GI Profile Stool Results Interpretation Guide

4U Health

PO Box 100083
Pittsburgh, PA 15233,USA
www.4uhealth.com

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Introduction

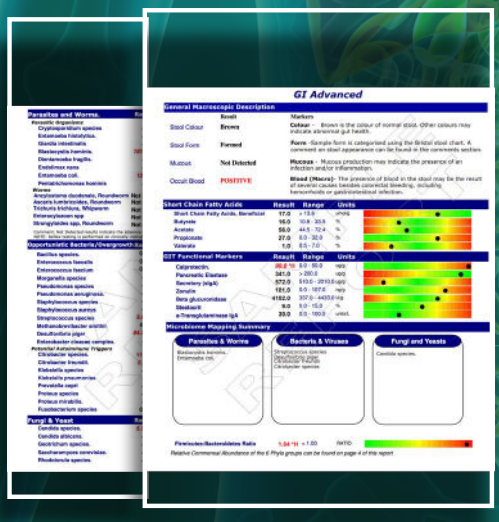
GI Profiles Overview

The GI profiles offered by **4U Health** provide a thorough functional evaluation of the gastrointestinal tract. The **GI Basic Profile** provides an overview of gut microbiome in the gastrointestinal tract using state-of-the-art PCR tests. The **GI Advanced Profile** includes all the PCR markers from the **GI Basic Profile** and adds clinically relevant markers for short-chain fatty acids, inflammation, and immunology. It also includes culture and sensitivity of live yeast, pathogens and dysbiotic bacteria. These live cultures are essential to determine sensitivities to antimicrobial pharmaceutical and natural agents and provide the highest standard of excellence in patient care.

The overview of the gut microbiome, immune functions, digestion and absorption provided by the **4U Health GI Profiles** may provide clinical insights and improve patient outcomes. Indications for stool analysis may include:

- Motility issues - loose stools, constipation
- Digestive symptoms – gas, bloating, indigestion, carbohydrate sensitivity, food intolerance or allergy, foul breath, blood or mucous in stools
- History of antibiotic use
- History of irritable bowel syndrome (IBS) or inflammatory bowel disease (IBD)
- Autoimmune or allergic responses – joint pain, skin conditions
- Suspicion of parasites - anal itching or history of risk factors
- Nutritional deficiencies (malabsorption)
- Behavior or mood disorders

Standard & Advanced



Detoxification
- ß-glucuronidase

Digestion
- Pancreatic elastase
- Steatocrit (fat stain)

Immunity
- Calprotectin
- Fecal secretory IgA
- Fecal Zonulin
- Anti-gliadin IgA
- Occult blood

Microbiome
- PCR identification of bacteria, viruses, parasites
- Culture and sensitivities on yeast, dysbiotic flora, etc.

Motility
- Macroscopic Stool Description
- Short-chain fatty acids

GI - Basic Profile

INCLUDES THE MARKERS LISTED BELOW

KEY PHYLA% COMMENSAL "ABONDANCE"
- Bacteriodetes
- Firmicutes
- Firmicutes/Bacteroidetes Ratio
- Proteobacteria
- Actinobacteria
- Verrucomicrobia
- Euryarchaeota

OPPORTUNISTIC BACTERIA
- Bacillus spp.
- *Enterococcus faecalis*
- *Enterococcus faecium*
- Morganella spp.
- Pseudomonas spp.
- *P. aeruginosa*
- Staphylococcus spp.
- *S. aureus*
- Streptococcus spp.
- Methanobacteriaceae
- *Desulfovibrio piger*
- *Oxalobacter formigenes*

POTENTIAL AUTOIMMUNE TRIGGERS
- Citrobacter spp.
- *Citrobacter freundii*
- Klebsiella spp.
- *Klebsiella pneumoniae*
- *Prevotella copri*
- Proteus spp.
- *Proteus mirabilis*
- Fusobacterium spp.

BACTERIAL PATHOGENS
- Aeromonas spp.
- Campylobacter spp.
- *C. difficile,* Toxin A
- *C. difficile,* Toxin B
- Enterohaemorrhagic *E. coli*
- Enteroinvasive *E. coli*/Shigella
- Enterotoxigenic *E. coli* LT/ST
- Shiga-like toxin *E. coli stx 1*
- Shiga-like toxin *E. coli stx 2*
- Salmonella spp.
- Vibrio spp.
- *Yersinia enterocolitica*
- *Helicobacter pylori*
- *H. pylori* virulence factors

VIRAL PATHOGENS
- Adenovirus 40/41
- Norovirus GI/II
- Rotavirus
- Sapovirus (I,II,IV,V)
- Astrovirus (hAstro)

PARASITES AND WORMS
- Cryptosporidium
- *E. Histolytica*
- *Giardia intestinalis*
- *Blastocystis hominis*
- *Dientamoeba fragilis*
- *Endolimax nana*
- *Entamoeba coli*
- *Pentatrichomonas hominis*
- *Ascaris lumbricoides,* roundworm
- *Necator americanus,* hookworm
- *Trichuris trichuria,* roundworm
- Taenia species, tapeworm
- *E. Vermicularis*
- *Strongyloides stercoralis*
- Enterocytozoon spp.
- Hymenolepis spp.

FUNGI & YEAST
- Candida spp.
- *Candida albicans*
- Geotrichum spp.
- *Saccharomyces cerevisiae*
- Rhodotorula spp.

