GI-MAP®

GI Microbial Assay Plus

Quantitative PCR Stool Technology for the
Integrative and Functional Medicine Practitioner




Diagnostic Solutions laboratory

RESEARCH. TECHNOLOGY. RESULTS.

2025
10th Anniversary Edition

Contents

The GI Microbial Assay Plus (GI-MAP)

Quantitative PCR Stool Technology for the Integrative and Functional Medicine Practitioner

Microbiology and DNA Analysis

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

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

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

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

Disruption of the Gastrointestinal Microbiome Can Cause:

Gastrointestinal Symptoms

- Abdominal 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 [13,17]
- Small Intestinal Bacterial Overgrowth (SIBO) [18]
- Ulcer [12]
- Ulcerative colitis [19]
- Vomiting [20]

Autoimmune Conditions

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

Allergic Disease

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

Molecular Methods Revolutionize Diagnostic Testing

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

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

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

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

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

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

Table 1. Comparison of Microbial Detection Methods

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

Performance Ranking of Each Microbial Detection Method

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

** Only GI-MAP is Fully Quantitative*

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

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

Setting the Bar for Microbial Detection and Quantitation

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

qPCR exceeds other DNA analysis techniques using multiplex polymerase chain reaction[29] and standard PCR for its ability to quantitate. In qPCR, all of the organisms are run separately, and in duplicate, which makes it possible to measure each organism accurately. There is no competition for chemical reagents in the same well that could lead to variation in the results. The GI-MAP has a larger analytical range, which means it measures higher and lower amounts of genomic DNA than other DNA analysis techniques. All results are quantitative (giving a precise measurement) instead of qualitative (positive or negative).

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

A "Primer" on Amplification and Hybridization

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

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

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

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

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

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

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

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

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

GI-MAP Analytes

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

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

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

Pathogens

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The ***E. coli* serotype O157:H7** has been implicated in many outbreaks and cases of bloody diarrhea and hemolytic uremic syndrome[50] and has a high prevalence worldwide.[54]

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

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

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

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

One of the most potent bacterial toxins known, shiga toxin (Stx) is made by *Shigella dysenteriae* 1. Some serogroups of *E. coli* make an identical toxin (called "Stx1") and a second type of shiga toxin (Stx2). Stx1 and Stx2 have the same mode of action but are antigenically distinct. Shiga toxins (Stx, Stx1, Stx2) cause bloody diarrhea and can cause hemolytic uremic syndrome (characterized by thrombocytopenia, hemolytic anemia, and kidney failure).[60]

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

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

Table 2. Food Sources of *Salmonella*

Sources
Poultry and Poulty Products
Meat
Dairy
Raw, Fresh, Ready-to-Eat Produce Such as: • Tomatoes • Berries • Leafy greens • Melons • Sprouts

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).[61]

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

Y. enterocolitica are iron-loving bacteria. Therefore, individuals with hereditary hemochromatosis are usually more susceptible to infection with *Yersinia*. Genito-urinary infection with *Y. enterocolitica* have been associated with inflammatory diseases such as reactive arthritis, most likely due to an immune-mediated mechanism.[22,23]

There is some evidence that *Y. enterocolitica* is associated with autoimmune thyroid disorders including Graves's disease and Hashimoto's thyroiditis in genetically susceptible individuals. Higher antibodies to *Y. enterocolitica* are often found in these patients.[68,69]

Infection is usually self-limiting and does not require treatment. However, for severe infections, pharmaceutical treatment with doxycycline in combination with an aminoglycoside may be warranted. Additionally, trimethoprim-sulfamethoxasole, chloramphenicol, and rifaximin may also be useful treatments.[70]

Parasitic Pathogens

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

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

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

Entamoeba histolytica (E. histolytica) is a disease-causing parasite that can affect anyone, although it is more common in those who lived or travelled in tropical areas with poor sanitary conditions. Diagnosis can be difficult since, under a microscope, it looks similar to other parasites such as *Entamoeba dispar* and *Entamoeba 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.[71] Treatment for infection with *E. histolytica* includes antiparasitic drug therapy and may include a combination based on the severity of infection.

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

Giardia Can Cause:[72]

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

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

Viral Pathogens

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

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

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

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

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

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

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

Helicobacter pylori

***H. pylori* and eight virulence genes** are included on the GI-MAP. *Helicobacter pylori* has been evolving with human beings for well over 50,000 years, since they migrated out of Africa.[12] *H. pylori* colonization has been implicated in a variety of gastroduodenal diseases including gastritis, gastric cancer, and duodenal and peptic ulcer.[83] *H. pylori* has also been detected by stool PCR in cases of dyspepsia, abdominal pain, and chronic gastrointestinal symptoms.[84-86] It is infamous for its causal link to ulcers and gastric cancer, which resulted in a Nobel prize awarded to Robin Warren and Barry Marshall in 2005. However, some sources are suggesting its role, at least in part, as a commensal organism. *H. pylori* may protect its host from certain atopic disorders,[16] as well as other diseases such as esophageal cancer[87] reflux, and obesity.[16]

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

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

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

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

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

Virulence Factors

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

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

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

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

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

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

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

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

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

Table 3. *Helicobacter pylori* Virulence Genes, Mechanisms, Interactions, and Disease Associations [91-100]

Gene Acronym and Name	Genetic Characteristics	Associations with Disease
BabA Blood group antigen binding adhesin	• Promotes DNA breakage in host cell • Improves *H. pylori* adherence ("stickiness") to epithelial cells • May promote other virulence factors, especially CagA	May promote carcinogenesis
CagA Cytotoxin-associated protein A	• Promotes *H. pylori* adhesion and colonization • Affects barrier function of gastric epithelial tight junctions • Promotes loss of cell polarity • Antagonizes VacA • Evades the immune system and affects the activity of dendritic cells and B-cells • Considered part of the "pathogenicity island" which includes VirB and VirD virulence factors. This is a closely-associated group of genes that work synergistically and often transfer as a unit.	Promotes carcinogenesis, strong association. Also associated with peptic ulcer disease
DupA Duodenal Ulcer-Promoting gene A	• Promotes Inflammation	Associated with duodenal ulcers, specifically
IceA Induced by contact with epithelium A	• Transcription of this gene is only initiated after adhesion to the gastric epithelium • Promotes inflammation and associated with elevated IL-8	Associated with dyspepsia and gastric & duodenal ulcers NOT associated with gastric cancer
OipA Outer inflammatory protein A	• Promotes Inflammation • Drives IL-8 production	Associated with carcinogenesis and peptic ulcer disease
VacA Vacuolating toxin A	• Enters the host cell by endocytosis • Affects mitochondrial function • Disrupts tight junctions • Causes a programmed necrosis by inducing the production of large vacuoles inside the host cells; inducing cellular swelling; disrupting cell barrier thus causing nutrient leakage • Facilitates nutrient acquisition (iron, minerals, amino acids, etc.) • Inhibits antigen presentation in vitro • Antagonizes CagA	Associated with gastric inflammation, peptic ulcer disease, and gastric cancers
VirB & VirD of the Cag pathogenicity island (Cag PAI)	• Part of the CagA "pathogenicity island" • Both genes can potentiate CagA virulence factor by aiding in its transmission to host epithelial cells • In the absence of CagA, these virulence factors are unlikely to change clinical outcome of *H. pylori* infections.	Evaluate in combination with CagA virulence factors. VirB & VirD, if positive, can potentiate CagA virulence and clinical associations

Commensal/Keystone Bacteria

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

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

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

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

B. fragilis has been the subject of rigorous investigation in recent years because it appears to have a protective effect against inflammation and possibly against autoimmune disorders. *B. fragilis* repairs defects in the gut barrier by influencing tight junction proteins and cytokine expression.[40] When autistic-like mice were given *Bacteroides fragilis,* it normalized intestinal permeability, restored microbial balance, and removed behavioral and cognitive symptoms.[40] *B. fragilis* has also been shown to correct gastrointestinal pathology in animal models of colitis[63] and inhibit neuroinflammation in mouse models of multiple sclerosis.[60] Its anti-inflammatory activity is attributed to a surface molecule called polysaccharide A

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

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

***Enterococcus* spp.** are gram-positive, lactate producing, facultative anaerobes that are part of the *Firmicutes* phyla.[122] *Enterococcus* colonize along the intestinal epithelium below the mucous layer. Residing in this location gives it the ability to increase intestinal mucosal barrier function.[123] *Enterococcus* play a key role in preventing foodborne infections with their production of bacteriocins providing inhibitory activity against the following organisms: *Shigella flexneri, Staphylococcus aureus, Listeria monocytogenes, Yersinia enterocolitica, Klebsiella pneumoniae, Escherichia coli,* and *Bacillus subtilis.*[124] Enterococcus species also produce B12 in the intestinal tract.[125] High levels may be reflective of small intestinal dysbiosis[126] and achlorhydria (or the lack of stomach acid). *Enterococcus faecalis* can survive in pancreatic juice and/or bile,[127] be associated with antibiotic resistance,[128] virulence factors,[129] and has the potential to be associated with pancreatitis.[127] For these reasons, it is wise to reference the *Enterococcus faecalis* measurement if elevated commensal Enterococcus species are detected on the report.[127] Enterococcus species are considered mucosa-associated microbiota. Therefore, low levels may be indicative of decreased mucosal and/or epithelial health.[130]

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

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

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

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

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

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

Phyla Microbiota

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

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

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

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

Opportunistic and Overgrowth Microbes

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

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

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

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

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

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

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

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

Autoimmune-Related Gastrointestinal Bacteria

Opportunistic gastrointestinal pathogens are gaining attention for their ability to initiate autoimmune thyroiditis and inflammatory arthritis such as rheumatoid arthritis and ankylosing spondylitis. *Klebsiella spp., Proteus mirabilis, Citrobacter spp.,* and *Yersinia* are bacteria that could contribute to inflammatory arthritis in susceptible individuals. *Yersinia enterocolitica* infection has been associated with Hashimoto's thyroiditis and Grave's disease[69] and higher antibodies to *Yersinia enterocolitica* have been found in these patients.[68] Enterovirus is also associated with immunogenic thyroiditis.[168] Analysis of gastrointestinal microbes is recommended in chronic autoimmune disorders that don't respond to the usual therapies.

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

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

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

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

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

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

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

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

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

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

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

As an opportunistic gastrointestinal pathogen, *P. mirabilis* may contribute to inflammatory arthritis in susceptible individuals[69] and there is substantial data supporting it as a causative role in rheumatoid arthritis.[24] Other data shows abnormal serum antibody responses to *P. mirabilis* in the spondyloarthropathies[174] and antibodies to *Proteus* in rheumatoid arthritis.[171]

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

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

Fungal Organisms

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

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

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

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

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

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

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

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

Viruses

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

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

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

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

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

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

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

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

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

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

Cordyceps may help to suppress the virus[207] and vitamin D may help prevent autoimmune sequellae of EBV.[208] Prevent transmission of EBV by washing hands, and avoiding people who have EBV infection, especially avoiding contact with saliva such as kissing, sharing drinks or food or toothbrushes.[206]

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

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

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

Parasites (Non-Pathogens)

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

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

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

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

Dientamoeba fragilis (D. fragilis) is found in the gastrointestinal tract of humans and pigs and is frequently associated with traveler's diarrhea. Found worldwide with high prevalence in developed countries, fecal-oral transmission via food and water is the most common source of exposure to *D. fragilis.* While generally considered non-pathogenic, in the presence of other parasites, and in the immunocompromised, symptoms may be more prevalent in infected individuals.[224] *D. fragilis* infection may present with abdominal pain, diarrhea, irritable bowel syndrome, or eosinophilic colitis.[225] *D. fragilis* and *Enterobius vermicularis* (pinworm) are often detected together[226] in stool samples and may cause symptoms of diarrhea, abdominal pain, and fatigue. Treatments for *D. fragilis* may include paromomycin or metronidazole; studies have found the former to me more effective against *D. fragilis* alone.[225] Co-infection with

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

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

Pentatrichomonas hominis (formerly Trichomonas hominis) is a protozoan found in the cecum[227] of the large intestine and generally considered to be non-pathogenic with most cases being asymptomatic. *P. hominis* is found in less than 2% of the population and is transmitted by fecal-oral contamination. *P. hominis* can be a sign of other dysbiosis, pathogenic infections, or weak digestion.[228] It is more common in children than adults, likely due to poor hygiene.[229] It also colonizes the gastrointestinal tract of many mammalian hosts and therefore it is possible to have zoonotic transmission, particularly from dogs or cats.[229]

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

Worms

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

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

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

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

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

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

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

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

Intestinal Health

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

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

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

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

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

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

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

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

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

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

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

Causes of Exocrine Pancreatic Insufficiency [254]

Pancreatic Disorders

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

Extrapancreatic Disorders

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

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

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

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

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

Secretory Immunoglobulin A (sIgA) is an antibody protein secreted into the gastrointestinal tract as a first line of immune defense against pathogenic microorganisms.[264] This immunoglobulin influences the gut microbiome[264] and helps to maintain barrier function[265] by forming complexes with gut pathogens and allergens, preventing them from penetrating the intestinal barrier. Impairment of sIgA may increase the risk of infectious, allergic, and inflammatory diseases of the intestine.[266] Chronic stress may also disrupt levels of sIgA. Elevated levels of sIgA may indicate an activated immune response to chronic infections or inflammatory reactions.

