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The GI Microbial Assay Plus (GI-MAP)

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. 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. 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) flora 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 multiplex, automated, DNA analysis to give integrative and functional medicine practitioners a better view into the gastrointestinal microbiome.

The GI-MAP measures pathogenic organisms that can cause hospital-acquired infections (HAI) such as C. difficile or norovirus, foodborne illness such as E. coli or Salmonella, and common causes of diarrhea such as Campylobacter, Shigella, and rotavirus A. 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, Yersinia enterocolitica, and Proteus mirabilis, associated with autoimmune molecular mimicry. It includes a panel of single-celled, amebic parasites such as Blastocystis hominis, Dientamoeba fragilis, and Entamoeba coli. Fungal organisms are measured by the GI-MAP such as Candida, Geotrichum, and Microsporidia, with the latter being a new addition to DNA stool analysis. Finally, the GI-MAP measures standard markers of immunity, inflammation and digestion including calprotectin, secretory immunoglobulin A (sIgA), anti-gliadin antibody, and pancreatic elastase 1.
Disruption of the gastrointestinal microbiome can cause:

**Gastrointestinal symptoms**
- Abdominal pain
- Bloating
- Constipation
- Crohn’s disease
- Diarrhea
- Food poisoning
- Gastric cancer
- Gastritis
- Gastroenteritis
- Gastroesophageal reflux
- Irritable bowel syndrome
- Small intestinal bacterial overgrowth (SIBO)

**Gastrointestinal symptoms, continued**
- Ulcer
- Ulcerative colitis
- Vomiting

**Autoimmune Conditions**
- Ankylosing spondylitis
- Reactive arthritis
- Rheumatoid arthritis

**Allergic Disease**
- Asthma
- Eczema

**Methodology**

Diagnostic Solutions Laboratory is using a novel DNA technique to detect a comprehensive list of stool bacteria, viruses, fungi, and parasites. The method and instrument used is FDA-cleared for the detection of 15 of the most common causes of gastroenteritis—bacteria, parasites, and viruses. It has been clinically validated for use with human stool samples and sensitivity and specificity data can be found below. This is an automated, multiplex DNA analysis method, allowing for the simultaneous measurement of multiple bacteria, fungi, parasites, and viruses, all from a single sample. Multiplex polymerase chain reaction (PCR) means that many genes are amplified at the same time, as though many separate PCR reactions were happening at once. This technique makes it possible to simultaneously detect many different organisms in one sample. Multiple primers and probes for each organism allow for enhanced sensitivity and specificity. The method measures the 16S or 23S ribosomal RNA (rRNA) regions, virulence factors, and viral targets for microbial detection. Pathogenic bacteria are reported as a qualitative result (positive/negative) whereas other microorganisms (opportunistic pathogens, fungi, and commensal flora) are reported with quantitative results.

Turn-around-time with this technique may be as low as only three to four 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. This is the only FDA-cleared DNA test for gastrointestinal microbes available. In contrast to molecular methodologies used by other laboratories, this method was not developed in-house. Independent clinical validation data on the method used by Diagnostic Solutions Laboratory shows that the test is reliable and reproducible. These added quality aspects are novel and give the clinician additional confidence in DNA analysis, not available elsewhere in the integrative and functional medicine market.

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
samples undergo multiplex PCR, which amplifies many gene targets. Amplification can generate thousands to millions of copies of a single target DNA sequence.

After amplifying the DNA targets found in the stool specimen, the specimen undergoes hybridization. It is treated with magnetic beads imbedded with DNA probes. A probe is a segment of DNA that seeks to join with its complementary match and is radioactively 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 bead has a unique DNA signature that will bind to the amplified target gene, if it is present in the stool specimen. This allows for accurate and sensitive detection of a target organism. Multiplex PCR uses this same molecular approach for many microbes at the same time, allowing for sensitive and specific differentiation of many organisms simultaneously.

In the past, drawbacks with DNA analysis have been its incredible sensitivity and potential for non-specific binding. Because of the tremendous sensitivity of DNA analysis, microbes might be detected in a patient specimen that were not actually present in high numbers at the time of stool collection. Specificity can be a problem with DNA analysis because microbes may be detected incorrectly due to cross-reactivity. With the GI-MAP method, probes are attached to different beads in such a way that non-specific binding is decreased and false positives are decreased. Diagnostic Solutions Laboratory uses several other method improvements to further improve accuracy and precision of the method.

