The GI Microbial Assay Plus

GI-MAP™

Quantitative PCR Stool Technology for the
Integrative and Functional Medicine Practitioner

Diagnostic Solutions laboratory




Contents

The GI Microbial Assay Plus (GI-MAP)

Quantitative PCR: Innovative Stool Testing for the Integrative and Functional Medicine Practitioner

Microbiology and DNA Analysis

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

The Gastrointestinal Microbial Assay Plus (GI-MAP) was designed to assess a patient's microbiome from a single stool sample, with particular attention to microbes that may be disturbing normal microbial balance and may contribute to perturbations in the gastrointestinal (GI) 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 *Campylobacte*r, Shigella, and rotavirus A.[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, Yersinia enterocolitica, and Proteus mirabilus*, 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[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]

Gastrointestinal symptoms, continued

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

New

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 Research and Development Department 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, 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).

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's 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 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.

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. 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 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 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 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.

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 flora can protect the host from infection. When gut microflora protects the intestines from pathogens and harmful microorganisms it is called, "colonization resistance." [32] 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.[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 with hundreds of specimens at Diagnostic Solutions Laboratory. The new qPCR assay has been calibrated against the previous assay to determine what quantity of genomic DNA constitutes a pathological threat. While the previous assay reported a "positive" or "negative" value only for pathogens, the qPCR assay will quantify the DNA and will indicate if it is high enough to cause pathology by medical standards.

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 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 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. 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.[45]

Multiplex PCR 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*.[46]

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.[46]

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.[47]

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. 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.[48]

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.[49] It is the largest health burden of all the bacterial pathogens.[50] *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. [49]

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.[49]

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. [49]

Food sources include:

Poultry	Tomatoes
Poultry products	Melons
Meat	Leafy greens
Dairy	Sprouts
Raw, fresh, ready-to-eat produce	Berries

Yersinia enterocolitica- food poisoning. Also an autoimmune trigger. Repeat below. Molecular mimicry.

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.[51] 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.[52] It may be asymptomatic or it can cause chornic 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:[52]

- 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%).[52]

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.[53] 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,[54] 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.[55] There are no standard treatments for viral gastroenteritis in healthy hosts. Antivirals are not recommended.[56] 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. [56]

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

Norovirus GI & GII, or Norwalk virus, is the most common cause of non-bacterial gastroenteritis in the world. It is widely known for causing the stomach flu on cruise ships.[57] 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.[58] 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.[59] 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.[60] 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.[61]

Helicobacter pylori

***H. pylori* and seven 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.[62] *H. pylori* has also been detected by stool PCR in cases of dyspepsia, abdominal pain, and chronic gastrointestinal symptoms.[63-65] 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[66] 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.[63,65,67] It has shown sensitivity and specificity reaching that of the diagnostic "gold standard," which is endoscopy with biopsy and urease test.[63-65] *H.pylori* genotyping may be useful for resistant *H. pylori* infections that have failed to respond to triple antibiotic therapy.[63] 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."[63]

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.[62] 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.[68] *H. pylori* may be asymptomatic and require no treatment or only supportive care to improve the intestinal mucosa and gastrointestinal lining. H. pylori may cause hypochlorhydria or hyperchlorhydria. 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.

Virulence Factors

New

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.[69]

CagA (Cytotoxin-associated protein A) presence in *H.pylori* strains has been significantly associated with gastric cancer and peptic ulcer.[70] 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.[70] These changes to normal cell signaling can initiate cancer.

Cag PAI (Cag pathogenicity island) 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 CagPAI is associated with highly virulent strains of H. pylori.[71]

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.[72]

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.[73,74]

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.[75]

Vacuolating toxin (vacA) has been associated with gastric cancer, peptic ulcer, and duodenal ulcer.[70] 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.[70]

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.[32] Research has demonstrated the microbiota's capacity to interact with the immune system as an important health benefit.[76] The microbiota also has anti-inflammatory and antioxidant activity.[77] 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.[78]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.[79] 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.[80]

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.[80]

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,[81] and help control intestinal pH.[82] *Bifidobacteria* and *Lactobacillus* help prevent the overgrowth of *Candida albicans*, *E. coli*, and other pathogenic bacteria. [34,83]

Clostridium spp.

Enterobacter spp.

New

Phyla Microbiota

New

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*.[84] 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, [85] 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.[86] 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.[85] 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.[84,85] 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.[87]

Table X. The bacterial species found in the *Firmicutes* and *Bacteroidetes* Phyla.

Firmicutes Phylum	*Bacteroidetes* Phylum
Clostridia spp.	Bacteroides spp.
Bacillus spp.	Prevotella spp.
Lactobacillus spp.	
Mycoplasma spp.	
Streptomyces spp.	

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[88] and higher antibodies to *Yersinia enterocolitica* have been found in these patients.[89] Enterovirus is also associated with immunogenic thyroiditis.[90] 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,[91] 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.[92,93] 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.[92]

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*[94,95] 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.[96,97] 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.[98,99]

Other data shows abnormal serum antibody responses to *Klebsiella* and *Proteus mirabilis* in the spondyloarthropathies,[97] 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.[93] 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.[100]

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.

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

Opportunistic Bacteria or Overgrowth Bacteria

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.[102] This bacterium exhibits enhanced virulence with stress, trauma, surgery, and cancer.[102] Symptoms of enteric infection include fever, dehydration, abdominal distention, diarrhea, and physical findings of Shanghai fever.[103] 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.

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.[104] 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.[104] Treatment often includes antifungal medications along with diet and nutritional interventions to help with chronic diarrhea.

Rhodotorula spp.

Trichosporon spp.

Viruses

New

Cytomegalovirus (CMV) is a herpes virus that has affected 60% of the US population.[105] 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. [106] 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.[107]

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. [105]

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.[106]

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.[108] 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.[109] The frequency of CMV infection in IBD patients was 10-36% and may contribute to colitis symptomology.[110] 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.[107] 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.[111]

CMV and Autoimmunity

CMV has been implicated in the development of autoimmune diseases: systemic lupus erythematosus, systemic sclerosis, diabetes mellitus type 1, and rheumatoid arthritis. In some autoimmune conditions, such as lupus and systemic sclerosis, patients have far higher antibodies against CMV than healthy controls. The high prevalence of CMV throughout the world's population (40-99%) makes it difficult to definitively prove a link between CMV and autoimmune conditions.[112]

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.[113] **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.[107]

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[114] and vitamin D may help prevent autoimmune sequellae of EBV.[115] 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.[116]

EBV blood antibodies are used to diagnose an EBV infection.[116] 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.[117] Past infections are usually characterized by antibodies to both VCA and EBNA and may be elevated years after the primary infection.[116]

EBV and Autoimmunity

Primary infection with EBV causes mononucleosis, Burkitt's lymphoma, gastric cancer, nasopharyngeal carcinoma, and autoimmune diseases.[68] EBV has a central role in the pathogenesis of systemic autoimmune diseases, specifically rheumatoid arthritis, systemic lupus erythematosus, and Sjogren's syndrome.[118] 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. [112] Other researchers suggest that EBV is a contributory factor in autoimmune thyroid disorders.[119]

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.[68] The frequency of EBV infection in IBD patients ranges from 30-64%.[107,110] 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.[110]

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.[120] 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.[121]

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[122] from tropical regions contaminated with feces have been known to be sources for outbreaks of Cyclosporiasis.[123] 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[124,125] such as chlorination. Cyclosporiasis is characterized by symptoms of prolonged watery diarrhea, intestinal distress, abdominal cramping, loss of appetite, weight loss, nausea, and vomiting.[126,127] 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.[126,128] In more persistent cases lasting more than seven days treatment with an antibiotic combination of trimethoprim and sulfamethoxazole,[129] 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.[130] There may also be an association between *Blastocystis* and chronic digestive disorders, such as irritable bowel syndrome.[131]

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.