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

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

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

Possible Causes of Elevated Eosinophil Activation Protein: [270-272]

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

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

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

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

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

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

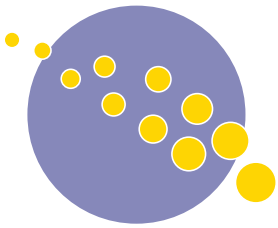
Commercially available fecal calprotectin ELISA kits historically suggest a 50 µg/g cut-off value for differentiation of IBD from non-IBD patients. Unfortunately, utilization of this "standard" value is not exceptionally helpful as it does not efficiently diagnose IBD for all patients and not across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

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

Add-On Tests

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

Zonulin



Zonulin is a protein secreted by intestinal cells that regulates intercellular tight junctions.[1,283] Tight junctions are the connections between epithelial cells that make up the gastrointestinal lining. Zonulin increases intestinal permeability in the jejunum and ileum[284] and is considered a biomarker for barrier permeability.[1,283] Tight junctions can be opened or closed, depending on the physiological need. Zonulin's role is to open tight junctions in the gut. In the case of enteric infections, high zonulin can "open the floodgates" and flush out bacteria and toxins.[1] Certain gut bacteria and gliadin (the main staple protein from wheat) can activate the zonulin system.[283,285]

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

Serum Zonulin is High in a Number of Immune-Mediated Conditions[1]

Autoimmune Diseases

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

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

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

Gluten Peptide

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

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

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

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

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

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

Fecal gluten monitoring is useful to:

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

Universal Antibiotic Resistance Genes

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

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

Universal Antibiotic Resistance Genes Panel
– Identifies Microbial Resistance to These Classes of Antibiotics

- β-lactams
- Fluoroquinolones
- Vancomycin
- Macrolides
- Ciprofloxacin
- 5-Nitroimidazoles *(non-Helicobacter pylori)*
- Trimethoprim
- Sulfonamides
- Methicillin
- Chloramphenicol

Tailoring a patient's pharmacotherapy using the Universal Antibiotic Resistance Genes[301] helps to: combat the rising public health threat of multidrug resistance, treat infections more efficiently, protect patients from unnecessary antibiotics, and reduce healthcare costs.[302,303]

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

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

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

Stool*OMX*™

The Stool*OMX*™ add-on to the GI-MAP measures 25 bile acids and 9 short chain fatty acids to assess the microbiome, diet, digestion, and the health of the gut lining. It can help pinpoint factors contributing to chronic diarrhea, constipation, intestinal permeability, GI inflammation, immune dysfunction, and metabolic syndrome.

Bile acids and short chain fatty acids are measured in stool using triple quadrupole tandem mass spectrometry (LC-MS/MS), a high-performance liquid chromatography separation followed by tandem mass spectrometry. The advantage of this method is its superior sensitivity and specificity, making it an accurate and highly reproducible test. It also allows for the quantification of a comprehensive list of analytes, not commonly available from other laboratories.

Bile Acids – Stool*OMX* measures fecal bile acids. Bile acids are metabolites that aid in the absorption of dietary lipids and fat-soluble vitamins.[304-307] They help regulate immunity, metabolism, intestinal motility, and have clinical associations with conditions such as irritable bowel syndrome and inflammatory bowel disease.[305,307-309] Primary bile acids are synthesized in the liver from cholesterol and conjugated with glycine or taurine before being stored in the gallbladder as bile salts.[304,310] During a meal, the small intestine releases the peptide hormone and neurotransmitter cholecystokinin which stimulates the release of primary bile acids into the duodenum to assist with dietary lipid digestion and absorption.[305-308]

Bacteria in the small intestine and colon convert primary bile acids to secondary bile acids.[306] Primary bile acids are first deconjugated, meaning glycine or taurine is removed, with bile salt hydrolase (BSH) and subsequently can be dehydroxylated with 7α-hydroxylase.[306,307,311-313] Approximately 95% of primary bile acids are reabsorbed by enterocytes in the distal ileum and returned to the liver via enterohepatic circulation, while approximately 5% are metabolized by bacteria to produce secondary bile acids.[305,306,311,312] Secondary bile acids are either passively absorbed in the colon or

excreted in stool.[305] Stool*OMX* measures total quantities and percentages of total bile acids, and primary and secondary bile acids, yielding insights into the stool metabolome and root causes of GI disturbances.

Physiological Functions of Bile Acids – Bile acids play essential roles in digestion, motility, microbial balance, and immune regulation, largely through their interactions with receptors located in the liver, intestines, gallbladder, and kidneys.[306,314-333] They aid in the digestion of dietary fats and fat-soluble vitamins and exhibit antimicrobial properties, including effects against *Clostridium difficile*.[304-306] Specific secondary bile acids inhibit germination, colonization, and Toxin B activity.[307,309] Imbalances, such as elevated primary bile acids and reduced secondary primary bile acids, are linked to antibiotic use and persistent *C. difficile* infection.[309] Malabsorption of bile acids can contribute to diarrheal symptoms and conditions such as bile acid diarrhea (BAD).[334]

Table 5. Abbreviations for Bile Acids and Short Chain Fatty Acids

Metabolite	Description and Clinical Use
Fecal Bile Acids	**Abbreviation**
Cholic Acid	CA
Chenodeoxycholic Acid	CDCA
Lithocholic Acid	LCA
Deoxycholic Acid	DCA
Fecal Short Chain Fatty Acids	**Abbreviation**
Short Chain Fatty Acids	SCFA*
Saccharolytic Straight Chain Fatty Acids	SCFA*
Branch Chain Fatty Acids	BCFA

Proper bile acid balance is crucial to gut homeostasis. Certain bile acids are more hydrophobic and imbalances can contribute to inflammation, cytotoxicity, and mitochondrial damage.[304-306,335] Cholic acid (CA) and chenodeoxycholic acid (CDCA) are the major primary bile acids.[308] Deoxycholic acid (DCA) and lithocholic acid (LCA) are the major secondary bile acids.[305,307] Cholic acid (CA) is converted to deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) is converted to lithocholic acid (LCA) through dehydroxylation.[306,307] Elevated secondary bile acids, particularly DCA and LCA, are associated with inflammation and increased risk of colon cancer.[309,335,336]

Bile Acids and Clinical Associations

Irritable Bowel Syndrome with Diarrhea (IBS-D) – In IBS-D, total and primary bile acids in stool are increased, contributing to visceral hypersensitivity, abdominal pain, and altered transit time.[334,337,338] Up to 30% of patients with IBS-D have bile acid diarrhea (BAD), a condition which is also implicated in pancreatic insufficiency,

* Note that short chain fatty acids and saccharolytic straight chain fatty acids are both referred to as "SCFA" in the literature. Most short chain fatty acids are saccharolytic straight chain fatty acids, hence the abbreviation SCFA may be used interchangeably in the literature.

postinfectious diarrhea, and post-cholecystectomy.[311,334,339] BAD arises from excess bile acid production or malabsorption and is associated with increased unconjugated and primary fecal bile acids.[305,311,334,340] In IBS-D, gut bacteria that modulate BSH and 7α-dehydroxylase may be reduced, leading to lower levels of secondary bile acids.[312,337,338]

Conventional treatment for BAD typically involves bile acid sequestrants (e.g. cholestyramine, colestipol, colesevelam), which may be poorly tolerated. A low-fat diet can offer additional support.[311,334,339] Stool*OMX* can offer deeper insights into the diagnostic workup of IBS-D and precision clinical management.

Inflammatory Bowel Disease – In inflammatory bowel disease (IBD), primary and conjugated bile acids are typically elevated, while secondary bile acids are reduced.[312,314,341] Bacteria that deconjugate primary bile acids are lower in IBD, leading to lower levels of secondary bile acids. This imbalance impacts immune regulation, leading to intestinal inflammation and Th17 and Treg cell dysregulation.[314,342] Secondary bile acids regulate colonic immune cells via receptors like farnesoid X receptor (FXR) and vitamin D receptor (VDR).[343] FXR activation inhibits pro-inflammatory cytokines and secondary bile acids suppress Th17 cells.[344,345] Therefore, lower levels of secondary bile acids can promote inflammation. Patients with IBD may have increased *Proteobacteria* and decreased *Firmicutes*, which contributes to the elevated primary bile acids together with decreased secondary bile acids, often seen in IBD.[306,309]

Metabolism and GLP-1 – Glucose and insulin influence bile acid synthesis by regulating CYP7A1 expression.[346] Enterocytes that produce the peptide hormone glucagon-like peptide-1 (GLP-1) have Takeda G protein coupled receptor 5 (TGR5) and FXR receptors. Bile acids activate these receptors to stimulate GLP-1 release.[347,348] LCA may dysregulate glucose regulation, so higher levels may be unfavorable.[348] Lower fecal hyocholic acid (HCA) levels are linked to prediabetes, suggesting HCA could be used as a potential marker of metabolic health.[349]

LCA/DCA Ratio – The ratio of LCA to DCA can serve as a valuable marker of assessing disease risk. LCA is considered more cytotoxic than DCA, and an elevated LCA/DCA ratio is associated with an increased risk for colon cancer and can be seen in patients after cholecystectomy.[330,336,342,350,351] Additionally, the ratio may provide insight into gastrointestinal motility, as lithocholic acid exerts a strong pro-motility effect and can accelerate intestinal transit time.[325,334]

The Microbiome Influence on Bile Acids

The microbiome composition influences the bile acid pool, impacting FXR receptor activation and the feedback loop that regulates bile acid synthesis.[306] DCA can inhibit the growth of certain bacteria, including *Lactobacillus* spp., *Clostridium perfringens, Bifidobacterium* spp., and *Bacteroides fragilis*, while CA suppresses *Roseburia* spp., *Lactobacillus* spp., and *Ruminococcus* spp.[352] In mouse models, greater diversity of BSH-producing bacteria and unconjugated bile acids correlated with increased transit times.[315] The bidirectional relationship between bile acids and the microbiome offers

Table 6. Microbes Found to Contribute to Secondary Bile Acid Production

Microbes Involved in Deconjugation of Primary Bile Acids to Secondary Bile Acids[305,306,313,335,353]	
Found on GI-MAP	**Other**
Firmicutes	*Proteobacteria*
Bacteroidetes	*Clostridium* spp.
Bacteroides spp.	*Eubacterium* spp.
Bifidobacterium spp.	*Methanobrevibacter smithii*
Enterococcus spp.	*Methanosphera stadmanae*
Lactobacillus spp.	

Bacteria Involved in 7α-dehydroxylation of Secondary Bile Acids[305-307]	
Found on GI-MAP	**Other**
Bacteroides spp.	*Lachnospiraceae*
Escherichia spp.	*Peptostreptococcaceae*
Lactobacillus spp.	*Clostridium* spp.
	Eubacterium spp.
	Ruminococcus spp.

insights into disease risk for conditions like diabetes, obesity, IBD, and metabolic-associated fatty liver disease (MAFLD).[342]

Medication and Diet Influence on Bile Acids

Antibiotic use greatly alters bile acid levels, increasing conjugated bile acids and reducing secondary bile acids.[309] Diet also plays a key role in bile acid composition. Increased fiber intake, particularly insoluble fiber, promotes bile acid excretion and may lead to lower bile acid levels in stool, whereas a high-fat diet may increase primary bile acid synthesis and elevate fecal secondary bile acids.[309,312,354]

Short Chain Fatty Acids

Stool*OMX* measures fecal short chain fatty acids. Short chain fatty acids (SCFA)* are produced by intestinal microbiota through the fermentation of carbohydrates or proteins. Total short chain fatty acids are comprised of approximately 90–95% saccharolytic straight chain fatty acids (SCFA)* and 5–10% branched chain fatty acids (BCFA). Saccharolytic straight chain fatty acids come from the fermentation of carbohydrates (starch, fibers, and polyphenols). This is known as saccharolytic fermentation. In contrast, branched chain fatty acids (BCFA) are generated through the fermentation of protein, or proteolytic fermentation. A balance of SCFA to BCFA reflects the diet composition, digestive capabilities, and the health of the

* Note that short chain fatty acids and saccharolytic straight chain fatty acids are both referred to as "SCFA" in the literature. Most short chain fatty acids are saccharolytic straight chain fatty acids, hence the abbreviation SCFA may be used interchangeably in the literature.

microbiome.[355] Stool*OMX* offers insight into this balance and can thus influence clinical interventions.

Saccharolytic Straight Chain Fatty Acids (SCFA) – Saccharolytic straight chain fatty acids (SCFA) are carboxylic acids produced in the cecum and colon through the fermentation of dietary fibers and resistant starch.[356-359] Approximately 95% of SCFAs are absorbed by colonocytes, with most passing through the portal vein and being metabolized into lipids by the liver. The remaining 5% are excreted in stool.[357,359-361] Acetate, propionate, and butyrate are the dominate SCFA in the colon in an approximate 60:20:20 ratio, while smaller amounts of valerate and caproate are typically reported.[356,358,360,362]

SCFA provide energy for colonocytes, regulate the microbiota, and support intestinal epithelial barrier function, mucus production, motility, fluid and electrolyte absorption, luminal pH balance, and nutrient absorption, including calcium, iron, and magnesium.[356-359,361-369] SCFA also modulate immune function by influencing monocytes, macrophages, neutrophils, and CD4+ T cell polarization.[357] A more acidic pH in the gut lumen promotes SCFA-producing bacteria, which inhibit the growth of pathogenic microbes such as *Escherichia coli (E. coli), Salmonella,* and *Campylobacter*.[361,362]

Table 7. Bacterial Production of Saccharolytic Straight Chain Fatty Acids

Saccharolytic Straight Chain Fatty Acid	SCFA-Producing Bacteria
Acetate	*Akkermansia muciniphila, Bacteroides* spp., *Barnesiella* spp., *Bifidobacterium* spp., *Blautia hydrogentrophica, Clostridium* spp., *Coprococcus* spp., *Firmicutes, Prevotella* spp., *Ruminococcus* spp., *Streptococcus* spp.[356,358,360,362,371]
Propionate	*Akkermansia muciniphila, Alistipes putredinis, Bacteroidetes, Bacteroides* spp., *Bacteroides fragilis, Bacteroides uniformis, Bacteroides vulgatus, Blautiaobeaum, Coprococcuscatus, Dialister* spp., *Eubacterium* spp., *Eubacterium hallii, Firmicutes, Lachnospiraceae, Megasphaera elsdenii, Prevotella* spp., *Roseburia* spp., *Roseburia inulinivorans, Ruminococcus obeum, Salmonella* spp., *Veillonella* spp.[356,358,362,372]
Butyrate	*Actinobacteria, Anaerostripes* spp., *Anaerostipes hadrus, Bacteroidetes, Bacteroides fragilis, Clostridium* clusters IV and XIVa, *Coprococcus catus, Coprococcus eutactus, Coprococcus comes, Coprococcuscatus, Eubacterium biforme, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Firmicutes, Fusobacteria, Lachnospiraceae, Roseburia* spp., *Roseburia inulinivorans, Roseburia intestinalis, Ruminococcus bromii, Spirochaetes, Subdoligranulum variabile, Thermotogae*[356-358,362] Lactic acid-producing bacteria such as *Lactobacillus* spp., *Enterococcus* spp., *Streptococcus* spp., *Escherichia/Shigella* spp., and *Bifidobacterium* spp. serves as cross-feeders for butyrate-producing bacteria[367]
Caproate	*Megasphaera elsdenii, Clostridium* spp., BS-1[368,373]