GI - Advanced Profile

INCLUDES ALL MARKERS LISTED IN THE **BASIC** PROFILE WITH THE ADDITION OF THE FOLLOWING MARKERS

MACROSCOPY
- Stool Colour
- Stool Form
- Mucous
- Occult Blood

SHORT CHAIN FATTY ACIDS
- Butyrate
- Acetate
- Propionate
- Valerate

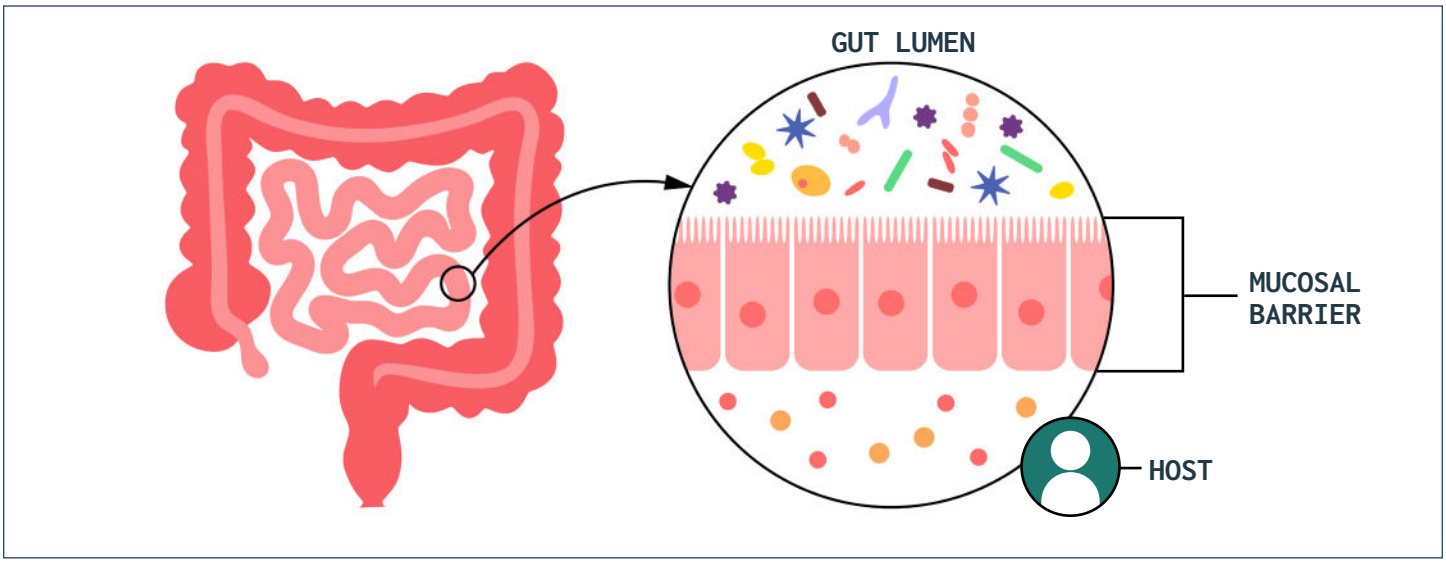
GIT FUNCTIONAL MARKERS
- Calprotectin
- Pancreatic Elastase
- Secretory IgA
- Zonulin
- b-Glucuronidase
- Steatocrit
- Gliadin IgA

MICROBIAL CULTURE
- Bacteria
- Yeasts

ANTIMICROBIAL SENSITIVITIES
- Antibiotics
- Natural Antimicrobial Agents
- Antifungals
- Natural Antifungals

Gastrointestinal Function, Permeability and Immune Tolerance

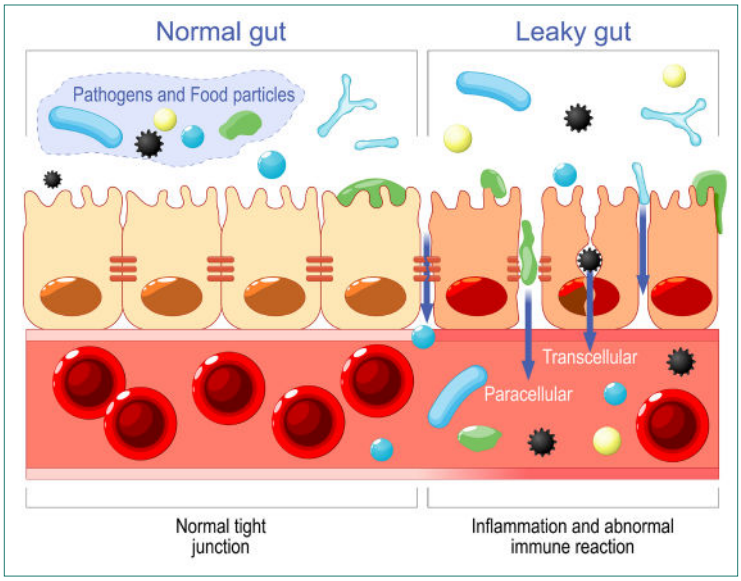
The mucosal lining of the gastrointestinal system begins with a single layer of epithelial cells. These cells must withstand the effects of corrosive digestive fluids, correctly identify self from pathogens, and recognize, assimilate and transport needed nutrients into circulation. The gut mucosa and its underlying tissues must also interact with the microbes that inhabit the gut lumen, our gut microbiome. Clinicians requiring a "refresher" on digestion, assimilation, gastrointestinal function are referred to "*Overview of the Digestive System*" by openstax.org https://openstax.org/books/anatomy-and-physiology-2e/pages/23-1-overview-of-the-digestive-system.



The presence or absence of specific groups of bacteria can have an adverse effect on intestinal permeability and host (human) health. Locally, the gut microbiome exchanges signal molecules and metabolites that can either support or inflame the mucosal lining and immune system. If the wrong bacteria populate the microbiome, or the right bacteria are lost, the cross-talk signals between the gut lining and the bacteria change, then the gut mucosa can become irritated and inflamed. An irritated mucosal lining will develop gaps between cells that can allow larger molecules or whole microbes into systemic circulation. Large molecules, such as undigested food molecules, bacterial or viral proteins, or toxins can act as antigens and/or stimulate inflammatory responses from the immune system. Eventually, such inflammatory responses can develop into allergies, sensitivities, or metabolic illnesses.

Both the gastrointestinal microbiome and the nutritional status of the host further modulate gut homeostasis. The beneficial/commensal bacteria, mucin layer and epithelial cell barriers are not just physical and chemical barriers to pathogenic infection but represent a clear communication system resulting in direct modulation of host-driven immune responses. Recent studies indicate that the gut microbiome plays an important role in shaping the T-cell repertoire in the gut and that certain bacterial species seem to be particularly efficient in promoting Treg cell differentiation and suppressive function. Some of this programming is delivered to the host via beneficial bacterial biofilms (exopolysaccharides).

Diet plays an important role in the maintenance of a healthy, diverse microbiome. Plant fibers (soluble and insoluble) are essential to develop the complex layers of feeding communities (guilds) that break down dietary components into substrates for other guilds of bacteria in the microbiome while also providing optimal nutrient extraction for the host. Evidence indicates that the loss of beneficial bacteria, and later colonization by commensal or dysbiotic species such as *Eschericia coli* can increase risk of allergies. Once allergies or sensitivities have developed, they can further contribute to local or systemic inflammatory reactions and cause further disruption of the gastrointestinal mucosal barrier, and result in a "leaky gut".