Worms

Ancylostoma duodenale and *Necatur americanus* are roundworms commonly known to cause hookworm infection by penetrating the skin.[132] 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.[133] Hookworm infected 12-15% of schoolchildren in the southeastern U.S. in the 1970s. *N. americanus* may still be found in pockets of the

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

southeastern U.S.[134] Hookworm infection is associated with poverty, poor sanitation, inadequate housing construction, and lack of access to medications.[134]

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.[135,136] Hookworm infection may affect physical and cognitive growth of children.[134] Hookworm is contracted via skin contact with soil 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;[137] therefore pets may also be a source of exposure. In cases of heavy hookworm infection, symptomatic individuals can be treated with albendazole or mebendazole.[138] Individuals presenting with anemia may benefit from iron supplements.[139]

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.[140] *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.[140] In cases of heavy infections, symptomatic indivduals can be treated with albendazole and mebendazole.[138] Individuals presenting with anemia may benefit from iron supplements.[141]

Taenia (tapeworm) is known to cause intestinal taeniasis after ingestion of contaminated or undercooked pork (*Taenia solium*) or beef (*Taenia saginata*).[142,143] *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.[143-145] Humans are the only definitive host for both *T. solium* and *T. saginata*.[146] PCR methods are sensitive and specific for detecting Taenia species in stool.[144,147]

Infection usually involves just a single tapeworm after ingestion of undercooked pork or beef from infected animals that have ingested eggs or tapeworm segments.[147] Individuals with taeniasis are usually asymptomatic or have mild symptoms.[148] 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.[136,148] Adult worms can be eliminated with albendazole or praziquantel.[149,150]

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.[151] This immunoglobulin influences the gut microbiome[151] and helps to maintain barrier function[152] 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.[153] 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,[154,155] 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. [156] This test also accurately predicts a patient's response to pancreatic enzyme supplementation, especially in patients with unexplained diarrhea and suspected pancreatic insufficiency.[157] 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.[158]

In patients with pancreatic insufficiency, 80% responded favorably to supplementation with pancreatic enzyme therapy; with an average dose of 120,000 units of lipase.[159]

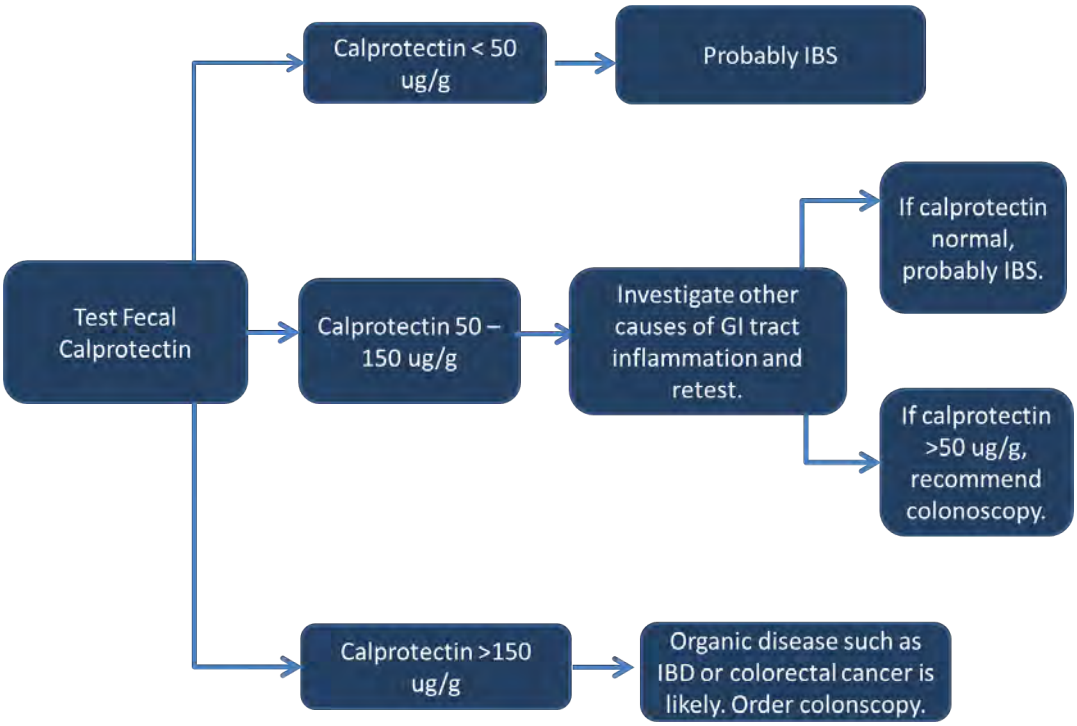
Table X. Staging of pancreatic insufficiency based on fecal elastase-1.

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

Fecal calprotectin is the most studied marker of gastrointestinal inflammation[160] and the gold standard marker for the diagnosis and monitoring of inflammatory bowel disease (IBD).[161] It is used to discriminate IBD from irritable bowel syndrome (IBS).[160,161] 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.[162] 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.[163] Fecal calprotectin levels are proportional to the level of neutrophil infiltration and inflammation in the gut.[162]

Calprotectin has been shown to correlate with histologic and endoscopic measures of inflammatory bowel disease severity.[163] It is non-invasive, stable,[161] and shows a considerable sensitivity and specificity of 93% and 96%, respectively, when used to screen for IBD activity.[164] High calprotectin can also be detected in colorectal cancers, diverticular disease, and infectious gastroenteritis.[160] 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).[160] Fecal calprotectin can elevate with enteropathy caused by excessive non-steroidal anti-inflammatory medication use.[162] For this reason, it may be beneficial to temporarily discontinue NSAIDs, when possible in select patients, prior to measuring fecal calprotectin.[162]

Figure 1. The algorithm used to differentiate IBD from IBS using fecal calprotectin. Adapted from Walsham et al.[160]



Beta-glucuronidase is an enzyme produced by cells in the liver, kidney, intestinal epithelium, endocrine, and reproductive organs.[165] 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.[166] 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.[166] However, this enzyme can also convert pro-carcinogens to carcinogenic compounds.[165]

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.[165] Evidence of increased enzymatic activity of intestinal microorganisms may suggest increased risk of digestive tract cancer.[167] 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.[165]

Steatocrit has been used widely since 1981 to detect steatorrhea in patients with pancreatic insufficiency and small intestinal malabsorption.[168] 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.[168] 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.[169,170]

Zonulin is a protein secreted by intestinal cells that regulates intercellular tight junctions.[1,171] Tight junctions are the connections between epithelial cells that make up the gastrointestinal lining. Zonulin increases intestinal permeability in the jejunum and ileum[172] and is considered a biomarker for barrier

permeability.[1,171] 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.[171,173]

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.[174] 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,[175] and obesity and metabolic disease.[176] In many cases, permeability precedes disease.[1]

Zonulin regulates barrier permeability. Serum zonulin correlates with intestinal permeability and lactulose/mannitol tests for intestinal permeability.[172,177] High serum zonulin has been associated with celiac disease, type 1 diabetes,[177] insulin resistance and type 2 diabetes,[172] cancers, neurological conditions, and autoimmune diseases.[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

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.[178] 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.[171] Treatment with zeolite lowered stool levels of zonulin in athletes and presumably improved intestinal barrier function.[179]

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

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,[180-182] 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.

