more sensitive and specific. It meets higher standards for scientific accuracy but does not use the same FDA-cleared pathogen assay of earlier years. The FDA-cleared assay did not quantify pathogens. It only reported a positive or negative (qualitative) finding. In contrast, the new GI-MAP will give practitioners quantitative information about *Giardia*, *Clostridium difficile*, *Salmonella*, and many more. Not all pathogens cause disease if they are present. Knowing exactly how much DNA is present can give the practitioner important information for better clinical decision-making.

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

The method measures the 16S or 23S ribosomal RNA (rRNA) regions and other target-specific gene fragments to detect bacteria. It also measures virulence factors and viral targets (RNA). Accurate measurement of DNA targets relies on two molecular methods: amplification and hybridization. Amplification is the process of making many copies of the target gene. Hybridization matches the target gene to a complementary DNA sequence in a lock-and-key manner.

qPCR, or real-time PCR, came by its name because it measures PCR amplification as it occurs, in real time. Data is collected throughout the PCR process instead of at the end of the PCR cycle. In qPCR, reactions depend on the detection of the target early in amplification, rather than the amount of DNA target that has accumulated after a fixed number of cycles.[29] This completely

revolutionizes PCR-based quantitation of DNA and RNA. Older PCR methods are semi-quantitative. In traditional PCR, results are collected after many rounds of amplification, so the starting concentration of target DNA is impossible to determine. It may be estimated by comparing it to a standard curve. Older PCR methods analyze the quality and yield of PCR products using gel electrophoresis, which is not quantitative.[29]

Diagnostic Solutions Laboratory decided to upgrade the DNA analysis techniques from multiplex polymerase chain reaction to qPCR because it is truly quantitative and more accurate.[29] 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 new GI-MAP can measure higher and lower amounts of genomic DNA than before *(also expressed as, "having a larger analytical range")*. All results are *quantitative* instead of qualitative (positive or negative).

The qPCR method used in the new GI-MAP is high throughput and is fully automated. Turn-around-time with this technique may be as low as three to five days.

Other stool testing options on the market can take weeks to deliver results. The automated nature of this method minimizes the chance for human error. DNA analysis is notorious for being highly labor intensive and there are chances for human error in extraction, hybridization, and amplification.

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 colorometrically 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

Other stool tests on the market primarily use Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) to identify stool microbes. *MALDI-TOF technology used by other laboratories for microbial detection 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 culture methods. 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 culture conditions cannot be identified. Collection of stool specimens for DNA analysis more closely represents the actual microbial populations of the patient's gastrointestinal tract at the time of collection.

In pyrosequencing or shotgun sequencing, the instrument sequences, or 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. Results are shown as a percent of total because they are not quantitative. Sequencing DNA is very slow. This method can give qualitative information about the microbes in stool but cannot be used to diagnose a pathogen. It can show patterns and relationships of microbes. Some pyrosequencing stool tests on the market only measure 16S rRNA regions and might miss microbes with DNA signatures in different regions. They cannot adjust for differences in genomic DNA in the stool samples. For example, some people have a lower amount of DNA in their fecal specimen than others and this must be accounted for in order to get accurate results.

Target Analytes

The human gastrointestinal microbiome houses trillions of bacteria and research shows that these microorganisms are essential for human metabolism,[30] nutrition, immune function,[31] and resistance to infection.[32] 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.[33] 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."*[32] 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.[32] 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.[34]

Commensal bacteria naturally inhabit the human gastrointestinal tract and do not cause disease. Many are beneficial; they produce enzymes,[35] vitamins,[36] short chain fatty acids,[37] 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.[38] 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 new GI-MAP qPCR technique was developed, verified, and validated by Diagnostic Solutions Laboratory. The new assay 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.[39] 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.[40]

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.[31,41]

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.