Continued exposure to allergy or sensitivity antigens, and other toxins such as mycotoxins, through diet, skin, or inhalation, maintains inflammation, even if attempts are made to correct gut function and microbiome balance. In such cases identification and elimination of the offending food, inhalant, or toxin is an essential part of treatment. Screening patients with gastrointestinal or chronic inflammation complaints for environmental allergy, sensitivity, toxin or mold exposure, can be an essential step in restoring health:

- Evaluate true food or mold allergy with **4U Health's IgE Allergy Panels**
- Evaluate non-IgE food sensitivity with **4U Health's IgG Food Panels**
  - $IgG_{1\text{-}3}$ can activate pro-inflammatory complement cascades associated with chronic conditions. High levels of $IgG_{1\text{-}3}$ antibodies overload receptors and drive the inflammatory reactions that can occur days after exposure.

The gut microbiome interacts with the gut-associated lymphoid tissues (GALT) found beneath the gut mucosal layer to program local and systemic immune responses. Most immune cell priming occurs in the gut, and depending upon levels of gastrointestinal tolerance or inflammation, cytokine signals program naïve immune cells as tolerant T-regulatory (Treg), or more inflammatory T helper (Th1 or Th2) or Th17 cells. Without proper cross-talk from the gut microbiome inflammation, irritation and loss of intestinal permeability can develop. The GALT system uses dietary components (such as healthy fats and vitamins) as modulatory molecules to maintain the gut mucosal homeostasis and immune tolerance until challenged by gut pathogens. Current evidence indicates that deficiencies in nutrients, such as vitamins A, C, D, E and zinc can adversely impact mitochondrial and immune function, particularly T-cell responses. Poor nutrition, mitochondrial dysfunction, or toxic exposures compromise the gut mucosal lining and can also have a negative impact on the bacteria of the gut microbiome. Microbiome disruptions and mucosal irritation can occur when the gut is exposed to food additives, pharmaceuticals, or toxic exposures such as chemicals, metals, or mold mycotoxins. If a background chemical exposure is suspected, consider **4U Health's Organic Acids Profile** and **Environmental Pollutants Profile**.

Mold contamination is ubiquitous in commercial food supplies (coffee, grains, dried fruits and nuts, etc.) and may result in local irritation and loss of barrier functions, allergic reactions, or an increased toxic burden from accumulated mycotoxins. It may be important to differentiate between mycotoxin burden and mold allergy, either of which can result in local or systemic symptoms. If mold exposure is suspected:

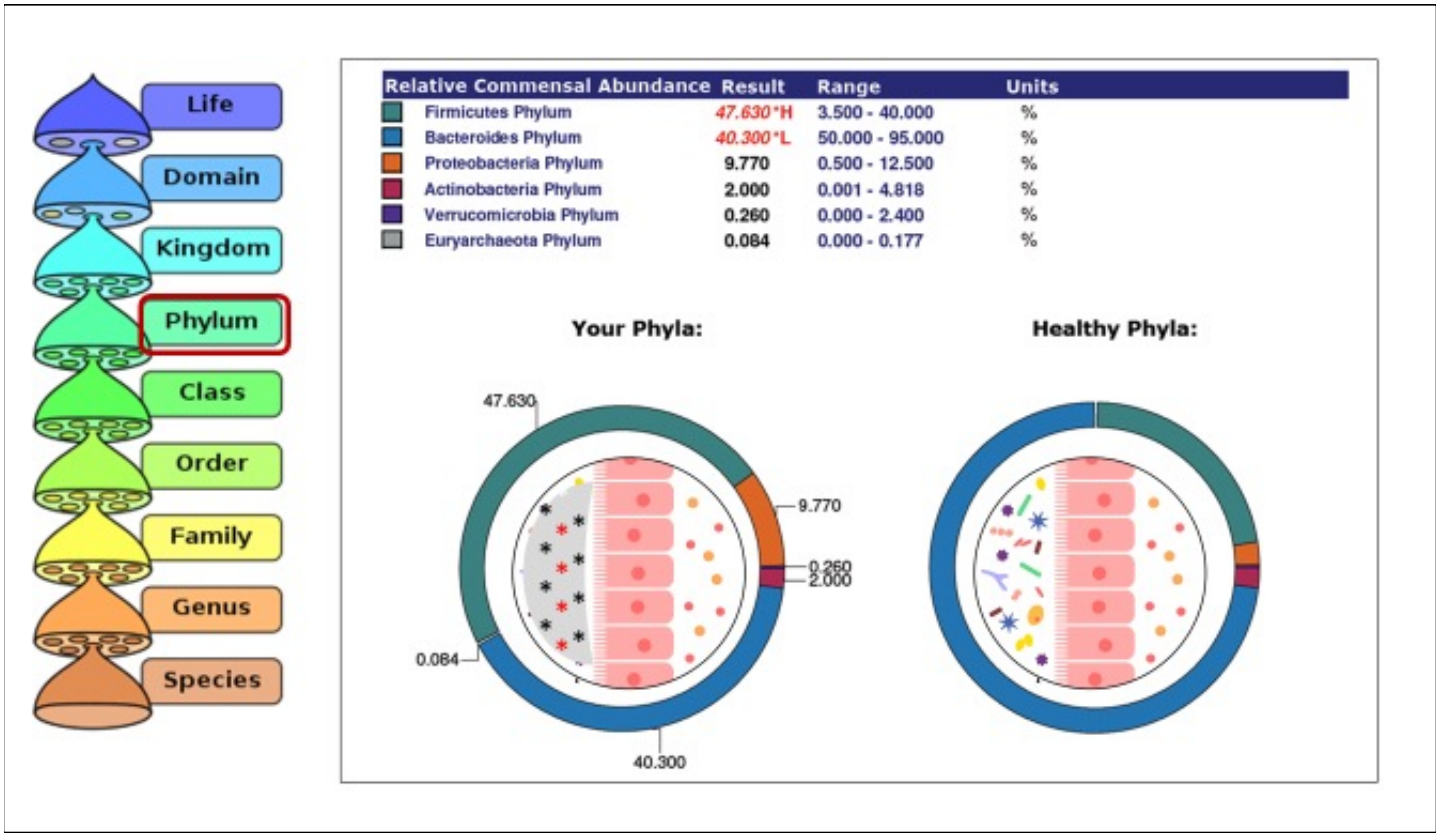
- Consider **4U Health's Mycotoxin Profile**

The Ecology of the Gastrointestinal Microbiome

Any discussion of bacterial populations, including human microbiomes, requires an understanding of some common terms, concepts, and scales of magnitude.