Antimicrobial Agent	Description and Clinical Use
Berberine	Berberine has shown effectiveness against ETEC-associated diarrhea and has been studied extensively for its antibacterial effect.[183] It shows antimicrobial activity against fungi, protozoans, helminths, viruses, and chlamydia.[184]
Garlic	Garlic has shown activity against bacteria, protozoa, helminths, viruses, and fungi.[185,186] It strongly suppressed gram-negative diarrheagenic pathogens (*Shigella, Salmonella, Proteus mirabilis*, and *E. coli*) isolated from stool samples.[187] Aqueous garlic extract inhibited *E.coli* O157:H7 and *E. coli* LF82 and enhanced the growth of *Lactobacillus reuteri in vitro*.[188] This suggests that antimicrobial herbs may spare beneficial flora.
Olive leaf	Olive leaf has antibacterial, antifungal,[189,190] and antiviral properties.[191-193]
Caprylic acid	Caprylic acid reduces *Campylobacter* and *Salmonella* in the gastrointestinal tract and stool of poultry when added to the feed or water. [194-196] Caprylic acid has antiviral and antifungal properties.[197,198]
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 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.[5] This technology has been used to identify and control pathogen outbreaks because of its rapid turn-around-time.[5] 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.[199]

Complete List of Target Analytes Measured on the GI-MAP

Bacterial pathogens:
Campylobacter
C. diff Toxin A
*C. diff Toxin B***
Enterohemorrhagic E. coli
*E. coli O157***
Enteroinvasive E. coli/Shigella
Enteropathogenic E. coli
*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
Norovirus GII**

H. pylori
Helicobacter pylori
Virulence Factor, babA
Virulence Factor, cagA**
Virulence Factor, cagPAI
Virulence Factor, dupA
Virulence Factor, iceA
Virulence Factor, opiA
Virulence Factor vacA**
Normal/Commensal Bacterial Flora:
Bacteroides fragilis

*Bifidobacter spp.***
Clostridium spp.
Enterobacter spp.
Enterococcus spp.
Escherichia spp. (E. coli)
*Lactobacillus spp.***
Phyla Microbiota
Bacteroidetes
Firmicutes
F/B Ratio
Opportunistic Bacteria
Potential Autoimmune Triggers
Citrobacter spp.
Citrobacter freundii
*Klebsiella spp.***
Klebsiella pneumoniae
Mycobacterium avium
Prevotella copri
Proteus spp.
Proteus mirabilus
Additional Dysbiotic/Overgrowth Bacteria
Bacillus spp.
Enterococcus faecalis
Enterococcus facium
Morganella morganii
*Pseudomonas spp.***
Pseudomonas aeruginosa
Staphylococcus spp.
Staphylococcus aureus
Streptococcus spp.

Fungi/yeast:
Candida albicans
Candida spp.
Cyclospora cayetanenensis
Geotrichum spp.
Microsporidia spp. including *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* **
Rhodotorula spp.
Trichosporon spp.

Viruses:
CMV-Cytomegalovirus
EBV- Epstein Bar Virus

Parasites:
Protozoa
*Blastocystis hominis***
Chilomastix mesnelli

Cyclospora cayetanenensis
Dientamoeba fragilis
Endolimax nana
*Entamoeba coli***
Pentatrichomonas hominis (formerly Trichomonas vaginalis)

Worms
Ancylostoma duodenale
Ascaris lumbricoides
Necatur americanus
Trichuris trichiura
Taenia solium/saginata

Intestinal Health:
Digestion
Pancreatic elastase 1
Steatocrit
Immune Response
Secretory IgA (sIgA)
Anti-gliadin sIgA
Inflammation
Calprotectin
GI Markers
Beta-glucuronidase
Occult blood

Add-on Test
Zonulin

Antibiotic Resistance Genes, phenotypes
Helicobacter
Antibiotic Resistance Genes, genotypes
Universal Microbiota Resistance Genes
B-lactamase
Fluoroquinolones
Macrolides
Vancomycin

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

Revision 2. Updated to include changes to the GI-MAP methodology released 12/2017.

References

1. Fasano A. Intestinal permeability and its regulation by zonulin: diagnostic and therapeutic implications. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2012;10(10):1096-1100.
2. Dora-Laskey A. Ascaris Lumbricoides. 2016; https://emedicine.medscape.com/article/788398-overview. Accessed Dec 8, 2017.
3. Segata N, Haake SK, Mannon P, et al. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. *Genome Biol.* 2012;13(6):R42.
4. Wade W. Unculturable bacteria--the uncharacterized organisms that cause oral infections. *Journal of the Royal Society of Medicine.* 2002;95(2):81-83.
5. xTAG gastrointestinal pathogen panel. *Luminex* http://www.luminexcorp.com/Products/Assays/ClinicalDiagnostics/xTAGGPP/. Accessed April 9, 2015.
6. Vandenberg O, Peek R, Souayah H, et al. Clinical and microbiological features of dientamoebiasis in patients suspected of suffering from a parasitic gastrointestinal illness: a comparison of Dientamoeba fragilis and Giardia lamblia infections. *Int J Infect Dis.* 2006;10(3):255-261.
7. Quigley EM. What is the evidence for the use of probiotics in functional disorders? *Current gastroenterology reports.* 2008;10(4):379-384.
8. Ewaschuk JB, Tejpar QZ, Soo I, Madsen K, Fedorak RN. The role of antibiotic and probiotic therapies in current and future management of inflammatory bowel disease. *Current gastroenterology reports.* 2006;8(6):486-498.
9. Fujimori S, Tatsuguchi A, Gudis K, et al. High dose probiotic and prebiotic cotherapy for remission induction of active Crohn's disease. *Journal of gastroenterology and hepatology.* 2007;22(8):1199-1204.
10. Iijima Y, Asako NT, Aihara M, Hayashi K. Improvement in the detection rate of diarrhoeagenic bacteria in human stool specimens by a rapid real-time PCR assay. *Journal of medical microbiology.* 2004;53(Pt 7):617-622.
11. [A rare outbreak of food poisoning caused by Salmonella enterica serovar. Oranienburg--a case report and features of isolates]. *Kansenshogaku Zasshi.* 2007;81(3):242-248.
12. Yamaoka Y. Mechanisms of disease: Helicobacter pylori virulence factors. *Nature reviews. Gastroenterology & hepatology.* 2010;7(11):629-641.
13. Reina J, Hervas J, Borrell N. Acute gastroenteritis caused by Hafnia alvei in children. *Clin Infect Dis.* 1993;16(3):443.
14. Othman M, Aguero R, Lin HC. Alterations in intestinal microbial flora and human disease. *Current opinion in gastroenterology.* 2008;24(1):11-16.
15. Fayed SB, Aref MI, Fathy HM, et al. Prevalence of celiac disease, Helicobacter pylori and gastroesophageal reflux in patients with refractory iron deficiency anemia. *Journal of tropical pediatrics.* 2008;54(1):43-53.
16. Walker MM, Talley NJ. Review article: bacteria and pathogenesis of disease in the upper gastrointestinal tract--beyond the era of Helicobacter pylori. *Alimentary pharmacology & therapeutics.* 2014;39(8):767-779.
17. Dahlqvist G, Piessevaux H. Irritable bowel syndrome: the role of the intestinal microbiota, pathogenesis and therapeutic targets. *Acta gastro-enterologica Belgica.* 2011;74(3):375-380.
18. Pimentel M. Review of rifaximin as treatment for SIBO and IBS. *Expert opinion on investigational drugs.* 2009;18(3):349-358.
19. Cummings JH, Macfarlane GT, Macfarlane S. Intestinal bacteria and ulcerative colitis. *Current issues in intestinal microbiology.* 2003;4(1):9-20.