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.[42]

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.[43] Real-time polymerase chain reaction is considered a gold standard diagnostic methodology for *C. diff*.[42]

Escherichia 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.[44] *E. coli* can cause infections outside of the GI tract such as urinary tract infections, meningitis, and intra-abdominal abscess.[45]

While there are many harmless, and even beneficial, *E.coli* strains, there are six strains that are notorious for their pathogenicity, especially for GI infections. Enterohemorrhagic *E. coli* can lead to hemorrhagic colitis or hemolytic-uremic syndrome. Enteroinvasive *E. coli (EIEC*) can lead to dysentery similar to that caused by *Shigella.* Enteropathogenic *E. coli* is a cause of childhood diarrhea. Enterotoxigenic *E. coli (ETEC)* can cause traveler's diarrhea. EIEC and EHEC colonize the colon while the others colonize the small intestines and subsequently initiate diarrhea.[45]

One of the most potent bacterial toxins known, shiga toxin (Stx) is made by *Shigella dysenteriae* 1. Some serogroups of *E. coli* make an identical toxin (called "Stx1") and a second type of shiga toxin (Stx2). Stx1 and Stx2 have the same mode of action but are antigenically distinct. Shiga toxins (Stx, Stx1, Stx2) cause bloody diarrhea and can cause hemolytic uremic syndrome *(characterized by thrombocytopenia, hemolytic anemia, and kidney failure)*.[46]

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. [47] 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.[48-50]

The ***serotype O157:H7*** has been implicated in many outbreaks and cases of bloody diarrhea and hemolytic uremic syndrome[45] and has a high prevalence worldwide.[50]

Enteroinvasive E. coli (EIEC)/Shigella 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. 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.[51] 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*.[52,53]

Enteropathogenic E. coli (EPEC) is an invasive bacteria most often known to cause diarrhea in infants and children. Foods contaminated with human feces from an ill individual, either directly or via contaminated water, could cause disease in others.[54] Outbreaks have been associated with hamburger meat and unpasteurized milk. Symptomatic individuals may experience watery, sometimes bloody, diarrhea. PCR methodology is noted to be useful to distinguish *E. coli* strains in infected patients.[53]

Enterotoxigenic E. coli 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.[55]

Shiga-like toxin producing E.coli (STEC) has been involved in foodborne illness outbreaks.[44] 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.[56]

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.[57] It is the largest health burden of all the bacterial pathogens.[58] *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. [57]

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.[57]

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 10^5 to 10^6 cells *(based on clinical studies)*. [57]

Table 1. Food Sources of *Salmonella.*

Poultry	
Poultry Products	
Meat	
Dairy	
Raw, fresh, ready-to-eat produce such as:	*Tomatoes, Leafy Greens, Sprouts, Berries, Melons*

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.[59] Common sources of exposure are contaminated water or undercooked pork, meats, dairy products.[60] 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.[61,62] *Y. enterocolitica* can mimic inflammatory bowel disease, especially Crohn's disease.[63]

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.[64,65]

Infection is usually self-limiting and does not require treatment. However, for severe infections, pharmaceutical treatment with doxycycline in combination with an aminoglycoside may be warranted. Additionally, trimethoprim-sulfamethoxasole, chloramphenicol, and rifaximin may also be useful treatments.[66]

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. 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 hartmanii*. 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.[67] Treatment for infection with *E. histolytica* includes antiparasitic drug therapy and may include a combination based on the severity of infection.

Giardia intestinalis (previously *Giardia lamblia*) is the most commonly identified intestinal parasite in the United States and the most commonly isolated protozoan worldwide.[68] 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:[68]

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

Metronidazole and tinidazole are approved pharmaceutical treatments for giardiasis. Stool ova & 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%).[68]

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.[69] 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,[70] 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.[71] There are no standard treatments for viral gastroenteritis in healthy hosts. Antivirals are not recommended.[72] 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.[72]

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.

NorovirusGI&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.[73] 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.[74] 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.[75] 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.[76] 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.[77]

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.[78] *H. pylori* has also been detected by stool PCR in cases of dyspepsia, abdominal pain, and chronic gastrointestinal symptoms.[79-81] 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 cancer[82] 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.[79,81,83] It has shown sensitivity and specificity reaching that of the diagnostic *"gold standard,"* which is endoscopy with biopsy and urease test.[79-81] *H.pylori* genotyping may be useful for resistant *H. pylori* infections that have failed to respond to triple antibiotic therapy.[79] 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."[79]

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.[78] 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.[84] *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.[85]

Virulence Factors

Positive virulence genes represent the potential for an *H. pylori* strain to create pathology. 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.[86]

CagA (Cytotoxin-associated protein A) presence in *H.pylori* strains has been significantly associated with gastric cancer and peptic ulcer.[87] 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.[87] These changes to normal cell signaling can initiate cancer.