Acting as signaling molecules, SCFA influence gut hormone release and can help regulate appetite and metabolic homeostasis.[363,370] High-fiber diets are linked to improved glycemic control and increased SCFA-producing microbes.[362,364]

Fecal SCFA concentrations are influenced by factors such as diet, genetics, and dysbiosis.[362] Elevated fecal SCFA result from malabsorption and increased motility, contributing to gut dysbiosis and permeability.[365] Patients with hypertension may have higher stool levels of acetate, butyrate, and propionate.[356] Increased fecal SCFA levels are linked to obesity, possibly due to higher *Firmicutes* diversity.[358,361] SCFA in stool are reduced in patients with Parkinson's disease and children with autism spectrum disorder.[356,362,366]

Proteolytic Branched Chain Fatty Acids (BCFA) – Branched chain fatty acids (BCFA), including iso-butyrate, iso-valerate, 2-methylbutyrate, and iso-caproate, are associated with dietary protein intake and serve as markers of protein fermentation.[359,374] When dietary fiber for SCFA production is limited, gut bacteria can metabolize amino acids like valine, leucine, and isoleucine into BCFA.[359,361,375,376] Protein fermentation also produces compounds like ammonia, amines, sulfides, and p-cresol, which can cause cell damage.[364,374]

BCFA are primarily produced by *Bacteroides* spp. and *Clostridium* spp.[374] Animal studies have shown that BCFAs play roles in energy homeostasis by inhibiting lipolysis in adipocytes and protecting against lipotoxicity.[375]

BCFA in Stool – Fecal BCFA concentrations are influenced by diet composition and digestive capacity. Elevated levels of iso-butyrate and iso-valerate may indicate impaired digestion, with excess unabsorbed protein (putrefactive) products reaching the colon.[362] A study of healthy individuals demonstrated that BCFA levels positively correlate with protein supplementation, but negatively correlate with fiber intake, particularly insoluble fiber.[374]

SCFA/BCFA Ratio – The SCFA/BCFA ratio serves as a valuable indicator of the balance between these fermentation processes, offering a deeper understanding of their implications in various disease states. Ideally, saccharolytic SCFA should make up approximately 90–95% of total SCFA, while BCFA should make up approximately 5–10% of total SCFA.[355,380] The SCFA/BCFA ratio may decline with age, primarily due to a significant decrease in SCFA levels in stool.[374] Microbial metabolism often shifts toward proteolytic fermentation in individuals with chronic kidney disease (CKD), and patients with IBD may experience reduced saccharolytic fermentation with an increase in proteolytic fermentation.[378,379]

* Note that short chain fatty acids and saccharolytic straight chain fatty acids are both referred to as "SCFA" in the literature. Most short chain fatty acids are saccharolytic straight chain fatty acids, hence the abbreviation SCFA may be used interchangeably in the literature.

Herbal Antimicrobial Agents

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

Table 8. Herbal Antimicrobial Agents Commonly Used by Integrative and Functional Medicine Practitioners to Correct Dysbiosis

Antimicrobial Agent	Description and Clinical Use
Berberine	Berberine has shown effectiveness against ETEC-associated diarrhea and has been studied extensively for its antibacterial effect.[384] It shows antimicrobial activity against fungi, protozoans, helminths, viruses, and chlamydia.[385]
Garlic	Garlic has shown activity against bacteria, protozoa, helminths, viruses, and fungi.[386,387] It strongly suppressed gram-negative diarrheagenic pathogens (*Shigella, Salmonella, Proteus mirabilis,* and *E. coli*) isolated from stool samples.[388] Aqueous garlic extract inhibited *E.coli* O157:H7 and *E. coli* LF82, and enhanced the growth of *Lactobacillus* reuteri in vitro.[389] This suggests that antimicrobial herbs may spare beneficial microbiota.
Olive leaf	Olive leaf has antibacterial, antifungal,[390,391] and antiviral properties.[392-394]
Caprylic acid	Caprylic acid reduces *Campylobacter* and *Salmonella* in the gastrointestinal tract and stool of poultry when added to the feed or water.[395-397] Caprylic acid has antiviral and antifungal properties.[398,399]
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.

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,[381-383] as compared to antibiotics, because multiple active ingredients from the whole plant work together in synchrony. Their long historical use suggests low risk of adverse effects.

Conclusions

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

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

This stool test offers integrative and functional medicine practitioners superior sensitivity and specificity to help resolve persistent and complex illnesses. Since the immune system, the intestinal barrier, and microbial diversity are intimately interwoven, thorough understanding of our gut microbiome holds promise for new approaches to treat and prevent disease.[400]

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*. Oct 2012;10(10):1096-100. doi:10.1016/j.cgh.2012.08.012
2. Dora-Laskey A. Ascaris Lumbricoides. Medscape. Accessed Dec 8, 2017. https://emedicine.medscape.com/article/788398-overview
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. doi:10.1186/gb-2012-13-6-r42
4. Wade W. Unculturable bacteria--the uncharacterized organisms that cause oral infections. *Journal of the Royal Society of Medicine*. Feb 2002;95(2):81-3.
5. xTAG gastrointestinal pathogen panel. Accessed April 9, 2015. http://www.luminexcorp.com/Products/Assays/ClinicalDiagnostics/xTAGGPP/
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*. May 2006;10(3):255-61. doi:S1201-9712(05)00208-0 [pii] 10.1016/j.ijid.2005.05.011
7. Quigley EM. What is the evidence for the use of probiotics in functional disorders? *Current gastroenterology reports*. Aug 2008;10(4):379-84.
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*. Dec 2006;8(6):486-98.
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*. Aug 2007;22(8):1199-204.
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*. Jul 2004;53(Pt 7):617-22.
11. [A rare outbreak of food poisoning caused by Salmonella enterica serovar. Oranienburg--a case report and features of isolates]. *Kansenshogaku Zasshi*. May 2007;81(3):242-8.
12. Yamaoka Y. Mechanisms of disease: Helicobacter pylori virulence factors. *Nature reviews Gastroenterology & hepatology*. Nov 2010;7(11):629-41. doi:10.1038/nrgastro.2010.154
13. Othman M, Aguero R, Lin HC. Alterations in intestinal microbial flora and human disease. *Current opinion in gastroenterology*. Jan 2008;24(1):11-6.
14. Reina J, Hervas J, Borrell N. Acute gastroenteritis caused by Hafnia alvei in children. *Clin Infect Dis*. Mar 1993;16(3):443.
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*. Feb 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*. Apr 2014;39(8):767-79. doi:10.1111/apt.12666
17. Dahlqvist G, Piessevaux H. Irritable bowel syndrome: the role of the intestinal microbiota, pathogenesis and therapeutic targets. *Acta gastro-enterologica Belgica*. Sep 2011;74(3):375-80.
18. Pimentel M. Review of rifaximin as treatment for SIBO and IBS. *Expert opinion on investigational drugs*. Mar 2009;18(3):349-58. doi:10.1517/13543780902780175
19. Cummings JH, Macfarlane GT, Macfarlane S. Intestinal bacteria and ulcerative colitis. *Current issues in intestinal microbiology*. Mar 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*. Mar 2009;28(2):130-3.
21. Brent LH, Diamond HS. Ankylosing spondylitis and undifferentiated spondyloarthropathy. Medscape. Updated May 14, 2014. Accessed May 14, 2015. http://emedicine.medscape.com/article/332945-overview
22. Leirisalo-Repo M, Hannu T, Mattila L. Microbial factors in spondyloarthropathies: insights from population studies. *Current opinion in rheumatology*. Jul 2003;15(4):408-12.
23. Palazzi C, Olivieri I, D'Amico E, Pennese E, Petricca A. Management of reactive arthritis. *Expert opinion on pharmacotherapy*. Jan 2004;5(1):61-70. doi:10.1517/14656566.5.1.61
24. Rashid T, Ebringer A. Autoimmunity in Rheumatic Diseases Is Induced by Microbial Infections via Crossreactivity or Molecular Mimicry. *Autoimmune Dis*. 2012;2012:539282. doi:10.1155/2012/539282
25. Panzer AR, Lynch SV. Influence and effect of the human microbiome in allergy and asthma. *Current opinion in rheumatology*. Jul 2015;27(4):373-80. doi:10.1097/BOR.0000000000000191

26. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. Controlled Clinical Trial Research Support, Non-U.S. Gov't. *The Journal of allergy and clinical immunology*. Feb 2012;129(2):434-40, 440 e1-2. doi:10.1016/j.jaci.2011.10.025

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*. Jan 2007;119(1):192-8.

28. West CE. Gut microbiota and allergic disease: new findings. *Current opinion in clinical nutrition and metabolic care*. May 2014;17(3):261-6. doi:10.1097/MCO.0000000000000044

29. qPCR vs. Digital PCR vs. Traditional PCR. ThermoFisher Scientific. http://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/qpcr-education/qpcr-vs-digital-pcr-vs-traditional-pcr.html

30. Rajilic-Stojanovic M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiol Rev*. Sep 2014;38(5):996-1047. doi:10.1111/1574-6976.12075

31. Rappe MS, Giovannoni SJ. The uncultured microbial majority. *Annual review of microbiology*. 2003;57:369-94. doi:10.1146/annurev.micro.57.030502.090759

32. Dewhirst FE, Chen T, Izard J, et al. The human oral microbiome. *J Bacteriol*. Oct 2010;192(19):5002-17. doi:10.1128/JB.00542-10

33. Piralla A, Lunghi G, Ardissino G, et al. FilmArray GI panel performance for the diagnosis of acute gastroenteritis or hemorragic diarrhea. *BMC Microbiol*. May 12 2017;17(1):111. doi:10.1186/s12866-017-1018-2

34. Zhang H, Morrison S, Tang YW. Multiplex polymerase chain reaction tests for detection of pathogens associated with gastroenteritis. *Clin Lab Med*. Jun 2015;35(2):461-86. doi:10.1016/j.cll.2015.02.006

35. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature*. Dec 21 2006;444(7122):1022-3.

36. Palva A. [Intestinal microorganisms and their significance for health]. *Duodecim; laaketieteellinen aikakauskirja*. 2009;125(6):685-94. Suolistomikrobit ja niiden merkitys terveydelle.

37. Kamada N, Seo SU, Chen GY, Nunez G. Role of the gut microbiota in immunity and inflammatory disease. *Nature reviews Immunology*. May 2013;13(5):321-35. doi:10.1038/nri3430

38. Canny GO, McCormick BA. Bacteria in the intestine, helpful residents or enemies from within? *Infection and immunity*. Aug 2008;76(8):3360-73. doi:10.1128/IAI.00187-08

39. Stecher B, Hardt WD. The role of microbiota in infectious disease. *Trends in microbiology*. Mar 2008;16(3):107-14.

40. Chow J. Probiotics and prebiotics: A brief overview. *J Ren Nutr*. Apr 2002;12(2):76-86.

41. Ramakrishna BS. Role of the gut microbiota in human nutrition and metabolism. *Journal of gastroenterology and hepatology*. Dec 2013;28 Suppl 4:9-17. doi:10.1111/jgh.12294

42. Hijova E, Chmelarova A. Short chain fatty acids and colonic health. *Bratislavske lekarske listy*. 2007;108(8):354-8.

43. Moore WE, Holdeman LV. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Comparative Study. *Appl Microbiol*. May 1974;27(5):961-79.

44. 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*. Sep 2007;11(36):1-216. doi:03/40/03 [pii]

45. Kahlau P, Malecki M, Schildgen V, et al. Utility of two novel multiplexing assays for the detection of gastrointestinal pathogens - a first experience. *SpringerPlus*. Dec 2013;2(1):106. doi:10.1186/2193-1801-2-106

46. 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*. Jun 30 1998;42(1-2):39-44.

47. Aberra F, Curry JA. Clostridium Difficile Colitis. Medscape. Updated December 18, 2014. Accessed April 20, 2015. http://emedicine.medscape.com/article/186458-overview#a0101

48. 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*. Jan 2010;1(1):58-64. doi:10.4161/gmic.1.1.10768

49. E. coli (Escherichia coli). Centers for Disease Control. Updated April 16, 2015. Accessed April 17, 2015. http://www.cdc.gov/ecoli/general/index.html

50. Madappa T, Hiong C. Escherichia Coli Infections. Medscape. Updated August 19, 2014. Accessed April 17, 2015. http://emedicine.medscape.com/article/217485-overview#a0104

51. Giacometti F, Bonilauri P, Piva S, et al. Paediatric HUS Cases Related to the Consumption of Raw Milk Sold by Vending Machines in Italy: Quantitative Risk Assessment Based on Escherichia coli O157 Official Controls over 7 years. *Zoonoses Public Health*. Nov 2017;64(7):505-516. doi:10.1111/zph.12331

52. Feng K, Hu W, Jiang A, et al. A Dual Filtration-Based Multiplex PCR Method for Simultaneous Detection of Viable Escherichia coli O157:H7, Listeria monocytogenes, and Staphylococcus aureus on Fresh-Cut Cantaloupe. *PLoS ONE*. 2016;11(12):e0166874. doi:10.1371/journal.pone.0166874

53. Hanabara Y, Ueda Y. A Rapid and Simple Real-Time PCR Assay for Detecting Foodborne Pathogenic Bacteria in Human Feces. *Jpn J Infect Dis*. Nov 22 2016;69(6):471-476. doi:10.7883/yoken.JJID.2015.205

54. 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*. Feb 2012;40(1):82-3. doi:10.1016/j.ajic.2011.07.019

55. van den Beld MJ, Reubsaet FA. Differentiation between Shigella, enteroinvasive Escherichia coli (EIEC) and noninvasive Escherichia coli. *Eur J Clin Microbiol Infect Dis*. Jun 2012;31(6):899-904. doi:10.1007/s10096-011-1395-7

56. Hahn A, Luetgehetmann M, Landt O, Schwarz NG, Frickmann H. Comparison of one commercial and two in-house TaqMan multiplex real-time PCR assays for detection of enteropathogenic, enterotoxigenic and enteroaggregative Escherichia coli. *Tropical medicine & international health : TM & IH*. Nov 2017;22(11):1371-1376. doi:10.1111/tmi.12976

57. Ud-Din A, Wahid S. Relationship among Shigella spp. and enteroinvasive Escherichia coli (EIEC) and their differentiation. *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*. 2014;45(4):1131-8.