20. Whong CM, Kwaga JK, Amber EI. Enteropathogenicity of Bacillus cereus isolated from some Nigerian foods. *West African journal of medicine.* 2009;28(2):130-133.
21. Brent LH, Diamond HS. Ankylosing spondylitis and undifferentiated spondyloarthropathy. *Drugs & Diseases* 2015; http://emedicine.medscape.com/article/332945-overview. Accessed May 14, 2015.
22. Palazzi C, Olivieri I, D'Amico E, Pennese E, Petricca A. Management of reactive arthritis. *Expert opinion on pharmacotherapy.* 2004;5(1):61-70.
23. Leirisalo-Repo M, Hannu T, Mattila L. Microbial factors in spondyloarthropathies: insights from population studies. *Current opinion in rheumatology.* 2003;15(4):408-412.
24. Rashid T, Ebringer A. Autoimmunity in Rheumatic Diseases Is Induced by Microbial Infections via Crossreactivity or Molecular Mimicry. *Autoimmune diseases.* 2012;2012:539282.
25. Panzer AR, Lynch SV. Influence and effect of the human microbiome in allergy and asthma. *Current opinion in rheumatology.* 2015;27(4):373-380.
26. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. *The Journal of allergy and clinical immunology.* 2012;129(2):434-440, 440 e431-432.
27. Kukkonen K, Savilahti E, Haahtela T, et al. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *The Journal of allergy and clinical immunology.* 2007;119(1):192-198.
28. West CE. Gut microbiota and allergic disease: new findings. *Current opinion in clinical nutrition and metabolic care.* 2014;17(3):261-266.
29. qPCR vs. Digital PCR vs. Traditional PCR. http://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/qpcr-education/qpcr-vs-digital-pcr-vs-traditional-pcr.html.
30. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature.* 2006;444(7122):1022-1023.
31. Palva A. [Intestinal microorganisms and their significance for health]. *Duodecim; laaketieteellinen aikakauskirja.* 2009;125(6):685-694.
32. Kamada N, Seo SU, Chen GY, Nunez G. Role of the gut microbiota in immunity and inflammatory disease. *Nature reviews. Immunology.* 2013;13(5):321-335.
33. Canny GO, McCormick BA. Bacteria in the intestine, helpful residents or enemies from within? *Infection and immunity.* 2008;76(8):3360-3373.
34. Stecher B, Hardt WD. The role of microbiota in infectious disease. *Trends in microbiology.* 2008;16(3):107-114.
35. Chow J. Probiotics and prebiotics: A brief overview. *J Ren Nutr.* 2002;12(2):76-86.
36. Ramakrishna BS. Role of the gut microbiota in human nutrition and metabolism. *Journal of gastroenterology and hepatology.* 2013;28 Suppl 4:9-17.
37. Hijova E, Chmelarova A. Short chain fatty acids and colonic health. *Bratislavske lekarske listy.* 2007;108(8):354-358.
38. Moore WE, Holdeman LV. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl Microbiol.* 1974;27(5):961-979.
39. Abubakar I, Irvine L, Aldus CF, et al. A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. *Health Technol Assess.* 2007;11(36):1-216.
40. Kahlau P, Malecki M, Schildgen V, et al. Utility of two novel multiplexing assays for the detection of gastrointestinal pathogens - a first experience. *SpringerPlus.* 2013;2(1):106.
41. Fukushima Y, Kawata Y, Hara H, Terada A, Mitsuoka T. Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. *International journal of food microbiology.* 1998;42(1-2):39-44.

42. Aberra F, Curry JA. Clostridium Difficile Colitis. *Drugs & Diseases* 2014; http://emedicine.medscape.com/article/186458-overview#a0101. Accessed April 20, 2015.
43. Carter GP, Rood JI, Lyras D. The role of toxin A and toxin B in Clostridium difficile-associated disease: Past and present perspectives. *Gut Microbes.* 2010;1(1):58-64.
44. E. coli (Escherichia coli). 2014; http://www.cdc.gov/ecoli/general/index.html. Accessed April 17, 2015.
45. Madappa T, Hiong C. Escherichia Coli Infections. *Drugs & Diseases* 2014; http://emedicine.medscape.com/article/217485-overview#a0104. Accessed April 17, 2015.
46. Malecki M, Schildgen V, Kamm M, Mattner F, Schildgen O. Rapid screening method for multiple gastroenteric pathogens also detects novel enterohemorrhagic Escherichia coli O104:H4. *Am J Infect Control.* 2012;40(1):82-83.
47. Hughes JM, Murad F, Chang B, Guerrant RL. Role of cyclic GMP in the action of heat-stable enterotoxin of Escherichia coli. *Nature.* 1978;271(5647):755-756.
48. Jinneman KC, Yoshitomi KJ, Weagant SD. Multiplex real-time PCR method to identify Shiga toxin genes stx1 and stx2 and Escherichia coli O157:H7/H- serotype. *Applied and environmental microbiology.* 2003;69(10):6327-6333.
49. Bell RL, Jarvis KG, Ottesen AR, McFarland MA, Brown EW. Recent and emerging innovations in Salmonella detection: a food and environmental perspective. *Microb Biotechnol.* 2016;9(3):279-292.
50. Zhang S, Yin Y, Jones MB, et al. Salmonella serotype determination utilizing high-throughput genome sequencing data. *Journal of clinical microbiology.* 2015;53(5):1685-1692.
51. Petri WA, Haque R. Entamoeba histolytica brain abscess. *Handbook of clinical neurology.* 2013;114:147-152.
52. Nazer H. Giardiasis. 2016; https://emedicine.medscape.com/article/176718-overview. Accessed Nov 27, 2017.
53. Amar CF, East CL, Gray J, Iturriza-Gomara M, Maclure EA, McLauchlin J. Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case-control Infectious Intestinal Disease Study (1993-1996). *Eur J Clin Microbiol Infect Dis.* 2007;26(5):311-323.
54. Huhulescu S, Kiss R, Brettlecker M, et al. Etiology of acute gastroenteritis in three sentinel general practices, Austria 2007. *Infection.* 2009;37(2):103-108.
55. Seitz SR, Leon JS, Schwab KJ, et al. Norovirus infectivity in humans and persistence in water. *Applied and environmental microbiology.* 2011;77(19):6884-6888.
56. Gompf SG, Cunha BA. Adenoviruses clinical presentation. *Drugs & Diseases* 2014; http://emedicine.medscape.com/article/211738-clinical. Accessed May 19, 2015.
57. Benaroch R. Norovirus: symptoms and treatment. *WebMD* 2012; http://www.webmd.com/children/norovirus-symptoms-and-treatment?page=2. Accessed 6/3/2015, 2015.
58. Noel JS, Fankhauser RL, Ando T, Monroe SS, Glass RI. Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution. *The Journal of infectious diseases.* 1999;179(6):1334-1344.
59. D'Souza DH, Sair A, Williams K, et al. Persistence of caliciviruses on environmental surfaces and their transfer to food. *International journal of food microbiology.* 2006;108(1):84-91.
60. Atmar RL, Estes MK. The epidemiologic and clinical importance of norovirus infection. *Gastroenterology clinics of North America.* 2006;35(2):275-290, viii.
61. Khan Z, Cunha BA. Norwalk virus. *Drugs & Diseases* 2013; http://emedicine.medscape.com/article/224225-overview. Accessed May 19, 2015.