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 immediately “freezes” the DNA, so that it more closely represents the actual microbial populations of the patient’s gastrointestinal tract at the time of collection.

<table>
<thead>
<tr>
<th>FDA-Cleared GI Pathogens Measured in the GI-MAP</th>
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<tbody>
<tr>
<td>Campylobacter</td>
</tr>
<tr>
<td>Clostridium difficile, Toxin A/B</td>
</tr>
<tr>
<td>Enteroaggregative E.coli (ETEC) LT/ST</td>
</tr>
<tr>
<td>Shiga-like Toxin producing E.coli (STEC) stx1/stx2</td>
</tr>
<tr>
<td>Salmonella</td>
</tr>
<tr>
<td>Shigella</td>
</tr>
<tr>
<td>Vibrio cholera</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
</tr>
<tr>
<td>Viruses</td>
</tr>
<tr>
<td>Adenovirus 40/41</td>
</tr>
<tr>
<td>Norovirus GI/GII</td>
</tr>
<tr>
<td>Rotavirus A</td>
</tr>
<tr>
<td>Parasites</td>
</tr>
<tr>
<td>Cryptosporidium</td>
</tr>
<tr>
<td>Entamoeba histolytica</td>
</tr>
<tr>
<td>Giardia</td>
</tr>
</tbody>
</table>
Target Analytes

The human gastrointestinal microbiome houses trillions of bacteria and research shows that these microorganisms are essential for human metabolism, nutrition, immune function, and resistance to infection. 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. 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 flora can protect the host from infection. When gut microflora protects the intestines from pathogens and harmful microorganisms it is called, “colonization resistance.” Animal models show that when normal gut microflora are lacking, the host is more susceptible to GI infections with Salmonella. Similarly, after antibiotic treatment there is increased risk of pathogenic infections. 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.

Commensal bacteria naturally inhabit the human gastrointestinal tract and do not cause disease. Many are beneficial; they produce enzymes, vitamins, short chain fatty acids, 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. Given the metabolic, nutritional, and immune-enhancing roles of these organisms, the microbiome deserves close analysis when treating patients with chronic illness.

Pathogens

The GI-MAP measures bacterial pathogens such as Campylobacter, Escherichia coli (E. coli) O157, Enterotoxigenic E. coli, Shiga-like toxin-producing E. coli, Clostridium difficile, Salmonella, Shigella, and Vibrio cholerae. The GI-MAP method has been validated and FDA-cleared for the measurement of these organisms. Sensitivity and specificity data have been determined on these pathogens in human stool samples and are listed below.

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. Multiplex PCR, using the same method as that used for the GI-MAP, 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.
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. 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 microflora, 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 have 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.

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. 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.

<table>
<thead>
<tr>
<th>Bacteria &amp; Bacterial Toxins in the GI-MAP</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigella</td>
<td>97.70%</td>
<td>97.80%</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>97.50%</td>
<td>97.80%</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>N/A</td>
<td>100.00%</td>
</tr>
<tr>
<td>Enterotoxigenic E. Coli (ETEC) LT/ST</td>
<td>N/A</td>
<td>97.30%</td>
</tr>
<tr>
<td>E. coli 0157</td>
<td>88.20%</td>
<td>98.80%</td>
</tr>
<tr>
<td>Shiga-like toxin producing E. Coli (STEC) stx 1/stx 2</td>
<td>100.00%</td>
<td>99%</td>
</tr>
<tr>
<td>Salmonella</td>
<td>82.10%</td>
<td>99.10%</td>
</tr>
<tr>
<td>Clostridium difficile Toxin A/B</td>
<td>97.70%</td>
<td>94.90%</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>N/A</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

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

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

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. Enterotoxigenic *E. coli* (ETEC) can cause traveler’s diarrhea. Enteropathogenic *E. coli* is a cause of childhood diarrhea. Enteroinvasive *E. coli* (EIEC) can lead to dysentery similar to that caused by *Shigella*. Enterohemorrhagic *E. coli* can lead to hemorrhagic colitis or hemolytic-uremic syndrome. EIEC and EHEC colonize the colon while the others colonize the small intestines and subsequently initiate diarrhea. The strains measured on the GI-MAP are: *E. coli* O157, Enterotoxigenic *E. coli* LT and ST (ETEC), and Shiga-like toxin-producing *E. coli* (STEC), targeting stx1 and stx2 genes.