Bacterial taxonomy is the hierarchical classification of different types of bacteria into groups. Traditionally, bacteria were grouped depending on appearance, metabolite production, nutrient preferences, chemical resistances, and observable functions. The emerging science of genome analysis has redefined the true nature and relationships of many bacteria – many bacteria important to humans now have an "alias". The original classification name is used in pre-genetics literature and if reclassified, a new name based upon genetic analysis in more recent literature using PCR (polymerase chain reaction) molecular assessments of bacterial populations. This guide will use the taxonomy names most commonly used in the current literature, however, as of October 2021 scientific literature may list different names for certain phyla of bacteria commonly found in the gastrointestinal microbiome:

PREVIOUS	NEW (AS OF OCTOBER 2021)
Firmicutes	Bacillota
Proteobacteria	Pseudomonata
Actinobacteria	Actinomycetota
Bacteroidetes	Bacteroidota
Thermodesulfobacteria	Thermodesulfobacteriota
Fusobacteria	Fusobacteriota
Verrucomicrobia	Verrucomicrobiota



A healthy microbiome contains approximately 1,000-1,500 different types of bacteria along with a variety of different types of fungus, viruses, parasites, and bacteriophages. While identifying and classifying individual bacteria

from a stool sample is important and certain bacteria have been associated with disease states, from a functional perspective the presence and activity of larger groups (guilds) of bacteria is far more important for host health. A bacterial guild may contain a variety of different classes, orders, genera, and species, all of whom serve the host and the larger gut ecosystem by performing a common function. Common functions may include protein metabolism, fiber breakdown, biochemical transformation, short-chain fatty acid (SCFA) production, and immune system "cross-talk" molecules that influence health, tolerance, and inflammatory responses. Just like individual bacteria, the guilds can be adversely affected by stress, poor diet, or toxic exposures.

Bacterial guild members will all have similar nutrient and environmental requirements, such as preferred nutrient substrates, acidity, oxygen levels, host signals, etc. Recent evidence suggests layered nutrient-processing guild networks. The primary trophic layers have the greatest microbial abundance. As with any ecosystem, the guild bacteria use the available raw materials, in this case provided by the host and its diet. Raw materials may include the mucus layer overlying the gut mucosal cells, products of digestion, substrates produced by other guilds, or even pharmaceutical or toxic substances. The guild bacteria will use these raw materials and release metabolites. These metabolic products are then used as raw materials and signals by other bacterial guilds with different functions (secondary trophic layers). Eventually, some of the products produced by the guilds of the microbiome are available to the host in the form of vitamins, short-chain fatty acids, amino acids, and immune system signal molecules. Some bacteria secrete "bacteriocins" – antimicrobial compounds that protect the host through regulation of microbiome bacterial populations. Bacteriocins regulate gut microbiome populations by preventing pathogen or fungal overgrowth.

Other bacteria will serve the host and the microbiome collective as they detoxify chemicals, metals, or other organic molecules. However, not all bacterial detoxification functions are beneficial for the host. For example, bacterial methylation of the toxic metal arsenic can increase host absorption of arsenic. Some bacteria produce an enzyme, ß-glucuronidase, that can reverse the effects of phase II liver detoxification. Most of the ß-glucuronidase genes are found in the bacteria of the Firmicutes phylum (Lactobacillus, Clostridium, Faecalibacterium, etc.). Microbial ß-glucuronidase in the gut breaks apart conjugated phase II liver metabolites intended for excretion in the stool. Once deconjugated, these active metabolites can pass back across the gut mucosa into the host's circulation where the active molecules can affect the levels of sex hormones, neurotransmitters, drug metabolites, plant polyphenol or isoflavone metabolites, and toxic metabolites from chemical exposures. Higher fecal ß-glucuronidase has been associated with an increased risk of colon cancer, while associations between fecal ß-glucuronidase levels and breast-cancer risk remains an active area of research. Fecal ß-glucuronidase levels can be evaluated with **4U Health's GI Advanced profile**.

Stool Chemistries

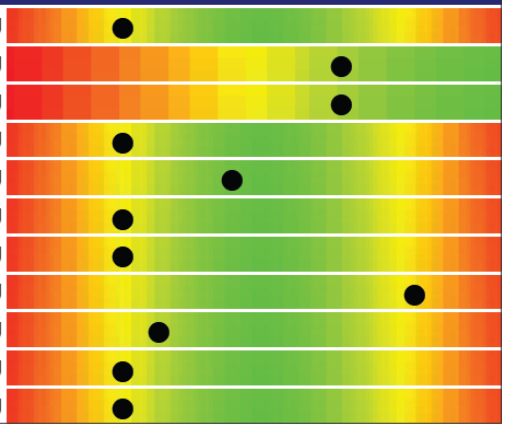
Interpretation Tips

The **GI Advanced profile** includes all the gut microbiome markers found on the **GI Basic profile**. The **GI Advanced** test sections will be reviewed in the order that they appear on the results; the information presented also applies to the same markers if they appear on the **GI Basic profile.**

The first step in successful interpretation is to understand that the different components work together to provide patterns of information. Those patterns, combined with patient signs, symptoms, and history may then be interpreted to uncover the cause of gastrointestinal symptoms. Clinicians requiring a "refresher" on digestion, assimilation, gastrointestinal function are referred to "*Overview of the Digestive System*" by openstax.org https://openstax.org/books/anatomy-and-physiology-2e/pages/23-1-overview-of-the-digestive-system ; clinicians requiring a review of the gastrointestinal microbiome are referred to intechopen.com "*Gut Microbiome: A New Organ System in Body*" https://www.intechopen.com/chapters/69898 .