62. Abadi AT, Taghvaei T, Wolfram L, Kusters JG. Infection with Helicobacter pylori strains lacking dupA is associated with an increased risk of gastric ulcer and gastric cancer development. *Journal of medical microbiology.* 2012;61(Pt 1):23-30.
63. Schabereiter-Gurtner C, Hirschl AM, Dragosics B, et al. Novel real-time PCR assay for detection of Helicobacter pylori infection and simultaneous clarithromycin susceptibility testing of stool and biopsy specimens. *Journal of clinical microbiology.* 2004;42(10):4512-4518.
64. Mishra S, Singh V, Rao GR, et al. Detection of Helicobacter pylori in stool specimens: comparative evaluation of nested PCR and antigen detection. *J Infect Dev Ctries.* 2008;2(3):206-210.
65. Weiss J, Tsang TK, Meng X, et al. Detection of Helicobacter pylori gastritis by PCR: correlation with inflammation scores and immunohistochemical and CLOtest findings. *Am J Clin Pathol.* 2008;129(1):89-96.
66. Thrift AP, Pandeya N, Smith KJ, et al. Helicobacter pylori infection and the risks of Barrett's oesophagus: a population-based case-control study. *Int J Cancer.* 2012;130(10):2407-2416.
67. Yan WH, Chen J, Yu JD, Li ZY, Huang XL, Zhang XP. [Rapid detection of clarithromycin resistant Helicobacter pylori from children's gastric biopsy specimens by polymerase chain reaction-restriction fragment length polymorphism]. *Zhonghua er ke za zhi.* 2009;47(11):848-851.
68. Singh S, Jha HC. Status of Epstein-Barr Virus Coinfection with Helicobacter pylori in Gastric Cancer. *J Oncol.* 2017;2017:3456264.
69. Ansari S, Yamaoka Y. Helicobacter pylori BabA in adaptation for gastric colonization. *World J Gastroenterol.* 2017;23(23):4158-4169.
70. Basso D, Zambon CF, Letley DP, et al. Clinical relevance of Helicobacter pylori cagA and vacA gene polymorphisms. *Gastroenterology.* 2008;135(1):91-99.
71. Naumann M, Sokolova O, Tegtmeyer N, Backert S. Helicobacter pylori: A Paradigm Pathogen for Subverting Host Cell Signal Transmission. *Trends in microbiology.* 2017;25(4):316-328.
72. Talebi Bezmin Abadi A, Perez-Perez G. Role of dupA in virulence of Helicobacter pylori. *World J Gastroenterol.* 2016;22(46):10118-10123.
73. Dabiri H, Jafari F, Baghaei K, et al. Prevalence of Helicobacter pylori vacA, cagA, cagE, oipA, iceA, babA2 and babB genotypes in Iranian dyspeptic patients. *Microbial pathogenesis.* 2017;105:226-230.
74. Yakoob J, Abbas Z, Khan R, et al. Helicobacter pylori: correlation of the virulence marker iceA allele with clinical outcome in a high prevalence area. *Br J Biomed Sci.* 2015;72(2):67-73.
75. Horridge DN, Begley AA, Kim J, Aravindan N, Fan K, Forsyth MH. Outer inflammatory protein a (OipA) of Helicobacter pylori is regulated by host cell contact and mediates CagA translocation and interleukin-8 response only in the presence of a functional cag pathogenicity island type IV secretion system. *Pathogens and disease.* 2017;75(8).
76. Martin R, Miquel S, Ulmer J, Kechaou N, Langella P, Bermudez-Humaran LG. Role of commensal and probiotic bacteria in human health: a focus on inflammatory bowel disease. *Microbial cell factories.* 2013;12:71.
77. Khanna S, Tosh PK. A clinician's primer on the role of the microbiome in human health and disease. *Mayo Clinic proceedings.* 2014;89(1):107-114.
78. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature.* 2012;489(7415):220-230.
79. Lobo LA, Benjamim CF, Oliveira AC. The interplay between microbiota and inflammation: lessons from peritonitis and sepsis. *Clin Transl Immunology.* 2016;5(7):e90.
80. Maier E, Anderson RC, Roy NC. Understanding how commensal obligate anaerobic bacteria regulate immune functions in the large intestine. *Nutrients.* 2014;7(1):45-73.

81. Sjogren YM, Tomicic S, Lundberg A, et al. Influence of early gut microbiota on the maturation of childhood mucosal and systemic immune responses. *Clin Exp Allergy.* 2009;39(12):1842-1851.
82. de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. *Advances in biochemical engineering/biotechnology.* 2008;111:1-66.
83. Sheil B, Shanahan F, O'Mahony L. Probiotic effects on inflammatory bowel disease. *J Nutr.* 2007;137(3 Suppl 2):819S-824S.
84. Abdallah Ismail N, Ragab SH, Abd Elbaky A, Shoeib AR, Alhosary Y, Fekry D. Frequency of Firmicutes and Bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. *Archives of medical science : AMS.* 2011;7(3):501-507.
85. Xiao L, Sonne SB, Feng Q, et al. High-fat feeding rather than obesity drives taxonomical and functional changes in the gut microbiota in mice. *Microbiome.* 2017;5(1):43.
86. Armougom F, Raoult D. Use of pyrosequencing and DNA barcodes to monitor variations in Firmicutes and Bacteroidetes communities in the gut microbiota of obese humans. *BMC genomics.* 2008;9:576.
87. de Souza AZ, Zambom AZ, Abboud KY, et al. Oral supplementation with L-glutamine alters gut microbiota of obese and overweight adults: A pilot study. *Nutrition.* 2015;31(6):884-889.
88. Tomer Y, Davies TF. Infection, thyroid disease, and autoimmunity. *Endocr Rev.* 1993;14(1):107-120.
89. Petru G, Stunzner D, Lind P, Eber O, Mose JR. [Antibodies to Yersinia enterocolitica in immunogenic thyroid diseases]. *Acta medica Austriaca.* 1987;14(1):11-14.
90. Desailloud R, Hober D. Viruses and thyroiditis: an update. *Virology journal.* 2009;6:5.
91. DeMeo MT, Mutlu EA, Keshavarzian A, Tobin MC. Intestinal permeation and gastrointestinal disease. *Journal of clinical gastroenterology.* 2002;34(4):385-396.
92. Fasano A, Shea-Donohue T. Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. *Nature clinical practice.* 2005;2(9):416-422.
93. Tiwana H, Wilson C, Walmsley RS, et al. Antibody responses to gut bacteria in ankylosing spondylitis, rheumatoid arthritis, Crohn's disease and ulcerative colitis. *Rheumatology international.* 1997;17(1):11-16.
94. Ebringer A, Rashid T. Rheumatoid arthritis is an autoimmune disease triggered by Proteus urinary tract infection. *Clinical & developmental immunology.* 2006;13(1):41-48.
95. Ebringer A, Rashid T, Wilson C. Rheumatoid arthritis: proposal for the use of anti-microbial therapy in early cases. *Scandinavian journal of rheumatology.* 2003;32(1):2-11.
96. Minerva P, Diamond HS. Enteropathic arthropathies. *Drugs & Diseases* 2014; http://emedicine.medscape.com/article/334746-overview#a0101. Accessed May 14, 2015.
97. Ebringer A, Wilson C. HLA molecules, bacteria and autoimmunity. *Journal of medical microbiology.* 2000;49(4):305-311.
98. Zambrano-Zaragoza JF, de Jesus Duran-Avelar M, Rodriguez-Ocampo AN, et al. The 30-kDa band from Salmonella typhimurium: IgM, IgA and IgG antibody response in patients with ankylosing spondylitis. *Rheumatology (Oxford, England).* 2009;48(7):748-754.
99. Duran-Avelar M, Vibanco-Perez N, Rodriguez-Ocampo A, Pena-Virgen S, Zambrano-Zaragoza J. Lymphoproliferative response to the 30-kDa protein and a crude lysate from Salmonella typhimurium in patients with ankylosing spondylitis. *Scandinavian journal of rheumatology.* 2013;42(3):232-234.
100. Lozada CJ, Diamond HS. Reactive arthritis. *Drugs & Diseases* 2014; http://emedicine.medscape.com/article/331347-overview#aw2aab6b2b2. Accessed May 14, 2015.