Shiga-like toxin producing *E. coli* (STEC) has been involved in foodborne illness outbreaks. It causes various GI illnesses, including bloody and non-bloody diarrhea. Shiga toxin (stx1) and Shiga toxin 2 (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.

The same method used in the GI-MAP was used to investigate the cause of an outbreak in German hospitals in patients suffering with hemolytic uremic syndrome, presumably induced by STEC. With rapid screening diagnostic methods they were able to identify a novel serotype—*E. coli* O104:H4 that had virulence factors characteristic of both enterohemorrhagic *E. coli* and enteroaggregative *E. coli*.

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

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.
Viral Pathogens

Adenovirus, norovirus, and rotavirus 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. 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, 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. There are no standard treatments for viral gastroenteritis in healthy hosts. Antivirals are not recommended. 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.

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.

<table>
<thead>
<tr>
<th>Viral Toxins in the GI-MAP</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus 40/41</td>
<td>100.00%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Rotavirus A</td>
<td>94.70%</td>
<td>99.80%</td>
</tr>
<tr>
<td>Norovirus GI/GII</td>
<td>93.50%</td>
<td>98%</td>
</tr>
</tbody>
</table>

**Norovirus GI & GII**, or Norwalk virus, is the most common cause of non-bacterial gastroenteritis in the world. It is widely known for causing the stomach flu on cruise ships. 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. 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. 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. 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.

Rotavirus is the most common cause of severe, watery diarrhea in infants and children, especially in the daycare setting. The most common type is rotavirus A, which accounts for more than 90% of infections in humans. Symptoms usually begin within 2 days of exposure to an infected person. Low-grade fever, vomiting, abdominal cramps, anorexia, and watery diarrhea are characteristic signs. Dehydration can be a major risk. The infection can persist for 3 to 8 days and usually resolves on its own. However, for those with weakened immune systems, the infection can cause hospitalization and death. By age five, almost every child has been infected. Adults can also become infected but the symptoms are generally less severe. Treatment consists mainly of rehydration therapy.

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

<table>
<thead>
<tr>
<th>Parasites in the GI-MAP</th>
<th>Sensitivity</th>
<th>Specificity</th>
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</thead>
<tbody>
<tr>
<td><em>Giardia lamblia</em></td>
<td>100.00%</td>
<td>98.20%</td>
</tr>
<tr>
<td><em>Cryptosporidium</em></td>
<td>87.50%</td>
<td>100.00%</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>100.00%</td>
<td>99.40%</td>
</tr>
</tbody>
</table>

**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. 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.

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

*Entamoeba coli* and *E. hartmanni* are intestinal amebae that are found in the large intestine. They generally are not considered pathogenic. However, when these amebae are found in stool samples it can indicate the presence of other potentially pathogenic organisms.
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 flora 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. Research has demonstrated the microbiota’s capacity to interact with the immune system as an important health benefit. The microbiota also has anti-inflammatory and antioxidant activity. 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. A limited list of commensal flora is included in the GI-MAP test as a general screen for levels of normal, protective flora or to monitor probiotic supplementation. These include Bacteroides fragilis, Lactobacillus and Bifidobacteria as well as E. coli.

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. 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.

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. When autistic-like mice were given Bacteroides fragilis, it normalized intestinal permeability, restored microbial balance, and removed behavioral and cognitive symptoms. B. fragilis has also been shown to correct gastrointestinal pathology in animal models of colitis and inhibit neuroinflammation in mouse models of multiple sclerosis. 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.

Bifidobacteria and Lactobacillus are a natural part of the flora 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, and help control intestinal pH. Bifidobacteria and Lactobacillus help prevent the overgrowth of Candida albicans, E. coli, and other pathogenic bacteria.
Opportunistic Pathogens

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. 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. *H.pylori* is one exception. Widely lauded as a pathogen, it is placed here in the category of Opportunistic Pathogens because of recent suggestions about its protective effects and dwindling evidence that it is pathogenic in all who are colonized.