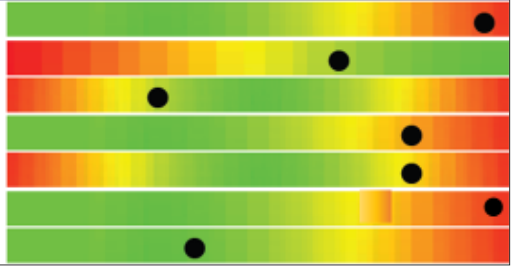
It is important to **consider the test as a whole.** Because all parts of the digestive system are interconnected, one change in the patient's homeostasis may impact multiple biomarkers on the results. For example:

Normal Bacterial GUT Flora	Result	Range	Units
Bacteroides fragilis	*1.2* *L	1.6 - 250.0	x10^9 CFU/g
Bifidobacterium species	752.0	> 6.7	x10^7 CFU/g
Bifidobacterium longum	385.0	> 5.2	x10^6 CFU/g
Enterococcus species	4.9	1.9 - 2000.0	x10^5 CFU/g
Escherichia species	1268.0	3.7 - 3800.0	x10^6 CFU/g
Lactobacillus species	11.0	8.6 - 6200.0	x10^5 CFU/g
Lactobacillus Rhamnosus	*6.3* *L	8.3 - 885.0	x10^4 CFU/g
Clostridium species	48.0	5.0 - 50.0	x10^6 CFU/g
Enterobacter species	9.0	1.0 - 50.0	x10^6 CFU/g
Akkermansia muciniphila	*<dl* *L	1.00 - 50.00	x10^3 CFU/g
Faecalibacterium prausnitzii	*122.0* *L	200.0 - 3500.0	x10^3 CFU/g



A loss of diversity in the normal bacterial gut microbiome flora (as seen above) may occur for a variety of reasons and result in an increased number of commensal or dysbiotic species. The change in species may then alter motility, pH, short-chain fatty acid synthesis, and immune responses. Alternately, digestive disorders can also alter the microbiome bacteria, pH, and short chain fatty acids. In the example below, inflammatory markers and a high steatocrit likely indicates an inability to assimilate fats, which will alter the bacteria of the gut microbiome.

GIT Functional Markers	Result	Range	Units
Calprotectin.	*65.0* *H	0.0 - 50.0	ug/g
Pancreatic Elastase	>500.0	> 200.0	ug/g
Faecal Secretory IgA	741.0	510.0 - 2010.0	ug/g
Faecal Zonulin	*109.0* *H	0.0 - 107.0	ng/g
Faecal B-Glucuronidase	*4566.0* *H	337.0 - 4433.0	U/g
Steatocrit	*22.5** H	0.0 - 15.0	%
anti-Gliadin IgA	22.0	0.0 - 100.0	units/L



Pre-existing medical conditions can alter results and gastrointestinal surgeries or medication use may alter the results of a GI profile. Removal of sections of the small or large intestine, or the use of stool from colostomy bags, may produce abnormal results in the microbiology, stool chemistries and short-chain fatty acids results. Many medications may affect GI function. This resource guide will review the **GI Advanced profile** section by section and review the clinically relevant information needed to interpret the results. Medications or natural therapies specifically designed to alter conditions in the gastrointestinal tract will affect GI functions and may also alter stool test results. Medications or natural therapies specifically designed to alter conditions in the gastrointestinal tract will affect GI functions and may also alter stool test results. This resource guide will review the **GI Advanced profile** section by section and review the clinically relevant information needed to interpret the results.

- Some medications may alter the gastrointestinal microbiome (bacteria and yeast):
  - Antibiotics and oral or injected corticosteroids may alter the composition of the normal gut flora.
  - Prescription anti-inflammatories, such as steroids or immunosuppressant medications, may depress the level of inflammatory markers on the **Advanced GI profile**. These medications may also alter the microbiome.
- Some medications may interfere with the performance of certain tests:
  - The steatocrit (fat stain) may show a false positive if the patient is using:
    - Rectal suppositories
    - Rectal creams and lubricants
    - Bismuth
    - Barium
    - Oily laxatives (mineral oil or castor oil)
  - Occult blood stool tests may show false positive if medications cause GI bleeding:
    - Anticoagulants
    - Aspirin
    - Colchicine
    - Iron supplements (in large doses)
    - NSAIDs
    - Corticosteroids
    - Oxidizing drugs (for example, iodine, bromides, and boric acid)
    - Reserpine
  - Occult stool tests may show false negatives with large amounts of vitamin C

General Macroscopic Description

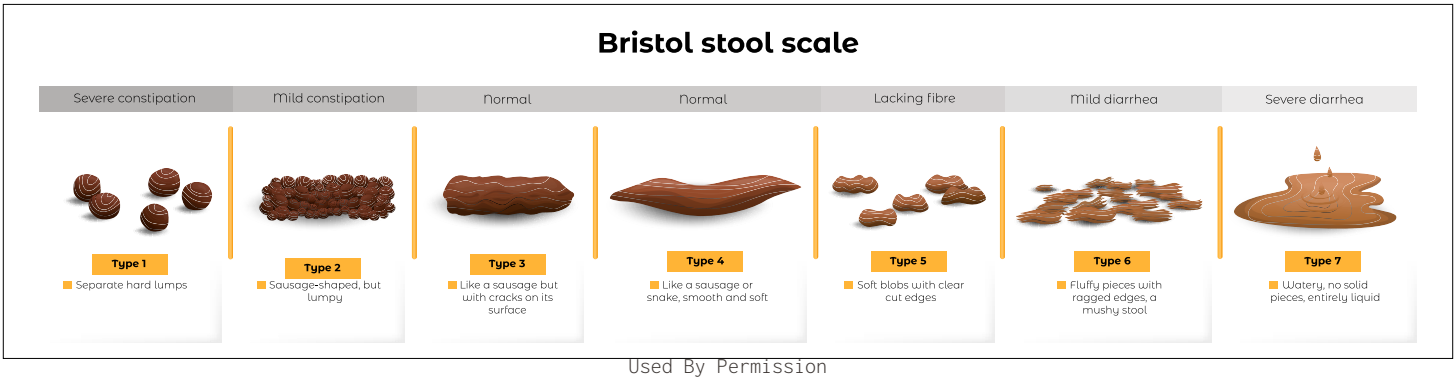
The stool's appearance may provide clues regarding motility and composition of stool.

Stool Color

- Colors other than brown may have clinical significance:
  - Black-colored stools indicate bleeding in the upper digestive tract. Iron supplements, bismuth medications, or black licorice may produce the same stool color.
  - Light or clay-colored stools may indicate a bile duct obstruction or a lack of bile synthesis due to liver or gallbladder disorders. Bismuth or anti-diarrheal medications may also lighten stool color.
  - Yellow, greasy, malodorous stool indicates either an excess of dietary fat or an inability to digest fat. Review the steatocrit results for confirmation.
  - Bright red stools indicate bleeding, either in the lower gastrointestinal tract or from hemorrhoids. Occasionally large quantities of red food dye, beets, cranberries, or tomatoes may turn stool red or dark red.
  - Green or orange stools may result if large quantities of greens or carrots are consumed (juicing, smoothies, etc.)