Cag PAI (Cag pathogenicity island) 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*.[88]

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.[89]

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.[90,91]

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.[92]

Vacuolating toxin A (vacA) has been associated with gastric cancer, peptic ulcer, and duodenal ulcer.[87] 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.[87]

Commensal 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 classes 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 *Penicillum*.

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.[32] Research has demonstrated the microbiota's capacity to interact with the immune system as an important health benefit.[93] The microbiota also has anti-inflammatory and antioxidant activity.[94] 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.[95] 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 serve important roles in the microbial community of the large intestine.[229] They are major producers of butyrate and other short-chain fatty acids (SCFA) from non-digestible carbohydrates.[230,231] SCFA are important regulators of mucosal integrity, immune balance, and pathogen resistance.[232,233] 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.

Faecalibacterium prausnitzii is a major butyrate-producing species, that is particularly abundant in the colon.[234] 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.[234,235]

Bifidobacteria and ***Lactobacillus*** 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,[98] and help control intestinal pH.[99] *Bifidobacteria* and *Lactobacillus* help prevent the overgrowth of *Candida albicans*, *E. coli*, and other pathogenic bacteria.[34,100] *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.[236]

Akkermansia muciniphila is a mucus-degrading bacterium that plays an important role in supporting the gut microbial ecosystem.[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.[234,237,238] 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, whereas high levels have been associated with several neurodegenerative diseases, such as Parkinson's disease and multiple sclerosis.[239,240]

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.[96] 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.[97]

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.[35] When autistic-like mice were given *Bacteroides fragilis*, it normalized intestinal permeability, restored microbial balance, and removed behavioral and cognitive symptoms.[35] *B. fragilis* has also been shown to correct gastrointestinal pathology in animal models of colitis[59] 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.[97]

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*.[101] 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, [102] 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.[103] And when obese subjects lost weight, there was a simultaneous change in the *Firmicutes* to *Bacteroidetes* ratio, favoring that of lean subjects.[30] Some authors have challenged those results, suggesting instead that obese subjects have lower microbial diversity.[102] 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.[101,102] 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.[104]

Gastrointestinal Bacteria as Potential Autoimmune Triggers

Opportunistic gastrointestinal pathogens are gaining attention for their ability to initiate autoimmune thyroiditis and inflammatory arthritis such as rheumatoid arthritis and ankylosing spondylitis. *Klebsiella* species, *Proteus mirabilis*, *Citrobacter* species, 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 disease[64] and higher antibodies to *Yersinia enterocolitica* have been found in these patients.[65] Enterovirus is also associated with immunogenic thyroiditis.[105] 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,[106] 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.[107,108] 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.[24] 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.[107]

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 miribalis*[109,110] 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.[111,112] Aggressive cases could evolve into ankylosing spondylitis.[22] 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.[113,114]

Other data shows abnormal serum antibody responses to *Klebsiella* and *Proteus mirabilis* in the spondyloarthropathies,[112] 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.[108] 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.[115]

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*.[241]

Fusobacterium species 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.[242-245] *Fusobacterium* spp. are considered normal members of the oral microbiome, but may also be 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.[242]

Klebsiella species 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.[116] Those with a history of long-term antibiotic use are at risk.

Proteus species 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.[117] *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.[117] 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 individuals[64] 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 spondyloarthropathies[112] and antibodies to *Proteus* in rheumatoid arthritis.[108]

As a urease-producing organism, *P. mirabilis* is known to be the cause of some genito-urinary infections,[118] specifically impacting the kidneys, bladder, and urethra. Patients with long-term

catheterization are particularly susceptible to urinary tract infections caused by *P. mirabilis.*[119] The most common infection involving *P. mirabilis* occurs when the bacteria moves to the urethra and urinary bladder.[120] In individuals with kidney stones, *P. mirabilis* can cause reinfection in individuals that have been treated with antibiotics.[118,120] *P. mirabilis* is generally susceptible to broad-spectrum penicillins or cephalosporins, except in severe cases.[121,122]

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.[34] 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 standard medical authorities to cause illness, and finding measurable quantities in the stool may be considered clinically insignificant. Examples are *Citrobacter* species or *Morganella* species.

However, certain opportunistic pathogens may be 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. *Klebsiella*, *Citrobacter* and *Yersinia* species are believed to set off systemic autoimmune disease in certain patients.