*Helicobacter pylori (H. pylori) and its virulence genes*, cagA (cytotoxin-associated protein A) and vacA (vacuolating toxin) 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. *H. pylori* colonization has been implicated in a variety of gastroduodenal diseases including gastritis, gastric cancer, and duodenal and peptic ulcer. *H. pylori* has also been detected by stool PCR in cases of dyspepsia, abdominal pain, and chronic gastrointestinal symptoms. 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, as well as other diseases such as esophageal cancer reflux, and obesity.

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. Host immune status and acid secretion seem to be other important factors contributing to *H. pylori’s* colonization and pathogenesis. The *H. pylori* virulence factors that are most well recognized are vacA and cagA.

The presence of cagA-positive *H.pylori* strains has been significantly associated with gastric cancer and peptic ulcer. 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. These changes to normal cell signaling can initiate cancer.

The presence of vacA has been associated with gastric cancer, peptic ulcer, and duodenal ulcer. 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.
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.\(^{72,74,77}\) It has shown sensitivity and specificity reaching that of the diagnostic “gold standard,” which is endoscopy with biopsy and urease test.\(^{72-74}\)

*H. pylori* genotyping may be useful for resistant *H. pylori* infections that have failed to respond to triple antibiotic therapy.\(^{72}\) 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.”\(^{72}\)

*H. pylori* may be asymptomatic and require no treatment or only supportive care to improve the intestinal mucosa and gastrointestinal lining. Information on the *cagA* and *vacA* genes may help determine whether treating a positive *H. pylori* result is necessary.

*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.\(^{78}\) This bacterium exhibits enhanced virulence with stress, trauma, surgery, and cancer.\(^{78}\) Symptoms of enteric infection include fever, dehydration, abdominal distention, diarrhea, and physical findings of Shanghai fever.\(^{79}\) 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.

*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.\(^{80}\) Those with a history of long-term antibiotic use are at risk.

**Gastrointestinal Bacteria as a Trigger for Autoimmunity**

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\(^{81}\) and higher antibodies to *Yersinia enterocolitica* have been found in these patients.\(^{82}\) Enterovirus is also associated with immunogenic thyroiditis.\(^{83}\) 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,\(^{84}\) good levels of commensal flora, 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.\(^{85,86}\)
Patients with these conditions or documented intestinal permeability may be at risk if gut microbiota are imbalanced.

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

Spondyloarthropathies are a family of chronic, multi-system, inflammatory diseases involving the sacroiliac joints and axial skeleton and they may have an infectious trigger. 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. 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.

Reactive arthritis can be brought on by genito-urinary infections with Proteus mirabilis or gastrointestinal infections with bacterial agents such as Chlamydia, Salmonella, Shigella, Campylobacter, Yersinia and Clostridium difficile. Parasites such as Strongyloides stercoralis, Giardia lamblia, Ascaris lumbricoides, and Cryptosporidium species can also result in reactive arthritis. Aggressive cases could evolve into ankylosing spondylitis. 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. Evidence of Salmonella has been found in cases of ankylosing spondylitis.

Other data shows abnormal serum antibody responses to Klebsiella and Proteus mirabilis in the spondyloarthopathies, 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. 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.

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.

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.

**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. 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, but the spectrum of related diseases due to these pathogens also includes sinusitis, bronchitis, pneumonia, nephritis, myositis, hepatitis, encephalitis, and other brain infections. Treatment often includes antifungal medications along with diet and nutritional interventions to help with chronic diarrhea.

### Additional Tests

The GI-MAP includes markers of immune function, inflammation, digestion, and gliadin sensitivity, and metabolic activity of the gastrointestinal biome. These markers were selected for their clinical utility. Calprotectin and elastase 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.

**Secretory Immunoglobulin A (sIgA)** is an antibody protein secreted into the gastrointestinal tract as a first line of immune defense against pathogenic microorganisms. This immunoglobulin influences the gut microbiome and helps to maintain barrier function 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. 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, 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 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). This can impair the body’s ability to absorb nutrients from food, including fat-soluble vitamins. This test also accurately predicts a patient’s response to pancreatic enzyme supplementation, especially in patients with unexplained diarrhea and suspected pancreatic insufficiency. 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.