101. Keyzer JJ, van Saene HK, van den Berg GA, Wolthers BG. Influence of decontamination of the digestive tract on the urinary excretion of histamine and some of its metabolites. *Agents and actions.* 1984;15(3-4):238-241.
102. Markou P, Apidianakis Y. Pathogenesis of intestinal Pseudomonas aeruginosa infection in patients with cancer. *Frontiers in cellular and infection microbiology.* 2014;3:115.
103. Chuang CH, Wang YH, Chang HJ, et al. Shanghai fever: a distinct Pseudomonas aeruginosa enteric disease. *Gut.* 2014;63(5):736-743.
104. Ghosh K, Weiss LM. Molecular diagnostic tests for microsporidia. *Interdisciplinary perspectives on infectious diseases.* 2009;2009:926521.
105. Akhter K. Cytomegalovirus Clinical Presentation. 2017; https://emedicine.medscape.com/article/215702-clinical. Accessed Nov 8, 2017.
106. Cytomegalovirus (CMV) and Congenital CMV Infection. https://www.cdc.gov/cmv/clinical/features.html.
107. Nahar S, Iraha A, Hokama A, et al. Evaluation of a multiplex PCR assay for detection of cytomegalovirus in stool samples from patients with ulcerative colitis. *World J Gastroenterol.* 2015;21(44):12667-12675.
108. Prachasitthisak N, Tanpowpong P, Lertudomphonwanit C, et al. Short article: Stool cytomegalovirus polymerase chain reaction for the diagnosis of cytomegalovirus-related gastrointestinal disease. *European journal of gastroenterology & hepatology.* 2017;29(9):1059-1063.
109. Thorn M, Rorsman F, Ronnblom A, et al. Active cytomegalovirus infection diagnosed by real-time PCR in patients with inflammatory bowel disease: a prospective, controlled observational study (.). *Scandinavian journal of gastroenterology.* 2016;51(9):1075-1080.
110. Ciccocioppo R, Racca F, Paolucci S, et al. Human cytomegalovirus and Epstein-Barr virus infection in inflammatory bowel disease: need for mucosal viral load measurement. *World J Gastroenterol.* 2015;21(6):1915-1926.
111. Chan KS, Lee WY, Yu WL. Coexisting cytomegalovirus infection in immunocompetent patients with Clostridium difficile colitis. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi.* 2016;49(6):829-836.
112. Halenius A, Hengel H. Human cytomegalovirus and autoimmune disease. *BioMed research international.* 2014;2014:472978.
113. !!! INVALID CITATION !!! {, #5805;Dittfeld, 2016 #5819}.
114. Ryu E, Son M, Lee M, et al. Cordycepin is a novel chemical suppressor of Epstein-Barr virus replication. *Oncoscience.* 2014;1(12):866-881.
115. Pender MP. CD8+ T-Cell Deficiency, Epstein-Barr Virus Infection, Vitamin D Deficiency, and Steps to Autoimmunity: A Unifying Hypothesis. *Autoimmune diseases.* 2012;2012:189096.
116. Epstein-Barr Virus and Infectious Mononucleosis. https://www.cdc.gov/epstein-barr/about-ebv.html. Accessed Nov 16, 2017.
117. Klutts JS, Ford BA, Perez NR, Gronowski AM. Evidence-based approach for interpretation of Epstein-Barr virus serological patterns. *Journal of clinical microbiology.* 2009;47(10):3204-3210.
118. Draborg AH, Duus K, Houen G. Epstein-Barr virus in systemic autoimmune diseases. *Clinical & developmental immunology.* 2013;2013:535738.
119. Dittfeld A, Gwizdek K, Michalski M, Wojnicz R. A possible link between the Epstein-Barr virus infection and autoimmune thyroid disorders. *Cent Eur J Immunol.* 2016;41(3):297-301.
120. Dagci H, Kurt O, Demirel M, et al. Epidemiological and diagnostic features of blastocystis infection in symptomatic patients in izmir province, Turkey. *Iranian journal of parasitology.* 2014;9(4):519-529.

121. Basak S, Rajurkar MN, Mallick SK. Detection of Blastocystis hominis: a controversial human pathogen. *Parasitology research.* 2014;113(1):261-265.
122. Abanyie F, Harvey RR, Harris JR, et al. 2013 multistate outbreaks of Cyclospora cayetanensis infections associated with fresh produce: focus on the Texas investigations. *Epidemiol Infect.* 2015;143(16):3451-3458.
123. Ghozzi K, Marangi M, Papini R, et al. First report of Tunisian coastal water contamination by protozoan parasites using mollusk bivalves as biological indicators. *Marine pollution bulletin.* 2017;117(1-2):197-202.
124. Giangaspero A, Marangi M, Koehler AV, et al. Molecular detection of Cyclospora in water, soil, vegetables and humans in southern Italy signals a need for improved monitoring by health authorities. *International journal of food microbiology.* 2015;211:95-100.
125. Kitajima M, Haramoto E, Iker BC, Gerba CP. Occurrence of Cryptosporidium, Giardia, and Cyclospora in influent and effluent water at wastewater treatment plants in Arizona. *Sci Total Environ.* 2014;484:129-136.
126. Milord F, Lampron-Goulet E, St-Amour M, Levac E, Ramsay D. Cyclospora cayetanensis: a description of clinical aspects of an outbreak in Quebec, Canada. *Epidemiol Infect.* 2012;140(4):626-632.
127. Orozco-Mosqueda GE, Martinez-Loya OA, Ortega YR. Cyclospora cayetanensis in a pediatric hospital in Morelia, Mexico. *Am J Trop Med Hyg.* 2014;91(3):537-540.
128. Kasper MR, Lescano AG, Lucas C, et al. Diarrhea outbreak during U.S. military training in El Salvador. *PLoS ONE.* 2012;7(7):e40404.
129. Tas Cengiz Z, Beyhan YE, Yilmaz H. Cyclospora cayetanensis, Opportunistic Protozoan Parasite, in Van Province, Turkey: A Report of Seven Cases. *Turkiye parazitolojii dergisi.* 2016;40(3):166-168.
130. Coyle CM, Varughese J, Weiss LM, Tanowitz HB. Blastocystis: to treat or not to treat. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2012;54(1):105-110.
131. Tan KS. New insights on classification, identification, and clinical relevance of Blastocystis spp. *Clinical microbiology reviews.* 2008;21(4):639-665.
132. Khurana S, Sethi S. Laboratory diagnosis of soil transmitted helminthiasis. *Tropical parasitology.* 2017;7(2):86-91.
133. Incani RN, Ferrer E, Hoek D, et al. Diagnosis of intestinal parasites in a rural community of Venezuela: Advantages and disadvantages of using microscopy or RT-PCR. *Acta tropica.* 2017;167:64-70.
134. Haburchak DR. Hookworm Disease. 2016; https://emedicine.medscape.com/article/218805-overview#a5. Accessed Dec 8, 2017.
135. Koh KH, Kim SW, Lee SY, et al. A case of parasite invasion of the intestinal tract: a missed diagnosis in irritable bowel syndrome. *Clinical endoscopy.* 2013;46(6):671-674.
136. Omran E, Mohammad AN. INTESTINAL PARASITES IN PATIENTS WITH CHRONIC ABDOMINAL PAIN. *Journal of the Egyptian Society of Parasitology.* 2015;45(2):389-396.
137. Chidambaram M, Parija SC, Toi PC, et al. Evaluation of the utility of conventional polymerase chain reaction for detection and species differentiation in human hookworm infections. *Tropical parasitology.* 2017;7(2):111-116.
138. Moser W, Schindler C, Keiser J. Efficacy of recommended drugs against soil transmitted helminths: systematic review and network meta-analysis. *BMJ (Clinical research ed.* 2017;358:j4307.
139. Wei KY, Yan Q, Tang B, et al. Hookworm Infection: A Neglected Cause of Overt Obscure Gastrointestinal Bleeding. *The Korean journal of parasitology.* 2017;55(4):391-398.