Pseudomonas species 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.[123] This bacterium exhibits enhanced virulence with stress, trauma, surgery, and cancer.[123] Symptoms of enteric infection include fever, dehydration, abdominal distention, diarrhea, and physical findings of Shanghai fever.[124] 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.

Methanobacteriaceae 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.[246] 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.[246–248] 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.[249]

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 species 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 is found in 40–80% of normal stool specimens.[125,126] 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. [125]

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.[127] 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.[127,128]

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.[125] Blood culture is used to detect disseminated candidiasis but it can miss 40–50% of cases.[125] 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*.

Microsporidia species 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.[129] 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.[129] *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)*.[130] *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.[131]

Viruses

Cytomegalovirus (CMV) is a herpes virus that has affected 60% of the US population.[133] 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.[134] 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 flulike 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.[135]

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.[133]

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.[134]

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.[136] High levels of CMV DNA were detected in 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.[137] The frequency of CMV infection in IBD patients was 10–36% and may contribute to colitis symptomology.[138] 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.[135] 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.[139]

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

MV 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.[140]

Epstein-Barr Virus (EBV) is one of the most common human viruses worldwide. Also known as herpesvirus 4, it is thought to infect 90- 95% of the population.[141] **The GI-MAP stool test detects *active* 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 immunocompromised patients than in immunocompetent patients.[135]

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 virus[142] and vitamin D may help prevent autoimmune sequellae of EBV.[143] 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.[144]

EBV blood antibodies are used to diagnose an EBV infection.[144] 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.[145] Past infections are usually characterized by antibodies to both VCA and EBNA and may be elevated years after the primary infection.[144]

EBV and Autoimmunity

Primary infection with EBV causes mononucleosis, Burkitt's lymphoma, gastric cancer, nasopharyngeal carcinoma, and autoimmune diseases.[84] EBV has a central role in the pathogenesis of systemic autoimmune diseases, specifically rheumatoid arthritis, systemic lupus erythematosus, and Sjogren's syndrome.[146] 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. [140] Other researchers suggest that EBV is a contributory factor in autoimmune thyroid disorders.[147]

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.[84] The frequency of EBV infection in IBD patients ranges from 30–64%.[135,138] 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.[138]

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.[148] 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.[149]

Cyclospora cayetanensis is a parasitic protozoan commonly associated with water- and food-borne outbreaks, often causing traveller's diarrhea in infected hosts via oral-fecal transmission of sporulated oocyst in its infectious stage. Travel to tropical regions and imported fresh produce[150] from tropical regions contaminated with feces have been known to be sources for outbreaks of cyclosporiasis.[151] 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[152,153] such as chlorination. Cyclosporiasis is characterized by symptoms of prolonged watery diarrhea, intestinal distress, abdominal cramping, loss of appetite, weight loss, nausea, and vomiting.[154,155] 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.[154,156] In more persistent cases lasting more than seven days treatment with an antibiotic combination of trimethoprim and sulfamethoxazole,[157] may be necessary.

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.[158] There may also be an association between *Blastocystis* and chronic digestive disorders, such as irritable bowel syndrome.[159]

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.

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*) generally does not cause symptoms in the gastrointestinal tract. It is not considered pathogenic. However, it can cause minor inflammation and long term overgrowth could cause reduction in the mucosal immune response, specifically secretory IgA, which could lead to secondary dysbiosis or parasitosis. Additionally, it is a major cause of vaginosis and therefore may require treatment in the GI tract for patients with recurring vaginal infections.
The specific microsporidia measured is Encephalitozoon intestinalis. It can be an opportunistic pathogen and cause diarrhea.

Worms

Worms detected with the GI-MAP will be labeled, “negative,” “detected,” or “detected high.” “Negative” indicates that no intact adult worm or egg DNA was found in the fecal sample. A “detected” finding means that there was DNA present consistent with an egg being detected in the sample. Many positive samples for worms fall in this category and it does not necessarily indicate that an adult worm is present in the GI tract. Finally, “detected high” signifies that a multicellular adult worm *(a few billion cells)* or a large volume of eggs *(likely generated by an adult worm)* is present in the stool.