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

Calprotectin has been shown to correlate with histologic and endoscopic measures of inflammatory bowel disease severity. It is non-invasive, stable, and shows a considerable sensitivity and specificity of 93% and 96%, respectively, when used to screen for IBD activity. High calprotectin can also be detected in colorectal cancers, diverticular disease, and infectious gastroenteritis. 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). Fecal calprotectin can elevate with enteropathy caused by excessive non-steroidal anti-inflammatory medication use. For this reason, it may be beneficial to temporarily discontinue NSAIDs, when possible in select patients, prior to measuring fecal calprotectin.

**Figure 1.** The algorithm used to differentiate IBD from IBS using fecal calprotectin. Adapted from Walsham et al.
**Beta-glucuronidase** is an enzyme produced by cells in the liver, kidney, intestinal epithelium, endocrine, and reproductive organs. 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. 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. However, this enzyme can also convert pro-carcinogens to carcinogenic compounds.

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. Evidence of increased enzymatic activity of intestinal microorganisms may suggest increased risk of digestive tract cancer. 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.

**Steatocrit** has been used widely since 1981 to detect steatorrhea in patients with pancreatic insufficiency and small intestinal malabsorption. 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. Acidification of the stool dramatically improved the performance of this method. The acid steatocrit method has been shown to correlate well with 24-hour and 72-hour fecal fats.

**Zonulin** is a protein secreted by intestinal cells that regulates intercellular tight junctions. Tight junctions are the connections between epithelial cells that make up the gastrointestinal lining. Zonulin increases intestinal permeability in the jejunum and ileum and is considered a biomarker for barrier dysfunction.
permeability.\textsuperscript{1,114} 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.\textsuperscript{1} Certain gut bacteria and gliadin (the main staple protein from wheat) can activate the zonulin system.\textsuperscript{114,116}

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.\textsuperscript{117} 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,\textsuperscript{118} and obesity and metabolic disease.\textsuperscript{119} In many cases, permeability precedes disease.\textsuperscript{1}

Zonulin regulates barrier permeability. Serum zonulin correlates with intestinal permeability and lactulose/mannitol tests for intestinal permeability.\textsuperscript{115,120} High serum zonulin has been associated with celiac disease, type 1 diabetes,\textsuperscript{120} insulin resistance and type 2 diabetes,\textsuperscript{115} cancers, neurological conditions, and autoimmune diseases.\textsuperscript{1}

<table>
<thead>
<tr>
<th>Serum zonulin is high in a number of immune-mediated conditions :\textsuperscript{1}</th>
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<tbody>
<tr>
<td><strong>Autoimmune diseases</strong></td>
</tr>
<tr>
<td>Celiac disease</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
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<tr>
<td>Inflammatory bowel disease</td>
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<tr>
<td>Type 1 diabetes</td>
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<tr>
<td>Rheumatoid arthritis</td>
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<tr>
<td>Systemic lupus erythematos</td>
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<tr>
<td><strong>Cancers</strong></td>
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<tr>
<td>Brain (gliomas)</td>
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<tr>
<td>Breast</td>
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<td>Lung adenocarcinoma</td>
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<td>Ovarian</td>
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<td>Pancreatic</td>
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<tr>
<td><strong>Neurological diseases</strong></td>
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<tr>
<td>Multiple sclerosis</td>
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<tr>
<td>Chronic inflammatory demyelinating polyneuropathy</td>
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<tr>
<td>Schizophrenia</td>
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</table>

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.\textsuperscript{121} 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.\textsuperscript{114} Treatment with zeolite lowered stool levels of zonulin in athletes and presumably improved intestinal barrier function.\textsuperscript{122}
Drug Resistance Genes

Drug resistance genes are genes carried by bacteria that confer a special resistance or protection from certain antibiotics. For most antibiotics there are several different genes. The gene type is dependent on the mode of resistance and the organism(s) it may be found in. In the GI-MAP, the antibiotic and drug resistance genes for an antibiotic are measured based on the pathogenic organism found to be positive in the fecal sample. Results will be reported for the genotypic resistance, meaning the AR gene specific to the positive organism, and globally, meaning all other AR Genes for that drug found throughout the microbiota.

Herbal Antimicrobial Agents

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 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.