58. Hughes JM, Murad F, Chang B, Guerrant RL. Role of cyclic GMP in the action of heat-stable enterotoxin of Escherichia coli. *Nature*. Feb 23 1978;271(5647):755-6.

59. 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*. Oct 2003;69(10):6327-33.

60. Melton-Celsa AR. Shiga Toxin (Stx) Classification, Structure, and Function. *Microbiology spectrum*. Aug 2014;2(4):Ehec-0024-2013. doi:10.1128/microbiolspec.EHEC-0024-2013

61. Bell RL, Jarvis KG, Ottesen AR, McFarland MA, Brown EW. Recent and emerging innovations in Salmonella detection: a food and environmental perspective. *Microb Biotechnol*. May 2016;9(3):279-92. doi:10.1111/1751-7915.12359

62. Zhang S, Yin Y, Jones MB, et al. Salmonella serotype determination utilizing high-throughput genome sequencing data. *Journal of clinical microbiology*. May 2015;53(5):1685-92. doi:10.1128/JCM.00323-15

63. Schmid A, Messelhausser U, Hormansdorfer S, Sauter-Louis C, Mansfeld R. Occurrence of zoonotic clostridia and Yersinia in healthy cattle. *Journal of food protection*. Oct 2013;76(10):1697-703. doi:10.4315/0362-028x.jfp-13-151

64. Bernardino-Varo L, Quinones-Ramirez EI, Fernandez FJ, Vazquez-Salinas C. Prevalence of Yersinia enterocolitica in raw cow's milk collected from stables of Mexico City. *Journal of food protection*. Apr 2013;76(4):694-8. doi:10.4315/0362-028x.jfp-12-325

65. Kaminska S, Sadkowska-Todys M. Yersiniosis in Poland in 2014. *Przegl Epidemiol*. 2016;70(3):367-374.

66. Laji N, Bowyer R, Jeyaratnam D, Zuckerman M. Another mistaken case of appendicitis. *BMJ case reports*. Oct 26 2015;2015doi:10.1136/bcr-2015-211861

67. Saebo A, Vik E, Lange OJ, Matuszkiewicz L. Inflammatory bowel disease associated with Yersinia enterocolitica O:3 infection. *Eur J Intern Med*. Jun 2005;16(3):176-182. doi:10.1016/j.ejim.2004.11.008

68. Petru G, Stunzner D, Lind P, Eber O, Mose JR. [Antibodies to Yersinia enterocolitica in immunogenic thyroid diseases]. *Acta medica Austriaca*. 1987;14(1):11-4. Antikorper gegen Yersinia enterocolitica bei immunogenen Schilddrusenerkrankungen.

69. Tomer Y, Davies TF. Infection, thyroid disease, and autoimmunity. *Endocr Rev*. Feb 1993;14(1):107-20. doi:10.1210/edrv-14-1-107

70. Novoa-Farias O, Frati-Munari AC, Peredo MA, et al. Susceptibility of bacteria isolated from acute gastrointestinal infections to rifaximin and other antimicrobial agents in Mexico. *Revista de gastroenterologia de Mexico*. Jan-Mar 2016;81(1):3-10. doi:10.1016/j.rgmx.2015.07.003

71. Petri W, R. H. Entamoeba histolytica brain abscess. *Handbook of clinical neurology*. 2013:147-152.

72. Nazer H. Giardiasis. Medscape. Accessed Nov 27, 2017. https://emedicine.medscape.com/article/176718-overview

73. Edling L RS, Eriksson S, Bohr J. Celiac disease and giardiasis: a case report. *European Journal of Gastroenterology and Hepatology*. 2012;24(8):984-987.

74. 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*. May 2007;26(5):311-23. doi:10.1007/s10096-007-0290-8

75. Huhulescu S, Kiss R, Brettlecker M, et al. Etiology of acute gastroenteritis in three sentinel general practices, Austria 2007. *Infection*. Apr 2009;37(2):103-8. doi:10.1007/s15010-008-8106-z

76. Seitz SR, Leon JS, Schwab KJ, et al. Norovirus infectivity in humans and persistence in water. *Applied and environmental microbiology*. Oct 2011;77(19):6884-8. doi:10.1128/AEM.05806-11

77. Gompf SG, Cunha BA. Adenoviruses clinical presentation. Medscape. Updated Jan 13, 2014. Accessed May 19, 2015. http://emedicine.medscape.com/article/211738-clinical

78. Benaroch R. Norovirus: symptoms and treatment. Accessed 6/3/2015, 2015. http://www.webmd.com/children/norovirus-symptoms-and-treatment?page=2

79. Noel JS FR, Ando T, Monroe SS, Glass RI. Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution. *Journal of infectious diseases*. 1999;179(6):1334-1344.

80. D'Souza DH, Sair A, Williams K, et al. Persistence of caliciviruses on environmental surfaces and their transfer to food. *Int J Food Microbiol*. Apr 15 2006;108(1):84-91. doi:10.1016/j.ijfoodmicro.2005.10.024

81. Atmar RL, Estes MK. The epidemiologic and clinical importance of norovirus infection. *Gastroenterol Clin North Am*. Jun 2006;35(2):275-90, viii. doi:10.1016/j.gtc.2006.03.001

82. Khan Z, Cunha BA. Norwalk virus. Medscape. Updated Feb 4, 2013. Accessed May 19, 2015. http://emedicine.medscape.com/article/224225-overview

83. 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*. Jan 2012;61(Pt 1):23-30. doi:10.1099/jmm.0.027052-0

84. 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):206-10.

85. 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*. Oct 2004;42(10):4512-8. doi:10.1128/JCM.42.10.4512-4518.2004

86. 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*. Jan 2008;129(1):89-96.

87. 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*. May 15 2012;130(10):2407-16. doi:10.1002/ijc.26242

88. 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*. Nov 2009;47(11):848-51.

89. Singh S, Jha HC. Status of Epstein-Barr Virus Coinfection with Helicobacter pylori in Gastric Cancer. *J Oncol*. 2017;2017:3456264. doi:10.1155/2017/3456264

90. Smolka AJ, Schubert ML. Helicobacter pylori-Induced Changes in Gastric Acid Secretion and Upper Gastrointestinal Disease. *Current topics in microbiology and immunology*. 2017;400:227-252. doi:10.1007/978-3-319-50520-6_10

91. Backert S, Clyne M. Pathogenesis of Helicobacter pylori infection. *Helicobacter*. Sep 2011;16 Suppl 1:19-25. doi:10.1111/j.1523-5378.2011.00876.x

92. Chung JM, Sheedlo MJ, Campbell AM, et al. Structure of the Helicobacter pylori Cag type IV secretion system. *Elife*. Jun 18 2019;8doi:10.7554/eLife.47644

93. Galmiche A, Rassow J. Targeting of Helicobacter pylori VacA to mitochondria. *Gut microbes*. Nov-Dec 2010;1(6):392-5. doi:10.4161/gmic.1.6.13894

94. Guttman JA, Finlay BB. Tight junctions as targets of infectious agents. *Biochim Biophys Acta*. Apr 2009;1788(4):832-41. doi:10.1016/j.bbamem.2008.10.028

95. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. *Clinical microbiology reviews*. Jul 2006;19(3):449-90. doi:10.1128/CMR.00054-05

96. Peek RM, Jr., Thompson SA, Donahue JP, et al. Adherence to gastric epithelial cells induces expression of a Helicobacter pylori gene, iceA, that is associated with clinical outcome. *Proc Assoc Am Physicians*. Nov-Dec 1998;110(6):531-44.

97. Salama NR, Hartung ML, Müller A. Life in the human stomach: persistence strategies of the bacterial pathogen Helicobacter pylori. *Nature reviews Microbiology*. Jun 2013;11(6):385-99. doi:10.1038/nrmicro3016

98. Shiota S, Watada M, Matsunari O, Iwatani S, Suzuki R, Yamaoka Y. Helicobacter pylori iceA, clinical outcomes, and correlation with cagA: a meta-analysis. *PLoS ONE*. 2012;7(1):e30354. doi:10.1371/journal.pone.0030354

99. Testerman TL, Morris J. Beyond the stomach: an updated view of Helicobacter pylori pathogenesis, diagnosis, and treatment. *World J Gastroenterol*. Sep 28 2014;20(36):12781-808. doi:10.3748/wjg.v20.i36.12781

100. van Doorn LJ, Figueiredo C, Sanna R, et al. Clinical relevance of the cagA, vacA, and iceA status of Helicobacter pylori. *Gastroenterology*. Jul 1998;115(1):58-66. doi:10.1016/s0016-5085(98)70365-8

101. Ansari S, Yamaoka Y. Helicobacter pylori BabA in adaptation for gastric colonization. *World J Gastroenterol*. Jun 21 2017;23(23):4158-4169. doi:10.3748/wjg.v23.i23.4158

102. Basso D, Zambon CF, Letley DP, et al. Clinical relevance of Helicobacter pylori cagA and vacA gene polymorphisms. *Gastroenterology*. Jul 2008;135(1):91-9. doi:10.1053/j.gastro.2008.03.041

103. Talebi Bezmin Abadi A, Perez-Perez G. Role of dupA in virulence of Helicobacter pylori. *World J Gastroenterol*. Dec 14 2016;22(46):10118-10123. doi:10.3748/wjg.v22.i46.10118

104. 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*. Apr 2017;105:226-230. doi:10.1016/j.micpath.2017.02.018

105. 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.

106. 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*. Nov 30 2017;75(8) doi:10.1093/femspd/ftx113

107. Naumann M, Sokolova O, Tegtmeyer N, Backert S. Helicobacter pylori: A Paradigm Pathogen for Subverting Host Cell Signal Transmission. *Trends in microbiology*. Apr 2017;25(4):316-328. doi:10.1016/j.tim.2016.12.004

108. Martín R, Miquel S, Ulmer J, Kechaou N, Langella P, Bermúdez-Humarán LG. Role of commensal and probiotic bacteria in human health: a focus on inflammatory bowel disease. *Microb Cell Fact*. Jul 23 2013;12:71. doi:10.1186/1475-2859-12-71

109. Khanna S, Tosh PK. A clinician's primer on the role of the microbiome in human health and disease. *Mayo Clin Proc*. Jan 2014;89(1):107-14. doi:10.1016/j.mayocp.2013.10.011

110. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature*. Sep 13 2012;489(7415):220-30. doi:10.1038/nature11550

111. Lopetuso LR, Scaldaferri F, Petito V, Gasbarrini A. Commensal Clostridia: leading players in the maintenance of gut homeostasis. *Gut pathogens*. Aug 13 2013;5(1):23. doi:10.1186/1757-4749-5-23

112. Fu X, Liu Z, Zhu C, Mou H, Kong Q. Nondigestible carbohydrates, butyrate, and butyrate-producing bacteria. *Crit Rev Food Sci Nutr*. 2019;59(sup1):S130-s152. doi:10.1080/10408398.2018.1542587

113. Oliphant K, Allen-Vercoe E. Macronutrient metabolism by the human gut microbiome: major fermentation by-products and their impact on host health. *Microbiome*. Jun 13 2019;7(1):91. doi:10.1186/s40168-019-0704-8

114. Parada Venegas D, De la Fuente MK, Landskron G, et al. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front Immunol*. 2019;10:277. doi:10.3389/fimmu.2019.00277

115. Rivera-Chávez F, Zhang LF, Faber F, et al. Depletion of Butyrate-Producing Clostridia from the Gut Microbiota Drives an Aerobic Luminal Expansion of Salmonella. *Cell Host Microbe*. Apr 13 2016;19(4):443-54. doi:10.1016/j.chom.2016.03.004

116. Lobo LA, Benjamim CF, Oliveira AC. The interplay between microbiota and inflammation: lessons from peritonitis and sepsis. *Clin Transl Immunology*. Jul 2016;5(7):e90. doi:10.1038/cti.2016.32

117. Maier E, Anderson RC, Roy NC. Understanding how commensal obligate anaerobic bacteria regulate immune functions in the large intestine. *Nutrients*. Dec 24 2014;7(1):45-73. doi:10.3390/nu7010045

118. 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*. Dec 2009;39(12):1842-51. doi:CEA3326 [pii] 10.1111/j.1365-2222.2009.03326.x

119. de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. *Advances in biochemical engineering/biotechnology*. 2008;111:1-66.

120. Sheil B, Shanahan F, O'Mahony L. Probiotic effects on inflammatory bowel disease. *J Nutr*. Mar 2007;137(3 Suppl 2):819S-24S.