Ancylostoma duodenale and Necatur americanus are roundworms commonly known to cause hookworm infection by penetrating the skin.[160] 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.[161] 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.[162] Hookworm infection is associated with poverty, poor sanitation, inadequate housing construction, and lack of access to medications.[162]

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.[163,164] Hookworm infection may affect physical and cognitive growth of children.[162] 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;[165] therefore pets may also be a source of exposure. In cases of heavy hookworm infection, symptomatic individuals can be treated with albendazole or mebendazole.[166] Individuals presenting with anemia may benefit from iron supplements.[167]

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.[168] *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.[168] In cases of heavy infections, symptomatic individuals can be treated with albendazole and mebendazole.[166] Individuals presenting with anemia may benefit from iron supplements.[169]

Taenia species (tapeworm) may be found in stool after ingestion of contaminated or undercooked pork (*Taenia solium*) or beef (*Taenia saginata*).[170,171] *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.[171-173] Humans are the only definitive host for both *T. solium* and *T. saginata*.[174] PCR methods are sensitive and specific for detecting Taenia species in stool.[172,175]

Infection usually involves just a single tapeworm after ingestion of undercooked pork or beef from infected animals that have ingested eggs or tapeworm segments.[175] Individuals with taeniasis are usually asymptomatic or have mild symptoms.[176] 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.[164,176] Adult worms can be eliminated with albendazole or praziquantel.[177,178]

Intestinal Health

The GI-MAP includes markers of immune function, inflammation, digestion, gliadin sensitivity, and metabolic activity of the gastrointestinal biome. These markers were selected for their clinical utility. Calprotectin and elastase-1 have a strong foundation of clinical evidence to support their use in clinical care. Calprotectin helps the integrative and functional medicine practitioner measure the level of immune activation in the gut, often associated with infection and/or inflammatory bowel disease.

Pancreatic elastase 1 is an excellent global marker of pancreatic exocrine function and can be an indicator of poor digestive capacity or pancreatitis when extremely low. Secretory IgA is the body's first line of defense in the gut. A portion of this immunoglobulin might be directed toward gliadin, indicating an immune reaction to the common protein in wheat and other field grass grains. Beta-glucuronidase is an enzyme produced naturally in cells of the liver, kidney, and intestinal epithelium. However, this enzyme is also produced excessively by bacteria known to be pathogenic, and high levels may be an indication of adverse metabolic activity of the intestinal microbiome.

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,[179,180]

This test also accurately predicts a patient's response to pancreatic enzyme supplementation, especially in patients with unexplained diarrhea and suspected pancreatic insufficiency.[181] In patients with pancreatic insufficiency, 80% responded favorably to supplementation with pancreatic enzyme therapy; with an average dose of 120,000 units of lipase.[182] Low elastase-1 may suggest an underlying hypochlorhydria or it may be seen in small intestinal bacterial overgrowth (SIBO). Consider elastase-1 levels in patients with symptomatic *H. pylori* infection.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.[183]

Table 2. Staging of Pancreatic Insufficiency Based on Fecal Elastase-1.

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

Steatocrit has been used widely since 1981 to detect steatorrhea in patients with pancreatic insufficiency and small intestinal malabsorption.[184] 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.[184] The acid steatocrit method has been shown to correlate well with 24-hour and 72-hour fecal fats.[185,186]

Secretory Immunoglobulin A (sIgA) is an antibody protein secreted into the gastrointestinal tract as a first line of immune defense against pathogenic microorganisms.[187] This immunoglobulin influences the gut microbiome[187] and helps to maintain barrier function[188] by forming complexes with gut pathogens and allergens, preventing them from penetrating the intestinal barrier. Impairment of secretory IgA may increase the risk of infectious, allergic, and inflammatory diseases of the intestine.[189] Chronic stress may also disrupt levels of sIgA. Elevated levels of sIgA may indicate an activated immune response to chronic infections or inflammatory reactions.