Stool form

- Patient descriptions of stool and laboratory evaluation of color and texture may differ, or the collected stool may not be typical for the patient. The use of the Bristol Stool chart in the clinic may improve discussions regarding stool consistency:



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Mucus

- Excess mucus may occur due to irritations of the gut mucosa, infections, parasites, IBD or mucus colitis. Less frequently, conditions such as spastic constipation, rectal cancer, or villous adenoma (polyps) of the colon may cause excess mucus.
- Chronic excess mucus production may decrease circulating levels of the amino acids needed to synthesize mucus: glutamine, threonine, serine, proline and cysteine.

Occult Blood

Occult Blood is the term used when hemoglobin is detected in the stool. High levels of hemoglobin indicate bleeding in the stomach or small intestines. If a repeat Occult Blood test is positive, the patient may be referred to a gastroenterologist for additional evaluation.

Symptoms associated with occult blood results include:

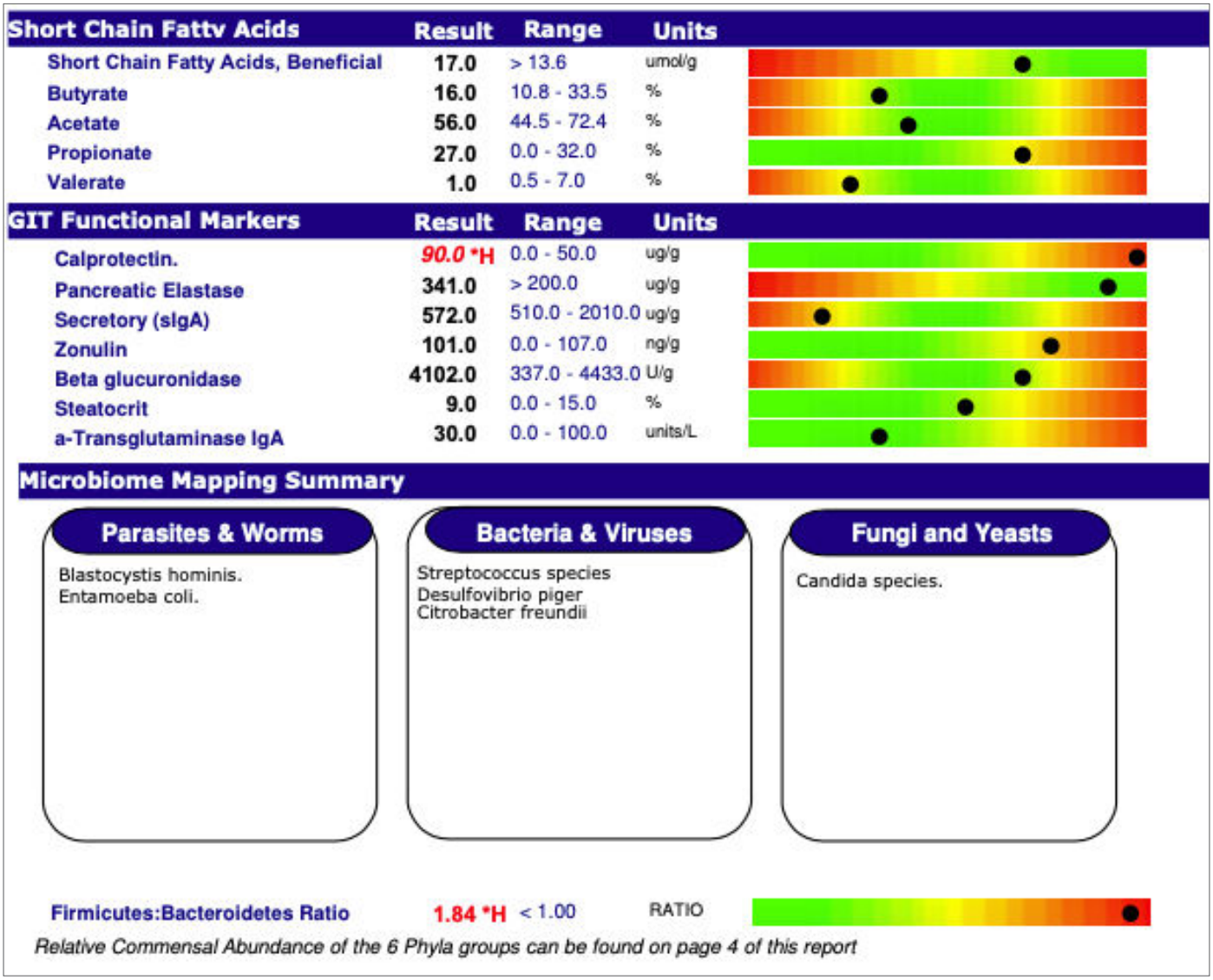
- A history of bloody or "coffee ground" vomiting indicates upper GI bleeding.
- A history of black, "tar-like" stools indicates bleeding in the small intestine.
- Chronic occult bleeding may be a cause of iron deficiency anemia.
- Complaints of fainting or light-headedness may indicate significant blood loss.
- Use of asprin, non-steroidal anti-inflammatories (NSAIDS), or cyclo-oxygenase-2 selective inhibitors (coxibs) may cause or exacerbate GI bleeding.

- False positive results may occur due to the use of medications:
  - Anticoagulants
  - Aspirin
  - Colchicine
  - Iron supplements (in large doses)
  - NSAIDs
  - Corticosteroids
  - Oxidizing drugs (for example, iodine, bromides, and boric acid)
  - Reserpine
- Occult stool tests may show false negatives if large amounts of vitamin C are consumed.

Short Chain Fatty Acids

Short Chain Fatty Acids (SCFAs) are produced when anaerobic gut bacteria ferment dietary fibers. SCFAs serve as anti-inflammatory signaling molecules and are metabolized by the gut mucosal cells for energy. Low levels of total SCFAs have been associated with higher levels of stool bile acids and increased cancer risk. The percentages reported reflect the relative proportions of the SCFAs.