121. Moens F, Verce M, De Vuyst L. Lactate- and acetate-based cross-feeding interactions between selected strains of lactobacilli, bifidobacteria and colon bacteria in the presence of inulin-type fructans. *Int J Food Microbiol*. Jan 16 2017;241:225-236. doi:10.1016/j.ijfoodmicro.2016.10.019

122. Franz CM, van Belkum MJ, Holzapfel WH, Abriouel H, Gálvez A. Diversity of enterococcal bacteriocins and their grouping in a new classification scheme. *FEMS Microbiol Rev*. Apr 2007;31(3):293-310. doi:10.1111/j.1574-6976.2007.00064.x

123. Barnes AMT, Dale JL, Chen Y, et al. Enterococcus faecalis readily colonizes the entire gastrointestinal tract and forms biofilms in a germ-free mouse model. *Virulence*. Apr 3 2017;8(3):282-296. doi:10.1080/21505594.2016.1208890

124. Nami Y, Vaseghi Bakhshayesh R, Mohammadzadeh Jalaly H, Lotfi H, Eslami S, Hejazi MA. Probiotic Properties of Enterococcus Isolated From Artisanal Dairy Products. *Front Microbiol*. 2019;10:300. doi:10.3389/fmicb.2019.00300

125. Li P, Gu Q, Wang Y, Yu Y, Yang L, Chen JV. Novel vitamin B(12)-producing Enterococcus spp. and preliminary in vitro evaluation of probiotic potentials. *Appl Microbiol Biotechnol*. Aug 2017;101(15):6155-6164. doi:10.1007/s00253-017-8373-7

126. Ghoshal UC, Shukla R, Ghoshal U. Small Intestinal Bacterial Overgrowth and Irritable Bowel Syndrome: A Bridge between Functional Organic Dichotomy. *Gut and liver*. Mar 15 2017;11(2):196-208. doi:10.5009/gnl16126

127. Maekawa T, Fukaya R, Takamatsu S, et al. Possible involvement of Enterococcus infection in the pathogenesis of chronic pancreatitis and cancer. *Biochem Biophys Res Commun*. Dec 2 2018;506(4):962-969. doi:10.1016/j.bbrc.2018.10.169

128. Strickertsson JA, Desler C, Martin-Bertelsen T, et al. Enterococcus faecalis infection causes inflammation, intracellular oxphos-independent ROS production, and DNA damage in human gastric cancer cells. *PLoS ONE*. 2013;8(4):e63147. doi:10.1371/journal.pone.0063147

129. Pillar CM, Gilmore MS. Enterococcal virulence--pathogenicity island of E. Faecalis. *Front Biosci*. Sep 1 2004;9:2335-46. doi:10.2741/1400

130. Gagliardi A, Totino V, Cacciotti F, et al. Rebuilding the Gut Microbiota Ecosystem. *International journal of environmental research and public health*. Aug 7 2018;15(8)doi:10.3390/ijerph15081679

131. Leitch C. How the E. coli Bacterium Can Benefit Us. labroots.com. 2022. https://www.labroots.com/trending/microbiology/12513/e-coli-bacterium-benefit

132. Christofi T, Panayidou S, Dieronitou I, Michael C, Apidianakis Y. Metabolic output defines Escherichia coli as a health-promoting microbe against intestinal Pseudomonas aeruginosa. *Sci Rep*. Oct 8 2019;9(1):14463. doi:10.1038/s41598-019-51058-3

133. Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG. The infant microbiome development: mom matters. *Trends Mol Med*. Feb 2015;21(2):109-17. doi:10.1016/j.molmed.2014.12.002

134. Winter SE, Winter MG, Xavier MN, et al. Host-derived nitrate boosts growth of E. coli in the inflamed gut. *Science*. Feb 8 2013;339(6120):708-11. doi:10.1126/science.1232467

135. Davin-Regli A, Lavigne JP, Pagès JM. Enterobacter spp.: Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance. *Clin Microbiol Rev*. Sep 18 2019;32(4)doi:10.1128/cmr.00002-19

136. Ramirez D, Giron M. Enterobacter Infections. *StatPearls*. StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC.; 2022.

137. Hiippala K, Jouhten H, Ronkainen A, et al. The Potential of Gut Commensals in Reinforcing Intestinal Barrier Function and Alleviating Inflammation. *Nutrients*. Jul 29 2018;10(8) doi:10.3390/nu10080988

138. Belzer C, Chia LW, Aalvink S, et al. Microbial Metabolic Networks at the Mucus Layer Lead to Diet-Independent Butyrate and Vitamin B(12) Production by Intestinal Symbionts. *mBio*. Sep 19 2017;8(5)doi:10.1128/mBio.00770-17

139. Chia LW, Hornung BVH, Aalvink S, et al. Deciphering the trophic interaction between Akkermansia muciniphila and the butyrogenic gut commensal Anaerostipes caccae using a metatranscriptomic approach. *Antonie Van Leeuwenhoek*. Jun 2018;111(6):859-873. doi:10.1007/s10482-018-1040-x

140. Cirstea M, Radisavljevic N, Finlay BB. Good Bug, Bad Bug: Breaking through Microbial Stereotypes. *Cell Host Microbe*. Jan 10 2018;23(1):10-13. doi:10.1016/j.chom.2017.12.008

141. Ottman N, Geerlings SY, Aalvink S, de Vos WM, Belzer C. Action and function of Akkermansia muciniphila in microbiome ecology, health and disease. *Best practice & research Clinical gastroenterology*. Dec 2017;31(6):637-642. doi:10.1016/j.bpg.2017.10.001

142. Jackson MA, Verdi S, Maxan ME, et al. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. *Nature communications*. Jul 9 2018;9(1):2655. doi:10.1038/s41467-018-05184-7

143. Cani PD, Everard A. Talking microbes: When gut bacteria interact with diet and host organs. *Mol Nutr Food Res*. Jan 2016;60(1):58-66. doi:10.1002/mnfr.201500406

144. Hernández MAG, Canfora EE, Jocken JWE, Blaak EE. The Short-Chain Fatty Acid Acetate in Body Weight Control and Insulin Sensitivity. *Nutrients*. Aug 18 2019;11(8)doi:10.3390/nu11081943

145. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol*. Jan 2017;19(1):29-41. doi:10.1111/1462-2920.13589

146. Tamanai-Shacoori Z, Smida I, Bousarghin L, et al. Roseburia spp.: a marker of health? *Future microbiology*. Feb 2017;12:157-170. doi:10.2217/fmb-2016-0130

147. 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*. Jun 2011;7(3):501-7. doi:10.5114/aoms.2011.23418

148. 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*. Apr 8 2017;5(1):43. doi:10.1186/s40168-017-0258-6

149. 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. doi:10.1186/1471-2164-9-576

150. 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*. Jun 2015;31(6):884-9. doi:10.1016/j.nut.2015.01.004

151. Markou P, Apidianakis Y. Pathogenesis of intestinal Pseudomonas aeruginosa infection in patients with cancer. *Front Cell Infect Microbiol*. 2014;3:115. doi:10.3389/fcimb.2013.00115

152. Chuang CH, Wang YH, Chang HJ, et al. Shanghai fever: a distinct Pseudomonas aeruginosa enteric disease. *Gut*. May 2014;63(5):736-43. doi:10.1136/gutjnl-2013-304786

153. Kushkevych I, Hýžová B, Vítězová M, Rittmann SKR. Microscopic Methods for Identification of Sulfate-Reducing Bacteria from Various Habitats. *Int J Mol Sci*. Apr 13 2021;22(8)doi:10.3390/ijms22084007

154. Braccia DJ, Jiang X, Pop M, Hall AB. The Capacity to Produce Hydrogen Sulfide (H(2)S) via Cysteine Degradation Is Ubiquitous in the Human Gut Microbiome. *Front Microbiol*. 2021;12:705583. doi:10.3389/fmicb.2021.705583

155. Carbonero F, Benefiel AC, Alizadeh-Ghamsari AH, Gaskins HR. Microbial pathways in colonic sulfur metabolism and links with health and disease. *Front Physiol*. 2012;3:448. doi:10.3389/fphys.2012.00448

156. Coffman CN, Varga MG, Alcock J, et al. Norepinephrine induces growth of Desulfovibrio vulgaris in an iron dependent manner. *Anaerobe*. Jun 2022;75:102582. doi:10.1016/j.anaerobe.2022.102582

157. Forsberg CW. Sulfide Production from Cysteine by Desulfovibrio desulfuricans. *Appl Environ Microbiol*. Feb 1980;39(2):453-5. doi:10.1128/aem.39.2.453-455.1980

158. Rey FE, Gonzalez MD, Cheng J, Wu M, Ahern PP, Gordon JI. Metabolic niche of a prominent sulfate-reducing human gut bacterium. *Proc Natl Acad Sci U S A*. Aug 13 2013;110(33):13582-7. doi:10.1073/pnas.1312524110

159. Goldstein EJ, Citron DM, Peraino VA, Cross SA. Desulfovibrio desulfuricans bacteremia and review of human Desulfovibrio infections. *J Clin Microbiol*. Jun 2003;41(6):2752-4. doi:10.1128/jcm.41.6.2752-2754.2003

160. Loubinoux J, Bronowicki JP, Pereira IA, Mougenel JL, Faou AE. Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. *FEMS Microbiol Ecol*. May 1 2002;40(2):107-12. doi:10.1111/j.1574-6941.2002.tb00942.x

161. Sun Y, Xie R, Li L, et al. Prenatal Maternal Stress Exacerbates Experimental Colitis of Offspring in Adulthood. *Front Immunol*. 2021;12:700995. doi:10.3389/fimmu.2021.700995

162. Kushkevych I, Castro Sangrador J, Dordević D, et al. Evaluation of Physiological Parameters of Intestinal Sulfate-Reducing Bacteria Isolated from Patients Suffering from IBD and Healthy People. *J Clin Med*. Jun 19 2020;9(6)doi:10.3390/jcm9061920

163. Chen YR, Jing QL, Chen FL, Zheng H, Chen LD, Yang ZC. Desulfovibrio is not always associated with adverse health effects in the Guangdong Gut Microbiome Project. *PeerJ*. 2021;9:e12033. doi:10.7717/peerj.12033

164. Chaudhary PP, Conway PL, Schlundt J. Methanogens in humans: potentially beneficial or harmful for health. *Appl Microbiol Biotechnol*. Apr 2018;102(7):3095-3104. doi:10.1007/s00253-018-8871-2

165. Ghavami SB, Rostami E, Sephay AA, et al. Alterations of the human gut Methanobrevibacter smithii as a biomarker for inflammatory bowel diseases. *Microbial pathogenesis*. Apr 2018;117:285-289. doi:10.1016/j.micpath.2018.01.029

166. Ghoshal U, Shukla R, Srivastava D, Ghoshal UC. Irritable Bowel Syndrome, Particularly the Constipation-Predominant Form, Involves an Increase in Methanobrevibacter smithii, Which Is Associated with Higher Methane Production. *Gut and liver*. Nov 15 2016;10(6):932-938. doi:10.5009/gnl15588

167. Beghini F, Pasolli E, Truong TD, Putignani L, Cacciò SM, Segata N. Large-scale comparative metagenomics of Blastocystis, a common member of the human gut microbiome. *Isme j*. Dec 2017;11(12):2848-2863. doi:10.1038/ismej.2017.139

168. Desailloud R, Hober D. Viruses and thyroiditis: an update. *Virology journal*. 2009;6:5. doi:10.1186/1743-422X-6-5

169. DeMeo MT, Mutlu EA, Keshavarzian A, Tobin MC. Intestinal permeation and gastrointestinal disease. *Journal of clinical gastroenterology*. Apr 2002;34(4):385-96.

170. Fasano A, Shea-Donohue T. Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. *Nature clinical practice*. Sep 2005;2(9):416-22.

171. 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):11-6.

172. Ebringer A, Rashid T. Rheumatoid arthritis is an autoimmune disease triggered by Proteus urinary tract infection. *Clin Dev Immunol*. Mar 2006;13(1):41-8. doi:10.1080/17402520600576578

173. 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.

174. Ebringer A, Wilson C. HLA molecules, bacteria and autoimmunity. *Journal of medical microbiology*. Apr 2000;49(4):305-11.

175. Minerva P, Diamond HS. Enteropathic arthropathies. Medscape. Updated December 30, 2014. Accessed May 14, 2015. http://emedicine.medscape.com/article/334746-overview#a0101

176. 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):232-4. doi:10.3109/03009742.2012.733960

177. 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)*. Jul 2009;48(7):748-54. doi:10.1093/rheumatology/kep113

178. Lozada CJ, Diamond HS. Reactive arthritis. Medscape. Updated Aug 20, 2018. Accessed Oct 31, 2018. http://emedicine.medscape.com/article/331347-overview#aw2aab6b2b2

179. Bellocchi C, Volkmann ER. Update on the Gastrointestinal Microbiome in Systemic Sclerosis. *Curr Rheumatol Rep*. Jun 25 2018;20(8):49. doi:10.1007/s11926-018-0758-9

180. Brennan CA, Garrett WS. Fusobacterium nucleatum - symbiont, opportunist and oncobacterium. *Nature reviews Microbiology*. Mar 2019;17(3):156-166. doi:10.1038/s41579-018-0129-6

181. Hayata M, Watanabe N, Tamura M, et al. The Periodontopathic Bacterium Fusobacterium nucleatum Induced Proinflammatory Cytokine Production by Human Respiratory Epithelial Cell Lines and in the Lower Respiratory Organs in Mice. *Cell Physiol Biochem*. 2019;53(1):49-61. doi:10.33594/000000120

182. He C, Wang H, Liao WD, et al. Characteristics of mucosa-associated gut microbiota during treatment in Crohn's disease. *World J Gastroenterol*. May 14 2019;25(18):2204-2216. doi:10.3748/wjg.v25.i18.2204

183. Karpiński TM. Role of Oral Microbiota in Cancer Development. *Microorganisms*. Jan 13 2019;7(1) doi:10.3390/microorganisms7010020

184. 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*. Oct 1984;15(3-4):238-41. doi:10.1007/bf01972355

185. Drzewiecka D. Significance and Roles of Proteus spp. Bacteria in Natural Environments. *Microbial ecology*. Nov 2016;72(4):741-758. doi:10.1007/s00248-015-0720-6

186. Norsworthy AN, Pearson MM. From Catheter to Kidney Stone: The Uropathogenic Lifestyle of Proteus mirabilis. *Trends in microbiology*. Apr 2017;25(4):304-315. doi:10.1016/j.tim.2016.11.015

187. Ansell T, Harari D. Urinary catheter-related visits to the emergency department and implications for community services. *British journal of nursing (Mark Allen Publishing)*. May 11 2017;26(9):S4-s11. doi:10.12968/bjon.2017.26.9.S4

188. Hobbs T, Schultz LN, Lauchnor EG, Gerlach R, Lange D. Evaluation of Biofilm Induced Urinary Infection Stone Formation in a Novel Laboratory Model System. *J Urol*. Jan 2018;199(1):178-185. doi:10.1016/j.juro.2017.08.083

189. Magyar A, Koves B, Nagy K, et al. Spectrum and antibiotic resistance of uropathogens between 2004 and 2015 in a tertiary care hospital in Hungary. *Journal of medical microbiology*. Jun 2017;66(6):788-797. doi:10.1099/jmm.0.000498

190. Sarker MM, Saha SK, Saha S, et al. Current Trends of Using Antimicrobials and Their Sensitivity Pattern in Infectious Cases at Department of Orthopedics in a Tertiary Care Hospital. *Mymensingh medical journal : MMJ*. Jul 2017;26(3):530-540.