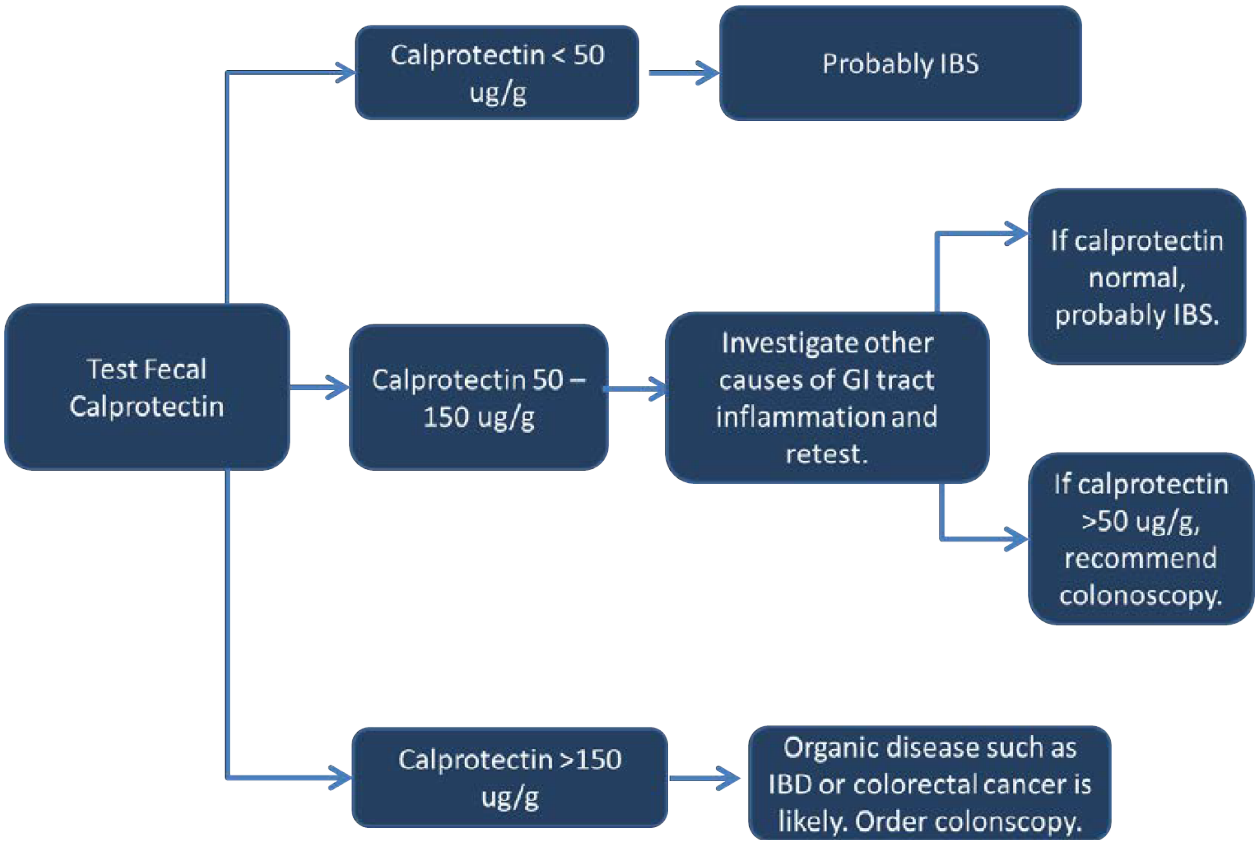
The presence of ***fecal anti-gliadin antibodies*** can indicate an immune response to gluten in the diet. Gliadin is a component of gluten, the protein found in wheat and other field grass grains such as barley, malt and rye. Because gliadin could stimulate intestinal immunity and increase levels of fecal anti-gliadin antibody even when serum concentrations are undetectable,[190,191] it is often used as marker for non-celiac gluten sensitivity. High levels of fecal anti-gliadin antibodies can provide clinicians with an effective treatment strategy: a gluten-free diet.

Fecal calprotectin is the most studied marker of gastrointestinal inflammation[192] and the gold standard marker for the diagnosis and monitoring of inflammatory bowel disease (IBD).[193] It is used to discriminate IBD from irritable bowel syndrome (IBS).[192,193] 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.[194] 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.[195] Fecal calprotectin levels are proportional to the level of neutrophil infiltration and inflammation in the gut.[194]

Calprotectin has been shown to correlate with histologic and endoscopic measures of inflammatory bowel disease severity.[195] It is non-invasive, stable,[193] and shows a considerable sensitivity and specificity of 93% and 96%, respectively, when used to screen for IBD activity.[196] High calprotectin can also be detected in colorectal cancers, diverticular disease, and infectious gastroenteritis.[192]