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Description and Clinical Use</th>
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<tbody>
<tr>
<td>Berberine</td>
<td>Berberine has shown effectiveness against ETEC-associated diarrhea and has been studied extensively for its antibacterial effect. It shows antimicrobial activity against fungi, protozoans, helminths, viruses, and chlamydia.</td>
</tr>
<tr>
<td>Garlic</td>
<td>Garlic has shown activity against bacteria, protozoa, helminths, viruses, and fungi. It strongly suppressed gram-negative diarrheagenic pathogens (Shigella, Salmonella, Proteus mirabilis, and E. coli) isolated from stool samples. Aqueous garlic extract inhibited E.coli O157:H7 and E. coli LF82 and enhanced the growth of Lactobacillus reuteri in vitro. This suggests that antimicrobial herbs may spare beneficial flora.</td>
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<tr>
<td>Olive leaf</td>
<td>Olive leaf has antibacterial, antifungal, and antiviral properties.</td>
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<tr>
<td>Caprylic acid</td>
<td>Caprylic acid reduces Campylobacter and Salmonella in the gastrointestinal tract and stool of poultry when added to the feed or water. Caprylic acid has antiviral and antifungal properties.</td>
</tr>
<tr>
<td>Wormwood</td>
<td>Artemisia annua (wormwood) demonstrates significant antimicrobial effects and has been used in the treatment of malaria and parasitic gastrointestinal infections.</td>
</tr>
<tr>
<td>Black walnut</td>
<td>Juglans nigra (Black Walnut) has a long history of use as an intestinal antiparasitic (i.e. vermicide, anthelminthic), antibacterial, and antifungal.</td>
</tr>
<tr>
<td>Uva ursi</td>
<td>Arctostaphylos uva-ursi leaves have been used worldwide as a diuretic, astringent, antiseptic, and treatment for urinary tract and gastrointestinal infections.</td>
</tr>
</tbody>
</table>
**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 can be used in the detection and identification of gastrointestinal microbial nucleic acids and has been clinically validated for the detection of gastrointestinal pathogens that cause infectious colitis or gastroenteritis. This technology has been used to identify and control pathogen outbreaks because of its rapid turn-around-time. It measures a substantial list of opportunistic pathogens as well as a list of FDA-cleared 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 to imbalanced gut microbes as a root cause. 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.

**Complete List of Target Analytes Measured on the GI-MAP**

**Bacterial pathogens:**
- *Campylobacter*
- *C. diff Toxin A & B***
- *E. coli* o157**
- *Enterotoxigenic* *E. coli* LT & ST (ETEC)**
- *Shiga-like Toxin producing* *E. coli* stx1 & stx2 (STEC)**
- *Salmonella*
- *Shigella*
- *Vibrio cholera*
- *Yersinia enterocolitica*

**Viral pathogens:**
- Adenovirus 40 & 41**
- Norovirus GI & GII**
- Rotavirus A**

**Parasitic pathogens**
- Cryptosporidium**
- *Entamoeba histolytica**
- *Giardia*

**Additional targets**
**Bacteria:**
*Helicobacter pylori and virulence factors, cagA and vacA***
*Enterococcus*
*Lactobacillus***
*Bifidobacter***
*Bacteroides spp.*
*Bacteroides fragilis grp*
*E. coli (total)*
*Citrobacter spp.*
*Citrobacter freundii*
*Proteus spp.*
*Proteus mirabilis*
*Proteus vulgaris*
*Pseudomonas spp.***
*Pseudomonas aeruginosa*
*Morganella spp.*
*Staphylococcus spp. (aureus)*
*Streptococcus spp.*
*Klebsiella spp.***
*Klebsiella pneumonieae*

**Parasites:**
*Blastocystis hominis***
*Dientamoeba fragilis*
*Endolimax nana*
*Entamoeba coli***
*Entamoeba hartmanni***
*Chilomastix mesnelli*
*Cyclospora cayetanenensis*
*Pentatrichomonas hominis*

**LDT fungi/yeast:**
*Microsporidia spp.* including *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis***
*Candida albicans*
*Candida spp.*
*Geotrichum spp.*
*Trichosporon spp.*

**Other tests:**
*Secretory IgA (sIgA)*
*Anti-gliadin sIgA*
*Pancreatic elastase 1*
*Beta glucuronidase*
*Calprotectin*
*Zonulin (available as an add-on test)*
*Steatocrit*
*Occult blood*

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