140. Zanwar VG, Pawar SV, Jain SS, Rathi SP, Contractor QQ, Rathi PM. An unusual cause of overt gastrointestinal bleeding in a malnourished child. *Tropical doctor.* 2016;46(2):100-102.
141. Arinola GO, Morenikeji OA, Akinwande KS, et al. Serum Micronutrients in Helminth-infected Pregnant Women and Children: Suggestions for Differential Supplementation During Anti-helminthic Treatment. *Annals of global health.* 2015;81(5):705-710.
142. Rostami S, Salavati R, Beech RN, Babaei Z, Sharbatkhori M, Harandi MF. Genetic variability of Taenia saginata inferred from mitochondrial DNA sequences. *Parasitol Res.* 2015;114(4):1365-1376.
143. Murrell KD. Zoonotic foodborne parasites and their surveillance. *Revue scientifique et technique (International Office of Epizootics).* 2013;32(2):559-569.
144. Ng-Nguyen D, Stevenson MA, Dorny P, et al. Comparison of a new multiplex real-time PCR with the Kato Katz thick smear and copro-antigen ELISA for the detection and differentiation of Taenia spp. in human stools. *PLoS Negl Trop Dis.* 2017;11(7):e0005743.
145. Hailemariam Z, Nakao M, Menkir S, et al. Molecular identification of species of Taenia causing bovine cysticercosis in Ethiopia. *Journal of helminthology.* 2014;88(3):376-380.
146. Ito A, Yanagida T, Nakao M. Recent advances and perspectives in molecular epidemiology of Taenia solium cysticercosis. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases.* 2016;40:357-367.
147. Roelfsema JH, Nozari N, Pinelli E, Kortbeek LM. Novel PCRs for differential diagnosis of cestodes. *Exp Parasitol.* 2016;161:20-26.
148. Nanjappa S. Taenia Infection Clinical Presentation. 2015; https://emedicine.medscape.com/article/999727-clinical?src=refgatesrc1. Accessed Dec 8, 2017.
149. Kungu JM, Dione MM, Ejobi F, Ocaido M, Grace D. Risk factors, perceptions and practices associated with Taenia solium cysticercosis and its control in the smallholder pig production systems in Uganda: a cross-sectional survey. *BMC infectious diseases.* 2017;17(1):1.
150. Braae UC, Magnussen P, Ndawi B, Harrison W, Lekule F, Johansen MV. Effect of repeated mass drug administration with praziquantel and track and treat of taeniosis cases on the prevalence of taeniosis in Taenia solium endemic rural communities of Tanzania. *Acta tropica.* 2017;165:246-251.
151. Rogier EW, Frantz AL, Bruno ME, Kaetzel CS. Secretory IgA is Concentrated in the Outer Layer of Colonic Mucus along with Gut Bacteria. *Pathogens.* 2014;3(2):390-403.
152. Corthesy B. Secretory immunoglobulin A: well beyond immune exclusion at mucosal surfaces. *Immunopharmacology and immunotoxicology.* 2009;31(2):174-179.
153. Kaetzel CS. Cooperativity among secretory IgA, the polymeric immunoglobulin receptor, and the gut microbiota promotes host-microbial mutualism. *Immunology letters.* 2014;162(2 Pt A):10-21.
154. Haas L, Meillet D, Kapel N, Rostoker G, Gobert JG. Increased concentrations of fecal anti-gliadin IgA antibodies in untreated celiac disease. *Clinical chemistry.* 1993;39(4):696-697.
155. Halblaub JM, Renno J, Kempf A, Bartel J, Schmidt-Gayk H. Comparison of different salivary and fecal antibodies for the diagnosis of celiac disease. *Clinical laboratory.* 2004;50(9-10):551-557.
156. Turner RC, McDermott R. Using faecal elastase-1 to screen for chronic pancreatitis in patients admitted with acute pancreatitis. *HPB : the official journal of the International Hepato Pancreato Biliary Association.* 2006;8(3):223-226.
157. Elphick DA, Kapur K. Comparing the urinary pancreolauryl ratio and faecal elastase-1 as indicators of pancreatic insufficiency in clinical practice. *Pancreatology : official journal of the International Association of Pancreatology.* 2005;5(2-3):196-200.