When IBD is suspected based on clinical presentation, a fecal calprotectin level <50 ug/g stool suggests IBS, not IBD. Calprotectin levels between 50 and 150 ug/g indicate GI inflammation and deserve treatment and follow-up testing. Calprotectin levels greater than 150 ug/g suggest organic disease such as IBD or colorectal cancer and follow-up colonoscopy is recommended (See Figure 1).[192] Fecal calprotectin can elevate with enteropathy caused by excessive non-steroidal anti-inflammatory medication use.[194] For this reason, it may be beneficial to temporarily discontinue NSAIDs, when possible in select patients, prior to measuring fecal calprotectin.[194]

Figure 1. The algorithm used to differentiate IBD from IBS using fecal calprotectin. Adapted from Walsham et al.[192]



Beta-glucuronidase is an enzyme produced by cells in the liver, kidney, intestinal epithelium, endocrine, and reproductive organs.[197] 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.[198] 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.[198] However, this enzyme can also convert pro-carcinogens to carcinogenic compounds.[197]

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.[197] Evidence of increased enzymatic activity of intestinal microorganisms may suggest increased risk of digestive tract cancer.[199] Toxins stimulate B-glucuronidase activity and dietary red meat and protein increases the enzyme. Antibiotics increase B-glucuronidase levels. A low-calorie, vegetarian diet can reduce fecal B-glucuronidase levels.[197]

Add-On Tests

Zonulin is a protein secreted by intestinal cells that regulates intercellular tight junctions.[1,200] Tight junctions are the connections between epithelial cells that make up the gastrointestinal lining. Zonulin increases intestinal permeability in the jejunum and ileum[201] and is considered a biomarker for barrier permeability.[1,200] 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.[200,202]

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.[203] 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,[204] and obesity and metabolic disease.[205] In many cases, permeability precedes disease.[1]

Serum zonulin is high in a number of immune-mediated conditions:[1]

Autoimmune Diseases

Celiac disease
Ankylosing spondylitis
Inflammatory bowel disease
Type 1 diabetes

Zonulin regulates barrier permeability. Serum zonulin correlates with intestinal permeability and lactulose/mannitol tests for intestinal permeability.[201,206] High serum zonulin has been associated with celiac disease, type 1 diabetes,[206] insulin resistance and type 2 diabetes,[201] 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.[207] 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.[200] Treatment with zeolite lowered stool levels of zonulin in athletes and presumably improved intestinal barrier function.[208]

Drug Resistance Genes

Drug resistance genes are genes carried by bacteria that confer a special resistance or protection from certain antibiotics. In the GI-MAP, drug resistance genes are measured in the bacterial genome of any pathogenic organism found to be positive in the fecal sample.

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.

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,[209-211] 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.

See Table 3 on following page.

Table 3. ***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.[212] It shows antimicrobial activity against fungi, protozoans, helminths, viruses, and chlamydia.[213]
Garlic	Garlic has shown activity against bacteria, protozoa, helminths, viruses, and fungi.[214,215] It strongly suppressed gram-negative diarrheagenic pathogens (*Shigella, Salmonella, Proteus mirabilis*, and *E. coli*) isolated from stool samples.[216] Aqueous garlic extract inhibited *E. coli* O157:H7 and *E. coli* LF82 and enhanced the growth of *Lactobacillus reuteri in vitro*.[217] This suggests that antimicrobial herbs may spare beneficial microbiota.
Olive leaf	Olive leaf has antibacterial, antifungal,[218,219] and antiviral properties.[220-222]
Caprylic acid	Caprylic acid reduces *Campylobacter* and *Salmonella* in the gastrointestinal tract and stool of poultry when added to the feed or water. [223-225] Caprylic acid has antiviral and antifungal properties.[226,227]
Wormwood	*Artemesia annua* (wormwood) demonstrates significant antimicrobial effects and has been used in the treatment of malaria and parasitic gastrointestinal infections.
Black walnut	*Juglans nigra* (Black Walnut) has a long history of use as an intestinal antiparasitic (i.e. vermifuge, anthelminthic), antibacterial, and antifungal.
Uva ursi	*Arctostaphylos uva-ursi* leaves have been used worldwide as a diuretic, astringent, antiseptic, and treatment for urinary tract and gastrointestinal infections.
Tribulus	*Tribulus terrestris* 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 *Candida* and *Geotrichum*. Citrus seed extract also has demonstrated antibacterial action, most notoriously with hemolytic coliform bacteria.