191. Hidalgo JA CB. Candidiasis. emedicine from WebMD. Accessed 5/14/2013, 2013. https://emedicine.medscape.com/article/213853-overview#showall

192. Santelmann H, Howard JM. Yeast metabolic products, yeast antigens and yeasts as possible triggers for irritable bowel syndrome. *Eur J Gastroenterol Hepatol*. Jan 2005;17(1):21-6. doi:10.1097/00042737-200501000-00005

193. Olmstead S MD, Ralson J. Candida, fungal-type dysbiosis and chronic disease: exploring the nature of the yeast connection. *Townsend Letter*. 2012;5

194. Cater RE, 2nd. Chronic intestinal candidiasis as a possible etiological factor in the chronic fatigue syndrome. *Med Hypotheses*. Jun 1995;44(6):507-15. doi:10.1016/0306-9877(95)90515-4

195. Ghosh K, Weiss LM. Molecular diagnostic tests for microsporidia. *Interdiscip Perspect Infect Dis*. 2009;2009:926521. doi:10.1155/2009/926521

196. Wojcik A, Blaszkowska J, Kurnatowski P, Goralska K. Sandpits as a reservoir of potentially pathogenic fungi for children. *Annals of agricultural and environmental medicine : AAEM*. Dec 23 2016;23(4):542-548. doi:10.5604/12321966.1226843

197. Guidara R, Trabelsi H, Neji S, et al. Rhodotorula fungemia: Report of two cases in Sfax (Tunisia). *Journal de mycologie medicale*. Jun 2016;26(2):178-181. doi:10.1016/j.mycmed.2016.02.020

198. Akhter K. Cytomegalovirus Clinical Presentation. Medscape. Accessed Nov 8, 2017. https://emedicine.medscape.com/article/215702-clinical

199. Cytomegalovirus (CMV) and Congenital CMV Infection. Centers for Disease Control and Prevention. Nov 7, 2017. https://www.cdc.gov/cmv/clinical/features.html

200. 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*. Nov 28 2015;21(44):12667-75. doi:10.3748/wjg.v21.i44.12667

201. 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*. Sep 2017;29(9):1059-1063. doi:10.1097/meg.0000000000000906

202. 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*. Sep 2016;51(9):1075-80. doi:10.3109/00365521.2016.1156154

203. 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*. Feb 14 2015;21(6):1915-26. doi:10.3748/wjg.v21.i6.1915

204. 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*. Dec 2016;49(6):829-836. doi:10.1016/j.jmii.2015.12.007

205. Halenius A, Hengel H. Human cytomegalovirus and autoimmune disease. *BioMed research international*. 2014;2014:472978. doi:10.1155/2014/472978

206. Epstein-Barr Virus and Infectious Mononucleosis. Centers for Disease Control and Prevention. Accessed Nov 16, 2017. https://www.cdc.gov/epstein-barr/about-ebv.html

207. 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.

208. 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. doi:10.1155/2012/189096

209. Klutts JS, Ford BA, Perez NR, Gronowski AM. Evidence-based approach for interpretation of Epstein-Barr virus serological patterns. *Journal of clinical microbiology*. Oct 2009;47(10):3204-10. doi:10.1128/JCM.00164-09

210. Draborg AH, Duus K, Houen G. Epstein-Barr virus in systemic autoimmune diseases. *Clinical & developmental immunology*. 2013;2013:535738. doi:10.1155/2013/535738

211. Houen G, Trier NH, Frederiksen JL. Epstein-Barr Virus and Multiple Sclerosis. *Front Immunol*. 2020;11:587078. doi:10.3389/fimmu.2020.587078

212. 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. doi:10.5114/ceji.2016.63130

213. Dagci H, Kurt Ö, Demirel M, et al. Epidemiological and diagnostic features of blastocystis infection in symptomatic patients in izmir province, Turkey. *Iran J Parasitol*. Oct-Dec 2014;9(4):519-29.

214. Basak S, Rajurkar MN, Mallick SK. Detection of Blastocystis hominis: a controversial human pathogen. *Parasitol Res*. Jan 2014;113(1):261-5. doi:10.1007/s00436-013-3652-4

215. Coyle CM, Varughese J, Weiss LM, Tanowitz HB. Blastocystis: to treat or not to treat. *Clin Infect Dis*. Jan 1 2012;54(1):105-10. doi:10.1093/cid/cir810

216. Tan KS. New insights on classification, identification, and clinical relevance of Blastocystis spp. *Clin Microbiol Rev*. Oct 2008;21(4):639-65. doi:10.1128/cmr.00022-08

217. 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*. Dec 2015;143(16):3451-8. doi:10.1017/s0950268815000370

218. 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*. Oct 15 2015;211:95-100. doi:10.1016/j.ijfoodmicro.2015.07.002

219. 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*. Jun 15 2014;484:129-36. doi:10.1016/j.scitotenv.2014.03.036

220. 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*. Apr 2012;140(4):626-32. doi:10.1017/s095026881100121x

221. Orozco-Mosqueda GE, Martinez-Loya OA, Ortega YR. Cyclospora cayetanensis in a pediatric hospital in Morelia, Mexico. *Am J Trop Med Hyg*. Sep 2014;91(3):537-40. doi:10.4269/ajtmh.13-0535

222. Kasper MR, Lescano AG, Lucas C, et al. Diarrhea outbreak during U.S. military training in El Salvador. *PLoS ONE*. 2012;7(7):e40404. doi:10.1371/journal.pone.0040404

223. Tas Cengiz Z, Beyhan YE, Yilmaz H. Cyclospora cayetanensis, Opportunistic Protozoan Parasite, in Van Province, Turkey: A Report of Seven Cases. *Turkiye parazitolojii dergisi*. Sep 2016;40(3):166-168. doi:10.5152/tpd.2016.4572

224. Sarzhanov F, Dogruman-Al F, Santin M, et al. Investigation of neglected protists Blastocystis sp. and Dientamoeba fragilis in immunocompetent and immunodeficient diarrheal patients using both conventional and molecular methods. *PLoS Negl Trop Dis*. Oct 2021;15(10):e0009779. doi:10.1371/journal.pntd.0009779

225. Burgaña A, Abellana R, Yordanov SZ, et al. Paromomycin is superior to metronidazole in Dientamoeba fragilis treatment. *Int J Parasitol Drugs Drug Resist*. Dec 2019;11:95-100. doi:10.1016/j.ijpddr.2019.10.005

226. Boga JA, Rojo S, Fernández J, et al. Is the treatment of Enterobius vermicularis co-infection necessary to eradicate Dientamoeba fragilis infection? *Int J Infect Dis*. Aug 2016;49:59-61. doi:10.1016/j.ijid.2016.05.027

227. Zhang H, Yu Y, Li J, et al. Changes of gut microbiota in colorectal cancer patients with Pentatrichomonas hominis infection. *Frontiers in cellular and infection microbiology*. 2022;12:961974. doi:10.3389/fcimb.2022.961974

228. Issa RM. Non-pathogenic Protozoa (Review Article). *International Journal of Pharmacy and Pharmaceutical Sciences*. 2014;6:30-40.

229. Meloni D, Mantini C, Goustille J, et al. Molecular identification of Pentatrichomonas hominis in two patients with gastrointestinal symptoms. *Journal of clinical pathology*. Oct 2011;64(10):933-5. doi:10.1136/jcp.2011.089326

230. Khurana S, Sethi S. Laboratory diagnosis of soil transmitted helminthiasis. *Tropical parasitology*. Jul-Dec 2017;7(2):86-91. doi:10.4103/tp.TP_29_17

231. 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*. Mar 2017;167:64-70. doi:10.1016/j.actatropica.2016.12.014

232. Haburchak DR. Hookworm Disease. Medscape. Accessed Dec 8, 2017. https://emedicine.medscape.com/article/218805-overview#a5

233. 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*. Nov 2013;46(6):671-4. doi:10.5946/ce.2013.46.6.671

234. Omran E, Mohammad AN. Intestinal parasites in patients with chronic abdominal pain. *Journal of the Egyptian Society of Parasitology*. Aug 2015;45(2):389-96.

235. 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*. Jul-Dec 2017;7(2):111-116. doi:10.4103/tp.TP_26_17

236. Moser W, Schindler C, Keiser J. Efficacy of recommended drugs against soil transmitted helminths: systematic review and network meta-analysis. *Bmj*. Sep 25 2017;358:j4307. doi:10.1136/bmj.j4307

237. Wei KY, Yan Q, Tang B, et al. Hookworm Infection: A Neglected Cause of Overt Obscure Gastrointestinal Bleeding. *The Korean journal of parasitology*. Aug 2017;55(4):391-398. doi:10.3347/kjp.2017.55.4.391

238. 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*. Apr 2016;46(2):100-2. doi:10.1177/0049475515598667

239. 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*. Sep-Oct 2015;81(5):705-10. doi:10.1016/j.aogh.2015.10.001

240. Murrell KD. Zoonotic foodborne parasites and their surveillance. *Revue scientifique et technique (International Office of Epizootics)*. Aug 2013;32(2):559-69.

241. Rostami S, Salavati R, Beech RN, Babaei Z, Sharbatkhori M, Harandi MF. Genetic variability of Taenia saginata inferred from mitochondrial DNA sequences. *Parasitol Res*. Apr 2015;114(4):1365-76. doi:10.1007/s00436-015-4314-5

242. Hailemariam Z, Nakao M, Menkir S, et al. Molecular identification of species of Taenia causing bovine cysticercosis in Ethiopia. *Journal of helminthology*. Sep 2014;88(3):376-80. doi:10.1017/s0022149x13000138

243. 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*. Jul 2017;11(7):e0005743. doi:10.1371/journal.pntd.0005743

244. 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*. Jun 2016;40:357-67. doi:10.1016/j.meegid.2015.06.022

245. Roelfsema JH, Nozari N, Pinelli E, Kortbeek LM. Novel PCRs for differential diagnosis of cestodes. *Exp Parasitol*. Feb 2016;161:20-6. doi:10.1016/j.exppara.2015.12.010

246. Nanjappa S. Taenia Infection Clinical Presentation. Medscape. Accessed Dec 8, 2017. https://emedicine.medscape.com/article/999727-clinical?src=refgatesrc1

247. 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*. Jan 2017;165:246-251. doi:10.1016/j.actatropica.2015.10.012

248. 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*. Jan 3 2017;17(1):1. doi:10.1186/s12879-016-2122-x

249. 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*. Dec 2009;28(6):195-7. doi:10.1007/s12664-009-0076-2

250. Amann ST, Josephson SA, Toskes PP. Acid steatocrit: a simple, rapid gravimetric method to determine steatorrhea. *The American journal of gastroenterology*. Dec 1997;92(12):2280-4.

251. Bijoor AR, Geetha S, Venkatesh T. Faecal fat content in healthy adults by the 'acid steatocrit method'. *Indian J Clin Biochem*. Jul 2004;19(2):20-2. doi:10.1007/BF02894252

252. Sperti C, Moletta L. Staging chronic pancreatitis with exocrine function tests: Are we better? *World J Gastroenterol*. Oct 14 2017;23(38):6927-6930. doi:10.3748/wjg.v23.i38.6927

253. Turner RC, McDermott R. Using faecal elastase-1 to screen for chronic pancreatitis in patients admitted with acute pancreatitis. *HPB (Oxford)*. 2006;8(3):223-6. doi:10.1080/13651820500539602

254. Elphick DA, Kapur K. Comparing the urinary pancreolauryl ratio and faecal elastase-1 as indicators of pancreatic insufficiency in clinical practice. *Pancreatology*. 2005;5(2-3):196-200. doi:10.1159/000085271

255. 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*. Sep 2016;25(3):303-9. doi:10.15403/jgld.2014.1121.253.uks

256. Walkowiak J, Wadolowska L, Szaflarska-Poplawska A, Lisowska A, Bugajewska A, Przyslawski J. The elimination of meat from the diet selectively decreases pancreatic elastase secretion. *Br J Nutr*. Jul 2007;98(1):154-8. doi:10.1017/s0007114507691764

257. Capurso G, Traini M, Piciucchi M, Signoretti M, Arcidiacono PG. Exocrine pancreatic insufficiency: prevalence, diagnosis, and management. *Clin Exp Gastroenterol*. 2019;12:129-139. doi:10.2147/ceg.S168266

258. 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*. Nov 2005;28(11):2810-1. doi:10.2337/diacare.28.11.2810

259. 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):319-25.

260. 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):5310-6.

261. 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):265-9.

262. Adamski KKJJ. Fecal Occult Blood Test. National Library of Medicine. 2021. https://www.ncbi.nlm.nih.gov/books/NBK537138/

263. Randel KR, Schult AL, Botteri E, et al. Colorectal Cancer Screening With Repeated Fecal Immunochemical Test Versus Sigmoidoscopy: Baseline Results From a Randomized Trial. *Gastroenterology*. Mar 2021;160(4):1085-1096.e5. doi:10.1053/j.gastro.2020.11.037

264. Rogier EW, Frantz AL, Bruno ME, Kaetzel CS. Secretory IgA is Concentrated in the Outer Layer of Colonic Mucus along with Gut Bacteria. *Pathogens*. Apr 29 2014;3(2):390-403. doi:10.3390/pathogens3020390

265. Corthësy B. Secretory immunoglobulin A: well beyond immune exclusion at mucosal surfaces. *Immunopharmacol Immunotoxicol*. Jun 2009;31(2):174-9. doi:10.1080/08923970802438441

266. Kaetzel CS. Cooperativity among secretory IgA, the polymeric immunoglobulin receptor, and the gut microbiota promotes host-microbial mutualism. *Immunology letters*. Dec 2014;162(2 Pt A):10-21. doi:10.1016/j.imlet.2014.05.008

267. Haas L, Meillet D, Kapel N, Rostoker G, Gobert JG. Increased concentrations of fecal anti-gliadin IgA antibodies in untreated celiac disease. *Clin Chem*. Apr 1993;39(4):696-7.

268. 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):551-7.