- Increased SCFAs may occur due to sugar/carbohydrate malabsorption, abnormal transit times, high protein diets or dysbiosis. Propionate and valerate percentages may increase due to high protein diets.
  - Correct the underlying cause to normalize SCFAs. Digestive enzymes for malabsorption, antimicrobials for dysbiosis, normalize transit times with increased fiber, relaxation, etc.
- Decreased SCFAs may occur due to insufficiency dysbiosis (low levels of expected and beneficial flora), low-fiber diet, or increased intestinal transit time. Butyrate levels may decrease due to chronic constipation.
  - Correct underlying cause to normalize SCFAs. Restore microbiome diversity (probiotics, diet, relaxation). Consider melatonin 3 mg hs for constipation.
  - Butyrate may be given if levels are low, but will not likely appear on results, as the gut mucosal cells higher in the GI tract will take it up and metabolize it quickly.
  - Most butyrate producers belong to the Clostridium cluster of the phylum Firmicutes, such as Faecalibacterium, Roseburia, Eubacterium, Anaerostipes, Coprococcus, Subdoligranulum, and Anaerobutyricum.



Short Chain Fatty Acids	Result	Range	Units
Short Chain Fatty Acids, Beneficial	17.0	> 13.6	umol/g
Butyrate	16.0	10.8 - 33.5	%
Acetate	56.0	44.5 - 72.4	%
Propionate	27.0	0.0 - 32.0	%
Valerate	1.0	0.5 - 7.0	%

GIT Functional Markers	Result	Range	Units
Calprotectin.	90.0 *H	0.0 - 50.0	ug/g
Pancreatic Elastase	341.0	> 200.0	ug/g
Secretory (sIgA)	572.0	510.0 - 2010.0	ug/g
Zonulin	101.0	0.0 - 107.0	ng/g
Beta glucuronidase	4102.0	337.0 - 4433.0	U/g
Steatocrit	9.0	0.0 - 15.0	%
a-Transglutaminase IgA	30.0	0.0 - 100.0	units/L

GIT Functional Markers

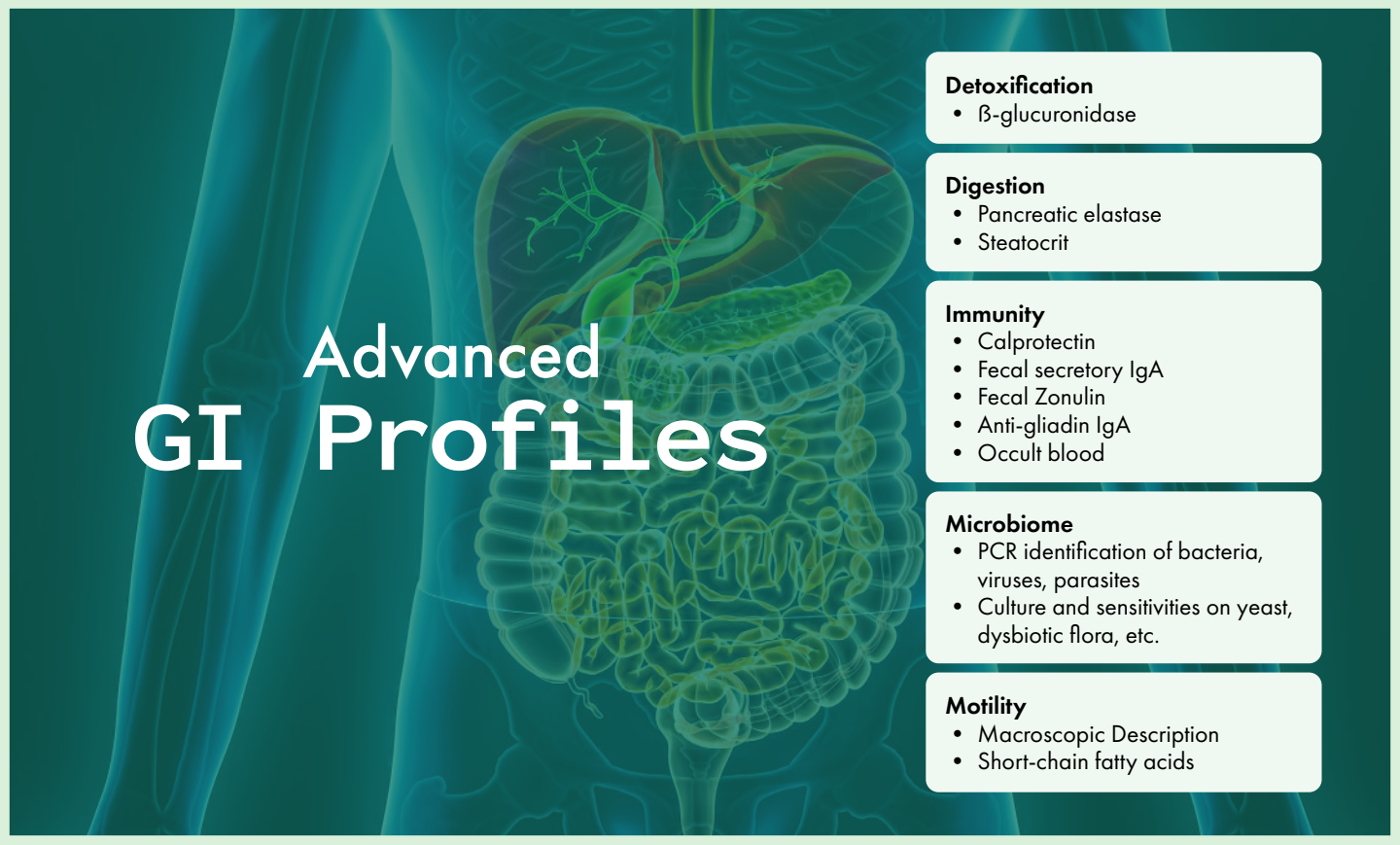
The markers in this section are included on the **GI Advanced profile**. These markers provide information about digestion and immune responses. Clinicians requiring a "refresher" on digestion, assimilation, gastrointestinal function are referred to "*Overview of the Digestive System*" by openstax.org https://openstax.org/books/anatomy-and-physiology-2e/pages/23-1-overview-of-the-digestive-system.