Conclusions

The GI-MAP has upgraded its stool testing methodology to RT-PCR, or qPCR, known as, “one of the most powerful and sensitive gene analysis techniques available.” It is more sensitive and specific than older methods for PCR detection of fecal microbes, detecting 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.[228]

Complete List of Target Analytes Measured on the GI-MAP

Pathogens:

Bacterial Pathogens –

- *Campylobacter*
- *C. diff Toxin A*
- *C. diff Toxin B***
- *Enterohemorrhagic E. coli*
- *E. coli O157***
- *Enteroinvasive E. coli/Shigella*
- *Enterotoxigenic E. coli LT/ST** (ETEC)*
- *Shiga-like Toxin producing E. coli stx1*
- *Shiga-like Toxin producing E. coli stx2 (STEC)***
- *Salmonella*
- *Vibrio cholerae*
- *Yersinia enterocolitica*

Parasitic Pathogens –

- *Cryptosporidium***
- *Entamoeba histolytica***
- *Giardia*

Viral Pathogens –

- Adenovirus 40/41**
- Norovirus GI/II**

H. pylori:

- *Helicobacter pylori*
- Virulence Factor, babA
- Virulence Factor, cagA**
- Virulence Factor, dupA
- Virulence Factor, iceA
- Virulence Factor, opiA
- Virulence Factor virA**
- Virulence Factor virB
- Virulence Factor virD

Normal/Commensal Bacterial Microbiota:

- *Akkermansia mucinophilia*
- *Bacteroides* spp.
- *Bifidobacterium* spp.**
- *Clostridia (class).*
- *Enterococcus* spp.
- *Escherichia* spp. *(E. coli)*
- *Faecalibacterium prausnitzii*
- *Lactobacillus* spp.
- *Enterobacter* spp.**

Phyla Microbiota –

- *Bacteroidetes*
- *Firmicutes*
- *Firmicutes/ Bacteroidetes Ratio*

Opportunistic Bacteria:

Additional Dysbiotic/Overgrowth Bacteria –

- *Bacillus* spp.
- *Enterococcus faecalis*
- *Enterococcus faecium*
- *Methanobacteriaceae (family)*
- *Morganella* spp.
- *Pseudomonas* spp.**
- *Pseudomonas aeruginosa*
- *Staphylococcus* spp.
- *Staphylococcus aureus*
- *Streptococcus* spp.

Potential Autoimmune Triggers –

- *Citrobacter* spp.
- *Citrobacter freundii*
- *Klebsiella* spp.**
- *Klebsiella pneumoniae*
- *M. avium subsp. paratuberculosis*
- *Prevotella copri*
- *Proteus* spp.
- *Proteus mirabilus*

Fungi/Yeast:

- *Candida* spp.
- *Candida albicans*
- *Geotrichum* spp.
- *Microsporidia* spp. including *Encephalitozoon intestinalis* **
- *Rhodotorula* spp.

Viruses:

- *CMV-Cytomegalovirus*
- *EBV- Epstein Bar Virus*

Parasites:

Protozoa –

- *Blastocystis hominis***
- *Chilomastix mesnelli*
- *Cyclospora* spp.
- *Dientamoeba fragilis*
- *Endolimax nana*
- *Entamoeba coli***
- *Pentatrichomonas hominis*

Worms –

- *Ancylostoma duodenale*
- *Ascaris lumbricoides*
- *Necatur americanus*
- *Trichuris trichiura*
- *Taenia* spp.

Intestinal Health:

Digestion –

- Pancreatic elastase 1
- Steatocrit

GI Markers –

- Beta-glucuronidase
- Occult blood

Immune Response –

- Secretory IgA (sIgA)
- Anti-gliadin sIgA

Inflammation –

- Calprotectin

Add-on Test –

Zonulin

Antibiotic Resistance Genes, phenotypes:

- Helicobacter
- Clarithromycin
- Fluoroquinolones
- Tetracycline

Antibiotic Resistance Genes, genotypes:

Universal Microbiota Resistance Genes –

- B-lactamase
- Fluoroquinolones
- Macrolides Vancomycin

***** Organisms with ** are listed with citations in this paper.***

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