158. Cavalot F, Bonomo K, Fiora E, Gaia E, Trovati M. Pancreatic elastase-1 in stools, a marker of exocrine pancreas function, correlates with both residual beta-cell secretion and metabolic control in type 1 diabetic subjects: response to Mueller et al. *Diabetes care.* 2005;28(11):2810-2811.
159. Campbell JA, Sanders DS, Francis KA, et al. Should we Investigate Gastroenterology Patients for Pancreatic Exocrine Insufficiency? A Dual Centre UK Study. *Journal of gastrointestinal and liver diseases : JGLD.* 2016;25(3):303-309.
160. Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. *Clinical and experimental gastroenterology.* 2016;9:21-29.
161. Siddiqui I, Majid H, Abid S. Update on clinical and research application of fecal biomarkers for gastrointestinal diseases. *World J Gastrointest Pharmacol Ther.* 2017;8(1):39-46.
162. Klingberg E, Strid H, Stahl A, et al. A longitudinal study of fecal calprotectin and the development of inflammatory bowel disease in ankylosing spondylitis. *Arthritis research & therapy.* 2017;19(1):21.
163. El-Matary W, Abej E, Deora V, Singh H, Bernstein CN. Impact of Fecal Calprotectin Measurement on Decision-making in Children with Inflammatory Bowel Disease. *Frontiers in pediatrics.* 2017;5:7.
164. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ (Clinical research ed.* 2010;341:c3369.
165. Mroczynska M, Galecka M, Szachta P, Kamoda D, Libudzisz Z, Roszak D. Beta-glucuronidase and Beta-glucosidase activity in stool specimens of children with inflammatory bowel disease. *Polish journal of microbiology / Polskie Towarzystwo Mikrobiologow = The Polish Society of Microbiologists.* 2013;62(3):319-325.
166. Li Y, Zhang X, Wang L, Zhou Y, Hassan JS, Li M. Distribution and gene mutation of enteric flora carrying beta-glucuronidase among patients with colorectal cancer. *Int J Clin Exp Med.* 2015;8(4):5310-5316.
167. Mroczynska M, Libudzisz Z. Beta-glucuronidase and beta-glucosidase activity of Lactobacillus and Enterococcus isolated from human feces. *Polish journal of microbiology / Polskie Towarzystwo Mikrobiologow = The Polish Society of Microbiologists.* 2010;59(4):265-269.
168. Ramakrishna BS. The steatocrit as a measure of fecal fat excretion: uses and pitfalls. *Indian journal of gastroenterology : official journal of the Indian Society of Gastroenterology.* 2009;28(6):195-197.
169. Amann ST, Josephson SA, Toskes PP. Acid steatocrit: a simple, rapid gravimetric method to determine steatorrhea. *The American journal of gastroenterology.* 1997;92(12):2280-2284.
170. Bijoor AR, Geetha S, Venkatesh T. Faecal fat content in healthy adults by the 'acid steatocrit method'. *Indian J Clin Biochem.* 2004;19(2):20-22.
171. Lamprecht M, Bogner S, Schippinger G, et al. Probiotic supplementation affects markers of intestinal barrier, oxidation, and inflammation in trained men; a randomized, double-blinded, placebo-controlled trial. *Journal of the International Society of Sports Nutrition.* 2012;9(1):45.
172. Stenman LK, Lehtinen MJ, Meland N, et al. Probiotic With or Without Fiber Controls Body Fat Mass, Associated With Serum Zonulin, in Overweight and Obese Adults-Randomized Controlled Trial. *EBioMedicine.* 2016;13:190-200.
173. Fasano A, Sapone A, Zevallos V, Schuppan D. Nonceliac gluten sensitivity. *Gastroenterology.* 2015;148(6):1195-1204.
174. Fasano A. Leaky gut and autoimmune diseases. *Clinical reviews in allergy & immunology.* 2012;42(1):71-78.

175. Fasano A. Physiological, pathological, and therapeutic implications of zonulin-mediated intestinal barrier modulation: living life on the edge of the wall. *The American journal of pathology.* 2008;173(5):1243-1252.
176. Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability--a new target for disease prevention and therapy. *BMC gastroenterology.* 2014;14:189.
177. Wang L, Llorente C, Hartmann P, Yang AM, Chen P, Schnabl B. Methods to determine intestinal permeability and bacterial translocation during liver disease. *J Immunol Methods.* 2015;421:44-53.
178. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. *Journal of cell science.* 2000;113 Pt 24:4435-4440.
179. Lamprecht M, Bogner S, Steinbauer K, et al. Effects of zeolite supplementation on parameters of intestinal barrier integrity, inflammation, redoxbiology and performance in aerobically trained subjects. *Journal of the International Society of Sports Nutrition.* 2015;12:40.
180. Hannan A, Ikram Ullah M, Usman M, Hussain S, Absar M, Javed K. Anti-mycobacterial activity of garlic (Allium sativum) against multi-drug resistant and non-multi-drug resistant mycobacterium tuberculosis. *Pakistan journal of pharmaceutical sciences.* 2011;24(1):81-85.
181. Karuppiah P, Rajaram S. Antibacterial effect of Allium sativum cloves and Zingiber officinale rhizomes against multiple-drug resistant clinical pathogens. *Asian Pacific journal of tropical biomedicine.* 2012;2(8):597-601.
182. Gull I, Saeed M, Shaukat H, Aslam SM, Samra ZQ, Athar AM. Inhibitory effect of Allium sativum and Zingiber officinale extracts on clinically important drug resistant pathogenic bacteria. *Ann Clin Microbiol Antimicrob.* 2012;11:8.
183. Rabbani GH, Butler T, Knight J, Sanyal SC, Alam K. Randomized controlled trial of berberine sulfate therapy for diarrhea due to enterotoxigenic Escherichia coli and Vibrio cholerae. *The Journal of infectious diseases.* 1987;155(5):979-984.
184. Berberine. *Altern Med Rev.* 2000;5(2):175-177.
185. Ibrahim AN. Comparison of in vitro activity of metronidazole and garlic-based product (Tomex(R)) on Trichomonas vaginalis. *Parasitol Res.* 2013;112(5):2063-2067.
186. Avato P, Tursil E, Vitali C, Miccolis V, Candido V. Allylsulfide constituents of garlic volatile oil as antimicrobial agents. *Phytomedicine.* 2000;7(3):239-243.
187. Eja ME, Asikong BE, Abriba C, Arikpo GE, Anwan EE, Enyi-Idoh KH. A comparative assessment of the antimicrobial effects of garlic (Allium sativum) and antibiotics on diarrheagenic organisms. *Southeast Asian J Trop Med Public Health.* 2007;38(2):343-348.
188. Sutherland J, Miles M, Hedderley D, et al. In vitro effects of food extracts on selected probiotic and pathogenic bacteria. *International journal of food sciences and nutrition.* 2009;60(8):717-727.
189. Markin D, Duek L, Berdicevsky I. In vitro antimicrobial activity of olive leaves. *Mycoses.* 2003;46(3-4):132-136.
190. Pereira AP, Ferreira IC, Marcelino F, et al. Phenolic compounds and antimicrobial activity of olive (Olea europaea L. Cv. Cobrancosa) leaves. *Molecules.* 2007;12(5):1153-1162.
191. Micol V, Caturla N, Perez-Fons L, Mas V, Perez L, Estepa A. The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV). *Antiviral research.* 2005;66(2-3):129-136.
192. Knipping K, Garssen J, van't Land B. An evaluation of the inhibitory effects against rotavirus infection of edible plant extracts. *Virology journal.* 2012;9:137.
193. Lee-Huang S, Zhang L, Huang PL, Chang YT, Huang PL. Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. *Biochem Biophys Res Commun.* 2003;307(4):1029-1037.

194. Metcalf JH, Donoghue AM, Venkitanarayanan K, et al. Water administration of the medium-chain fatty acid caprylic acid produced variable efficacy against enteric Campylobacter colonization in broilers. *Poult Sci.* 2011;90(2):494-497.
195. Molatova Z, Skrivanova E, Bare J, Houf K, Bruggeman G, Marounek M. Effect of coated and non-coated fatty acid supplementation on broiler chickens experimentally infected with Campylobacter jejuni. *Journal of animal physiology and animal nutrition.* 2011;95(6):701-706.
196. Hermans D, Martel A, Garmyn A, et al. Application of medium-chain fatty acids in drinking water increases Campylobacter jejuni colonization threshold in broiler chicks. *Poult Sci.* 2012;91(7):1733-1738.
197. Burnouf T, Terpstra F, Habib G, Seddik S. Assessment of viral inactivation during pH 3.3 pepsin digestion and caprylic acid treatment of antivenoms. *Biologicals : journal of the International Association of Biological Standardization.* 2007;35(4):329-334.
198. Takahashi M, Inoue S, Hayama K, Ninomiya K, Abe S. [Inhibition of Candida mycelia growth by a medium chain fatty acids, capric acid in vitro and its therapeutic efficacy in murine oral candidiasis]. *Medical mycology journal.* 2012;53(4):255-261.
199. Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. *Nature.* 2012;489(7415):231-241.