269. Abedin N, Seemann T, Kleinfeld S, et al. Fecal Eosinophil Cationic Protein Is a Diagnostic and Predictive Biomarker in Young Adults with Inflammatory Bowel Disease. *J Clin Med*. Nov 20 2019;8(12)doi:10.3390/jcm8122025

270. Bentz S, Hausmann M, Piberger H, et al. Clinical relevance of IgG antibodies against food antigens in Crohn's disease: a double-blind cross-over diet intervention study. *Digestion*. 2010;81(4):252-64. doi:10.1159/000264649

271. Kalach N, Kapel N, Waligora-Dupriet AJ, et al. Intestinal permeability and fecal eosinophil-derived neurotoxin are the best diagnosis tools for digestive non-IgE-mediated cow's milk allergy in toddlers. *Clin Chem Lab Med*. Feb 2013;51(2):351-61. doi:10.1515/cclm-2012-0083

272. Konikoff MR, Blanchard C, Kirby C, et al. Potential of blood eosinophils, eosinophil-derived neurotoxin, and eotaxin-3 as biomarkers of eosinophilic esophagitis. *Clin Gastroenterol Hepatol*. Nov 2006;4(11):1328-36. doi:10.1016/j.cgh.2006.08.013

273. Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. *Clinical and experimental gastroenterology*. 2016;9:21-9. doi:10.2147/CEG.S51902

274. Siddiqui I, Majid H, Abid S. Update on clinical and research application of fecal biomarkers for gastrointestinal diseases. *World J Gastrointest Pharmacol Ther*. Feb 06 2017;8(1):39-46. doi:10.4292/wjgpt.v8.i1.39

275. 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*. Feb 02 2017;19(1):21. doi:10.1186/s13075-017-1223-2

276. 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. doi:10.3389/fped.2017.00007

277. 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*. Jul 15 2010;341:c3369. doi:10.1136/bmj.c3369

278. Pathirana WGW, Chubb SP, Gillett MJ, Vasikaran SD. Faecal Calprotectin. *Clin Biochem Rev*. Aug 2018;39(3):77-90.

279. Gisbert JP, McNicholl AG, Gomollon F. Questions and answers on the role of fecal lactoferrin as a biological marker in inflammatory bowel disease. *Inflamm Bowel Dis*. Nov 2009;15(11):1746-54. doi:10.1002/ibd.20920

280. Jukic A, Bakiri L, Wagner EF, Tilg H, Adolph TE. Calprotectin: from biomarker to biological function. *Gut*. Oct 2021;70(10):1978-1988. doi:10.1136/gutjnl-2021-324855

281. Lewis JD. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. *Gastroenterology*. May 2011;140(6):1817-1826.e2. doi:10.1053/j.gastro.2010.11.058

282. Mumolo MG, Bertani L, Ceccarelli L, et al. From bench to bedside: Fecal calprotectin in inflammatory bowel diseases clinical setting. *World J Gastroenterol*. Sep 7 2018;24(33):3681-3694. doi:10.3748/wjg.v24.i33.3681

283. 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. *J Int Soc Sports Nutr*. Sep 20 2012;9(1):45. doi:10.1186/1550-2783-9-45

284. 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*. Nov 2016;13:190-200. doi:10.1016/j.ebiom.2016.10.036

285. Fasano A, Sapone A, Zevallos V, Schuppan D. Nonceliac gluten sensitivity. *Gastroenterology*. May 2015;148(6):1195-204. doi:10.1053/j.gastro.2014.12.049

286. Fasano A. Leaky gut and autoimmune diseases. *Clinical reviews in allergy & immunology*. Feb 2012;42(1):71-8. doi:10.1007/s12016-011-8291-x

287. 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*. Nov 2008;173(5):1243-52. doi:10.2353/ajpath.2008.080192

288. Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability--a new target for disease prevention and therapy. *BMC Gastroenterol*. Nov 18 2014;14:189. doi:10.1186/s12876-014-0189-7

289. 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*. Jun 2015;421:44-53. doi:10.1016/j.jim.2014.12.015

290. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. *Journal of cell science*. Dec 2000;113 Pt 24:4435-40.

291. 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. doi:10.1186/s12970-015-0101-z

292. Shan L, Molberg Ø, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. *Science*. Sep 27 2002;297(5590):2275-9. doi:10.1126/science.1074129

293. Schalk K, Lang C, Wieser H, Koehler P, Scherf KA. Quantitation of the immunodominant 33-mer peptide from alpha-gliadin in wheat flours by liquid chromatography tandem mass spectrometry. *Scientific reports*. Mar 22 2017;7:45092. doi:10.1038/srep45092

294. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiological reviews*. Jan 2011;91(1):151-75. doi:10.1152/physrev.00003.2008

295. Balakireva AV, Zamyatnin AA. Properties of Gluten Intolerance: Gluten Structure, Evolution, Pathogenicity and Detoxification Capabilities. *Nutrients*. Oct 18 2016;8(10)doi:10.3390/nu8100644

296. Moreno ML, Rodríguez-Herrera A, Sousa C, Comino I. Biomarkers to Monitor Gluten-Free Diet Compliance in Celiac Patients. *Nutrients*. Jan 6 2017;9(1)doi:10.3390/nu9010046

297. Pietzak MM. Follow-up of patients with celiac disease: achieving compliance with treatment. *Gastroenterology*. Apr 2005;128(4 Suppl 1):S135-41. doi:10.1053/j.gastro.2005.02.025

298. Lerner BA, Phan Vo LT, Yates S, Rundle AG, Green PHR, Lebwohl B. Detection of Gluten in Gluten-Free Labeled Restaurant Food: Analysis of Crowd-Sourced Data. *Am J Gastroenterol*. May 2019;114(5):792-797. doi:10.14309/ajg.0000000000000202

299. Hollon JR, Cureton PA, Martin ML, Puppa EL, Fasano A. Trace gluten contamination may play a role in mucosal and clinical recovery in a subgroup of diet-adherent non-responsive celiac disease patients. *BMC gastroenterology*. Feb 28 2013;13:40. doi:10.1186/1471-230X-13-40

300. Comino I, Real A, Vivas S, et al. Monitoring of gluten-free diet compliance in celiac patients by assessment of gliadin 33-mer equivalent epitopes in feces. *Am J Clin Nutr*. Mar 2012;95(3):670-7. doi:10.3945/ajcn.111.026708

301. Watkins RR. Antibiotic stewardship in the era of precision medicine. *JAC Antimicrob Resist*. Jun 2022;4(3):dlac066. doi:10.1093/jacamr/dlac066

302. Bankar NJ, Ugemuge S, Ambad RS, Hawale DV, Timilsina DR. Implementation of Antimicrobial Stewardship in the Healthcare Setting. *Cureus*. Jul 2022;14(7):e26664. doi:10.7759/cureus.26664

303. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile Genetic Elements Associated with Antimicrobial Resistance. *Clinical microbiology reviews*. Oct 2018;31(4)doi:10.1128/CMR.00088-17

304. Xiang J ZZ, Xie H, et al. Effect of different bile acids on the intestine through enterohepatic circulation based on FXR. *Mucosal Immunol*. 2021;13(1):1949095. doi:10.1080/19490976.2021.1949095

305. Chen ML TK, Sundrud MS. Emerging roles of bile acids in mucosal immunity and inflammation. *Mucosal Immunol*. 2019;12(4):851-861. doi:10.1038/s41385-019-0162-4

306. Larabi AB MH, Bäumler AJ. Bile acids as modulators of gut microbiota composition and function. *Gut Microbes*. 2023;15(1):2172671. doi:10.1080/19490976.2023.2172671

307. Vital M RT, Rath S, Pieper DH, Schlüter D. Diversity of Bacteria Exhibiting Bile Acid-inducible 7α-dehydroxylation Genes in the Human Gut. *Comput Struct Biotechnol J.* 2019;17:1016-1019. doi:10.1016/j.csbj.2019.07.012

308. MahmoudianDehkordi S AM, Nho K, et al. Altered bile acid profile associates with cognitive impairment in Alzheimer's disease—An emerging role for gut microbiome. *Alzheimers Dement.* 2019;15(1):76-92. doi:10.1016/j.jalz.2018.07.217

309. Collins SL SJ, Bisanz JE, Okafor CD, Patterson AD. Bile acids and the gut microbiota: metabolic interactions and impacts on disease. *Nat Rev Microbiol.* 2023;21(4):236-247. doi:10.1038/s41579-022-00805-x

310. Caliceti C PA, Silla A, Simoni P, Roda G, Hrelia S. New Insights into Bile Acids Related Signaling Pathways in the Onset of Colorectal Cancer. *Nutrients*. 2022;14(14):2964. doi:10.3390/nu14142964

311. Farrugia A AR. Bile acid diarrhoea: pathophysiology, diagnosis and management. *Frontline Gastroenterol.* 2020;12(6):500-507. doi:10.1136/flgastro-2020-101436

312. Thomas JP MD, Rushbrook SM, Powell N, Korcsmaros T. The Emerging Role of Bile Acids in the Pathogenesis of Inflammatory Bowel Disease. *Front Immunol.* 2022;13:829525. doi:10.3389/fimmu.2022.829525

313. Sinha SR HY, Nguyen LP, et al. Dysbiosis-Induced Secondary Bile Acid Deficiency Promotes Intestinal Inflammation. *Cell Host Microbe.* 2020;27(4):659-670.e5. doi:10.1016/j.chom.2020.01.021

314. Yang ZH LF, Zhu XR, Suo FY, Jia ZJ, Yao SK. Altered profiles of fecal bile acids correlate with gut microbiota and inflammatory responses in patients with ulcerative colitis. *World J Gastroenterol.* 2021;27(24):3609-3629. doi:10.3748/wjg.v27.i24.3609

315. Li N KS, Lachance DM, Dutta M, Cui JY, Dey N. Microbiome-encoded bile acid metabolism modulates colonic transit times. *iScience.* 2021;24(6):102508. doi:10.1016/j.isci.2021.102508

316. Yang Z, Guo, Y., Huang, J., Gao, Y., Peng, F., Xu, R., ... Zhang, P. Isomaltulose exhibits prebiotic activity, and modulates gut microbiota, the production of short chain fatty acids, and secondary bile acids in rats. . *Molecules.* 2021;26(9):2464. doi:10.3390/molecules26092464

317. Sun X, Winglee, K., Gharaibeh, RZ., Gauthier, J., He, Z., Tripathi, P., ... Jobin, C. Microbiota-derived metabolic factors reduce campylobacteriosis in mice. *Gastroenterology.* 2018;154(6):1751-1763.e2. doi:10.1053/j.gastro.2018.01.042

318. Triantos C, Koukias, NM., Nikolopoulou, VN., Burroughs AK. Meta-analysis: ursodeoxycholic acid for primary sclerosing cholangitis. alimentary pharmacology & therapeutics. *Aliment Pharmacol Ther.* 2011;34:901-910.

319. Chiang J. Bile acid metabolism and signaling. *Comprehensive Physiology.* 2013;

320. Chen B. BY, Tong F., Yan J., Zhang R., Zhong Y., ... Ma X. Glycoursodeoxycholic acid regulates bile acids level and alters gut microbiota and glycolipid metabolism to attenuate diabetes. *Gut Microbes.* 2023;15(1)

321. Yang I. JG, Patil B. In vitro bile acid binding capacities of red leaf lettuce and cruciferous vegetables. *Journal of Agricultural and Food Chemistry.* 2017;65(36):8054-8062.

322. Ziegler F. SA, Pizio A., Behrens M. Physiological activation of human and mouse bitter taste receptors by bile acids. *Communications Biology.* 2023;6(1)

323. Beuers U. DG, Soroka C., Wimmer R., Rust C., Paumgartner G., ... Boyer J. Taurolithocholic acid exerts cholestatic effects via phosphatidylinositol 3-kinase-dependent mechanisms in perfused rat livers and rat hepatocyte couplets. *Journal of Biological Chemistry.* 2003;278(20):17810-17818.

324. Vandewynckel Y LD, Devisscher L, Paridaens A, Bogaerts E, Verhelst X, ... & Vlierberghe H. Tauroursodeoxycholic acid dampens oncogenic apoptosis induced by endoplasmic reticulum stress during hepatocarcinogen exposure. *Oncotarget.* 2015;6(29):28011-28025.

325. Li N YC, Zhou S, Song S, Jin Y, Wang D, ... & Chen Z. Combination of plasma-based metabolomics and machine learning algorithm provides a novel diagnostic strategy for malignant mesothelioma. *Diagnostics.* 2021;11(7):1281.

326. Roma MG TF, Boaglio AC, Basiglio CL, Crocenzi FA, & Pozzi EJS. Ursodeoxycholic acid in cholestasis: linking action mechanisms to therapeutic applications. *Clinical Science.* 2011;121(12):523-544.

327. Yan Y YL, Qu Y, Fan Z, Zhang T, Xu Y, ... & Zhang E. Bacteroides uniformis-induced perturbations in colonic microbiota and bile acid levels inhibit th17 differentiation and ameliorate colitis developments. *NPJ Biofilms and Microbiomes.* 2023;9(1)

328. Sun Z WY, Su X, Yang X, & Luo Q. Proteomic characterization of human gut habitual bacteroides intestinalis against common intestinal bile acid stress. *Advanced Gut & Microbiome Research.* 2023;2023(1)

329. Xu J ZZ, Li X, Sun X, Wang X, Qin F, ... & Yan R. Effect of gegen qinlian decoction on the regulation of gut microbiota and metabolites in type II diabetic rats. *Frontiers in Microbiology*. 2024;15

330. Chen S WC, Zou X, Li H, Yang G, Su X, ... & Mo, Z. Multi-omics insights implicate the remodeling of the intestinal structure and microbiome in aging. *Frontiers in Genetics.* 2024;15

331. Shih DM SZ, Meng Y, Rosales M, Wang X, Wu J, ... & Lusis AJ. Hyodeoxycholic acid improves hdl function and inhibits atherosclerotic lesion formation in ldlr-knockout mice. *The FASEB Journal.* 2013;27(9):3805-3817.

332. Sato Y AK, Plichta DR, et al. Novel bile acid biosynthetic pathways are enriched in the microbiome of centenarians. *Nature.* 2021;599(7885):458-464. doi:10.1038/s41586-021-03832-5

333. Smirnova E MM, Narayan N, Siddiqui MS, Puri P, Luketic VA, ... & Sanyal AJ. Metabolic reprogramming of the intestinal microbiome with functional bile acid changes underlie the development of NAFLD. *Hepatology.* 2022;76(6):1811-1824.

334. Camilleri M BJ. New Developments in Bile Acid Diarrhea. *Gastroenterol Hepatol (N Y).* 2023;19(9):520-537.

335. Zeng H US, Rust B, Lazarova D, Bordonaro M. Secondary Bile Acids and Short Chain Fatty Acids in the Colon: A Focus on Colonic Microbiome, Cell Proliferation, Inflammation, and Cancer. *Int J Mol Sci.* 2019;20(5):1214. doi:10.3390/ijms20051214

336. Saito Y ST. Defecation status, intestinal microbiota, and habitual diet are associated with the fecal bile acid composition: a cross-sectional study in community-dwelling young participants. *Eur J Nutr.* 2023;62(5):2015-2016. doi:10.1007/s00394-023-03126-8

337. Zhan K WH, Xu Y, et al. The function of the gut microbiota-bile acid-TGR5 axis in diarrhea-predominant irritable bowel syndrome. *mSystems.* 2024;9(3):e0129923. doi:10.1128/msystems.01299-23

338. Janssen AWF HT, Katiraei S, et al. Modulation of the gut microbiota impacts nonalcoholic fatty liver disease: a potential role for bile acids. *J Lipid Res.* 2017;58(7):1399-1416. doi:10.1194/jlr.M075713

339. Zhao L FX, Jia W, Bian ZX. Managing Chronic Diarrhea From a Gut Microbiota-Bile Acid Perspective. *Clin Transl Gastroenterol.* 2020;11(8):e00208. doi:10.14309/ctg.0000000000000208

340. Sagar NM DH, Kay GL, et al. The pathophysiology of bile acid diarrhoea: differences in the colonic microbiome, metabolome and bile acids. *Sci Rep.* 2020;10(1):20436. doi:10.1038/s41598-020-77374-7

341. Sommersberger S GS, Elger T, et al. Altered fecal bile acid composition in active ulcerative colitis. *Lipids Health Dis.* 2023;22(1):199. doi:10.1186/s12944-023-01971-4

342. Li L LT, Gu Y, et al. Regulation of gut microbiota-bile acids axis by probiotics in inflammatory bowel disease. *Front Immunol.* 2022;13:974305. doi:10.3389/fimmu.2022.974305

343. Li N MP, Li Y, et al. Gut microbiota-derived 12-ketolithocholic acid suppresses the IL-17A secretion from colonic group 3 innate lymphoid cells to prevent the acute exacerbation of ulcerative colitis. *Gut Microbes.* 2023;15(2):2290315. doi:10.1080/19490976.2023.2290315

344. Pavlidis P PN, Vincent RP, Ehrlich D, Bjarnason I, Hayee B. Systematic review: bile acids and intestinal inflammation-luminal aggressors or regulators of mucosal defence? *Aliment Pharmacol Ther.* 2015;42(7):802-817. doi:10.1111/apt.13333

345. Paik D YL, Zhang Y, et al. Human gut bacteria produce TH17-modulating bile acid metabolites. *Nature.* 2022;603(7903):907-912. doi:10.1038/s41586-022-04480-z

346. Mercer KE MA, Pack LM, et al. Exercise training and diet-induced weight loss increase markers of hepatic bile acid (BA) synthesis and reduce serum total BA concentrations in obese women. *Am J Physiol Endocrinol Metab.* 2021;320(5):E864-E873. doi:10.1152/ajpendo.00644.2020

347. Calderon G MA, Rievaj J, et al. Ileo-colonic delivery of conjugated bile acids improves glucose homeostasis via colonic GLP-1-producing enteroendocrine cells in human obesity and diabetes. *EBioMedicine.* 2020;55:102759. doi:10.1016/j.ebiom.2020.102759

348. Chen Y CS, Harris DA, et al. A small intestinal bile acid modulates the gut microbiome to improve host metabolic phenotypes following bariatric surgery. *Cell Host Microbe.* 2024;32(8):1315-1330.e5. doi:10.1016/j.chom.2024.06.014

349. Zheng X CT, Zhao A, et al. Hyocholic acid species as novel biomarkers for metabolic disorders. *Nat Commun.* 2021;12(1):1487. doi:10.1038/s41467-021-21744-w

350. Tong JL, Ran ZH, Shen J, Fan GQ, Xiao SD. Association between fecal bile acids and colorectal cancer: a meta-analysis of observational studies. *Yonsei Med J.* Oct 31 2008;49(5):792-803. doi:10.3349/ymj.2008.49.5.792

351. Zuccato E, Venturi M, Di Leo G, et al. Role of bile acids and metabolic activity of colonic bacteria in increased risk of colon cancer after cholecystectomy. *Dig Dis Sci.* Mar 1993;38(3):514-9. doi:10.1007/bf01316508

352. Zhan K ZH, Li J, et al. Gut Microbiota-Bile Acid Crosstalk in Diarrhea-Irritable Bowel Syndrome. *Biomed Res Int.* 2020;2020:3828249. doi:10.1155/2020/3828249

353. Lucas LN BK, Kerby RL, et al. Dominant Bacterial Phyla from the Human Gut Show Widespread Ability To Transform and Conjugate Bile Acids. *mSystems.* 2021;doi:10.1128/mSystems.00805-21

354. Shalon D CR, Grembi JA, et al. Profiling the human intestinal environment under physiological conditions. *Nature.* 2023;617(7961):581-591. doi:10.1038/s41586-023-05989-7

355. Cook SI SJ. Review article: short chain fatty acids in health and disease. *Aliment Pharmacol Ther.* 1998;12(6):499-507.

356. Zhang D JY, Zhang YN, et al. Short-chain fatty acids in diseases. *Cell Commun Signal*. 2023;21(1):212. doi:10.1186/s12964-023-01219-9

357. Ohira H TW, Fujioka Y. Are Short Chain Fatty Acids in Gut Microbiota Defensive Players for Inflammation and Atherosclerosis? *J Atheroscler Thromb.* 2017;24(7):660-672. doi:10.5551/jat.RV17006

358. Xu Y, Zhu, Y, Li, X, Sun, B. Dynamic balancing of intestinal short-chain fatty acids: The crucial role of bacterial metabolism. *Trends in Food Science & Technology.* 2020;100:118-130. doi:10.1016/j.tifs.2020.02.026

359. Trefflich I DS, Braune A, Abraham K, Weikert C. Short- and Branched-Chain Fatty Acids as Fecal Markers for Microbiota Activity in Vegans and Omnivores. *Nutrients.* 2021;13(6):1808. doi:10.3390/nu13061808

360. Sivaprakasam S PP, Singh N. Benefits of short-chain fatty acids and their receptors in inflammation and carcinogenesis. *Pharmacol Ther.* 2016;164:144-151. doi:10.1016/j.pharmthera.2016.04.007

361. Ríos-Covían D R-MP, Margolles A, Gueimonde M, de los Reyes-Gavilá, Salazar N. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. *Front Microbiol.* 2016;7doi:10.3389/fmicb.2016.00185

362. Markowiak-Kopeć P ŚK. The Effect of Probiotics on the Production of Short-Chain Fatty Acids by Human Intestinal Microbiome. *Nutrients.* 2020;12(4):1107. doi:10.3390/nu12041107

363. Birkeland E GS, Birkeland KI, et al. Prebiotic effect of inulin-type fructans on faecal microbiota and short-chain fatty acids in type 2 diabetes: a randomised controlled trial [published correction appears in *Eur J Nutr. Eur J Nutr.* 2020;59(7):3325-3338. doi:10.1007/s00394-020-02282-5

364. Hernández MAG CE, Jocken JWE, Blaak EE. The Short-Chain Fatty Acid Acetate in Body Weight Control and Insulin Sensitivity. *Nutrients.* 2019;11(8):1943. doi:10.3390/nu11081943

365. Ju X JZ, Ma J, Yang D. Changes in Fecal Short-Chain Fatty Acids in IBS Patients and Effects of Different Interventions: A Systematic Review and Meta-Analysis. *Nutrients.* 2024;16(11):1727. doi:10.3390/nu16111727

366. Gao C LB, He Y, et al. Early changes of fecal short-chain fatty acid levels in patients with mild cognitive impairments. *CNS Neurosci Ther*. 2023;29(11):3657-3666. doi:10.1111/cns.14252

367. McGuinness AJ DJ, Dawson SL, et al. A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. *Mol Psychiatry.* 2022;27(4):1920-1935. doi:10.1038/s41380-022-01456-3

368. Zhu X ZY, Wang Y, et al. Production of high-concentration n-caproic acid from lactate through fermentation using a newly isolated Ruminococcaceae bacterium CPB6. *Biotechnol Biofuels*. 2017;10:102. doi:10.1186/s13068-017-0788-y

369. Cheng HY C, JCY, Yap GC, Huang C-H, Kioh DYQ, Tham EH, Loo EXL, Shek LPC, Karnani N, Goh A, Van Bever HPS, Teoh OH, Chan YH, Lay C, Knol J, Yap F, Tan KH, Chong Y-S, Godfrey KM, Chan ECY, Lee BW, Ta LDH. Evaluation of Stool Short Chain Fatty Acids Profile in the First Year of Life with Childhood Atopy-Related Outcomes. *Front Allergy.* 2022;3doi:10.3389/falgy.2022.873168

370. Kasubuchi M HS, Hiramatsu T, Ichimura A, Kimura I. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients.* 2015;7(4):2839-2849. doi:10.3390/nu7042839

371. Nogal A LP, Zhang X, Wells PM, Steves CJ, Spector TD, Falchi M, Valdes AM, Menni C. Circulating Levels of the Short-Chain Fatty Acid Acetate Mediate the Effect of the Gut Microbiome on Visceral Fat. *Front Microbiol.* 2021;12doi:10.3389/fmicb.2021.711359

372. Langfeld LQ DK, Bereswill S, Heimesaat MM. A review of the antimicrobial and immune-modulatory properties of the gut microbiota-derived short chain fatty acid propionate - What is new? *Eur J Microbiol Immunol (Bp).* 2021;11(2):50-56. doi:10.1556/1886.2021.00005

373. Fan L ZX, Sun S, et al. Ca:Mg ratio, medium-chain fatty acids, and the gut microbiome. *Clin Nutr.* 2022;41(11):2490-2499. doi:10.1016/j.clnu.2022.08.031

374. Ríos-Covían D, González S, Nogacka AM, Arboleya S, Salazar N, Gueimonde M, de los Reyes-Gavilán CG. An Overview on Fecal Branched Short-Chain Fatty Acids Along Human Life and as Related With Body Mass Index: Associated Dietary and Anthropometric Factors. *Front Microbiol.* 2020;11:973. doi:10.3389/fmicb.2020.00973

375. Heimann E NM, Pålbrink AK, Lindkvist-Petersson K, Degerman E. Branched short-chain fatty acids modulate glucose and lipid metabolism in primary adipocytes. *Adipocyte.* 2016;5(4):359-368. doi:10.1080/21623945.2016.1252011

376. Faujan NH AA, Fatimah AB, Anas OM, Shuhaimi M, Manap MYA, ... & Loong YY. The impact of the level of the intestinal short chain fatty acids in inflammatory bowel disease patients versus healthy subjects. *The Open Biochemistry Journal.* 2010;4:53-58.

377. Szczesniak O HK, Hanssen JF, & Rudi K. Isovaleric acid in stool correlates with human depression. *Nutritional Neuroscience.* 2015;19(7):279-283.

378. Evenepoel P, Poesen, R. & Meijers, B. The gut–kidney axis. *Pediatr Nephrol.* 2017;32(11):2005-2014. doi:10.1007/s00467-016-3527-x

379. Vich Vila A HS, Andreu-Sánchez S, et al. Faecal metabolome and its determinants in inflammatory bowel disease. *Gut.* 2023;72(8):1472-1485. doi:10.1136/gutjnl-2022-328048

380. McKay MJ CM, Catania S, Charles KA, Shanahan E, Clarke SJ, Engel A, Varelis P, Molloy MP. Quantification of short-chain fatty acids in human stool samples by LC-MS/MS following derivatization with aniline analogues. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2023;15(1217):123618.

381. 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. doi:10.1186/1476-0711-11-8

382. 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.* Jan 2011;24(1):81-5.

383. 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.* Aug 2012;2(8):597-601. doi:10.1016/S2221-1691(12)60104-X

384. 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.* May 1987;155(5):979-84.

385. Berberine. *Altern Med Rev.* Apr 2000;5(2):175-7.

386. Avato P, Tursil E, Vitali C, Miccolis V, Candido V. Allylsulfide constituents of garlic volatile oil as antimicrobial agents. *Phytomedicine.* Jun 2000;7(3):239-43.

387. Ibrahim AN. Comparison of in vitro activity of metronidazole and garlic-based product (Tomex(R)) on Trichomonas vaginalis. *Parasitol Res.* May 2013;112(5):2063-7. doi:10.1007/s00436-013-3367-6

388. 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.* Mar 2007;38(2):343-8.

389. 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.* Dec 2009;60(8):717-27. doi:10.3109/09637480802165650

390. Markin D, Duek L, Berdicevsky I. In vitro antimicrobial activity of olive leaves. *Mycoses.* Apr 2003;46(3-4):132-6.

391. 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):1153-62.

392. 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. doi:10.1186/1743-422X-9-137

393. 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.* Aug 8 2003;307(4):1029-37.

394. 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.* Jun 2005;66(2-3):129-36. doi:10.1016/j.antiviral.2005.02.005

395. 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.* Jul 2012;91(7):1733-8. doi:10.3382/ps.2011-02106

396. 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.* Feb 2011;90(2):494-7. doi:10.3382/ps.2010-00891

397. 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.* Dec 2011;95(6):701-6. doi:10.1111/j.1439-0396.2010.01100.x

398. 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.* Oct 2007;35(4):329-34. doi:10.1016/j.biologicals.2006.11.003

399. 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]. 2012;53(4):255-61.

400. Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. Sep 13 2012;489(7415):231-41. doi:10.1038/nature11551

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