Immune Response Overview:

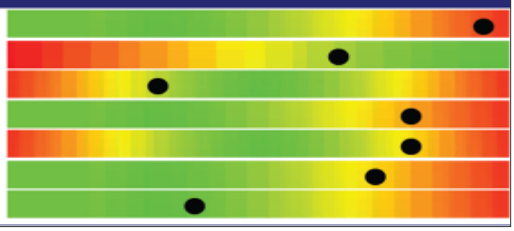
The inflammatory fecal markers calprotectin, secretory IgA (sIgA), zonulin, ß-glucuronidase, and anti-gliadin IgA help clinicians to distinguish between autoimmune inflammatory bowel disorders (IBD) and other inflammatory gastrointestinal disorders. Early diagnosis is required to optimize the management of IBD patients. The use of immunosuppressive therapies or chemotherapy may neutralize normal immune responses and prevent the expected elevation of inflammatory and immunology markers on the stool test. Prescription anti-inflammatories, such as steroids or immunosuppressant medications, may depress the level of inflammatory markers on the **GI profiles** and the medications may also alter the microbiome.

Digestion/Absorption Overview:

The biomarkers pancreatic elastase and steatocrit (fat stain) provide information on the patient's digestion of protein and fats. If digestion is compromised or incomplete, gastrointestinal symptoms and nutrient deficiencies may occur. Digestive disorders may have a mechanical or psychological origin. Psychological stress is considered a contributing factor in functional gastrointestinal disorders. Stress may inhibit normal digestive functions, and decrease levels of expected and beneficial Lactobacillus, Enterococcus, and Clostridium bacteria. Quiet, mindful, distraction-free mealtimes and thorough chewing are necessary to promote the proper neurological and hormonal signaling required for normal digestion and assimilation.



GIT Functional Markers	Result	Range	Units
Calprotectin.	65.0 *H	0.0 - 50.0	ug/g
Pancreatic Elastase	>500.0	> 200.0	ug/g
Faecal Secretory IgA	741.0	510.0 - 2010.0	ug/g
Faecal Zonulin	109.0 *H	0.0 - 107.0	ng/g
Faecal B-Glucuronidase	4566.0 *H	337.0 - 4433.0	U/g
Steatocrit	14.0	0.0 - 15.0	%
anti-Gliadin IgA	22.0	0.0 - 100.0	units/L



Calprotectin

Calprotectin is a calcium-binding protein released by neutrophils, monocytes and reactive macrophages. Calprotectin is very stable in stool, and multiple studies have associated increased levels of Calprotectin with the degree of IBD activity in children and adults. Liver disease or the use of aspirin or nonsteroidal anti-inflammatory (NSAID) medications may elevate Calprotectin levels. Red blood cell transfusions may increase fecal Calprotectin levels. Fecal Calprotectin levels may also be increased in newborns, and may not indicate IBD in this population.

- High levels of Calprotectin (> 200 µg/gm) are associated with active IBD and gastrointestinal inflammation; elevation may also occur due to bacterial infection, colitis or sometimes, cancer. Fecal Calprotectin should be reassessed after about 4 weeks for confirmation. Use of non-steroidal anti-inflammatory medications (NSAIDS) may elevate Calprotectin levels.
  - Patient supports for IBD may include probiotics, omega-3 fatty acids, N-acetylglucosamine, and anti-inflammatory agents such as curcumin. Modification of diet and alteration of the consistency of fibrous foods may provide symptom relief and play a role in patient support during active disease.
    - Improve fiber tolerance via texture modification to decrease bloating/gas. Clinicians interested in IBD diet modifications are referred to the article *"An anti-inflammatory diet as treatment for inflammatory bowel disease: a case series report"* (Oldenzki et al. 2014).
      - Plant fibers in fruits and vegetables can be modified by cooking, grinding, or purée
      - Consider gradual increase in plant fiber length and complexity over weeks to months:
        - Cooked purées → cooked/raw purées and cooked chopped (salsa consistency) → cooked/raw purées and cooked/raw chopped (carrots & peas consistency) → cooked/raw purées and cooked/raw chopped and cooked chunked (cubes or floret-sized) → cooked/raw purées and cooked/raw chopped and cooked/raw chunks or sticks
    - Once fruits and vegetables are tolerated, consider a similar texture modification program for legumes (if desired)
- Moderate Calprotectin (50-200 µg/gm) is an indicator of chronic inflammation. Inflammation at this level may be due to IBD in remission or inflammation caused by non-steroidal anti-inflammatories (NSAIDs). Levels should be reassessed after about 4 weeks.
- Low levels of Calprotectin (< 50 µg/gm) are usually associated with viral GI infections or non-inflammatory bowel conditions such as IBS.

Elastase

Elastase is produced by the pancreas and is a biomarker for protein digestion and pancreatic exocrine function. The **GI Profiles** measure only human elastase; the presence of supplemental pancreatic enzymes will not be reported. Low levels of stomach hydrochloric acid and intestinal elastase may prevent normal protein and fat digestion. Maldigestion may result in diarrhea or loose stools.

- Supplement low levels of pancreatic elastase with elastase supplements if clinically indicated.
- Reduce or eliminate alcohol use and smoking.
- Low elastase levels may prohibit the assimilation of fatty vitamins A, D, E and K. Consider fatty vitamin supplementation if clinically indicated.
- Elastase levels may be decreased by chronic pancreatitis or other pancreatic disorders.
  - Genetic inheritance may affect the risk for chronic pancreatitis. Animal studies indicate that inheritance may

also affect levels of digestive enzymes.

Secretory IgA (sIgA)

Secretory IgA (sIgA) is considered the first line of defense for the intestinal epithelium. sIgA prevents antigens and pathogens from binding to the receptors of the gut epithelial cells. Levels of sIgA may influence the programming of the immune system and the composition of the gastrointestinal microbiome.

- Increased levels of sIgA may indicate infection, inflammation, antigen, or autoimmune responses. Levels may remain high 4-6 weeks after the resolution of an infection.
  - Acute, temporary stress may transiently elevate sIgA
  - Breast-feeding may result in elevated sIgA in infants due to maternal donation
  - Use of bovine colostrum may artificially elevate sIgA
  - The acquisition of a tattoo may stimulate the immune response and increase sIgA levels
  - Mucosal antigen responses (allergy or sensitivity) may elevate IgA
    - Consider evaluation of true food or mold allergy with **4U Health's IgE Allergy Panels**
    - Consider evaluation of non-IgE food sensitivity with **4U Health's IgG Food Panels**
- Decreased levels of sIgA have been associated with frequent infections, renal damage, leukemia, ataxia-telangiectasia, chronic atopy, or allergic reactions; rarely a low sIgA may indicate gastrointestinal epithelial neoplasia. Low sIgA levels may occur for a variety of reasons:
  - Inherited IgA deficiency (low serum IgA)
  - Degradation of sIgA by bacteria, yeast or Blastocystis enzymes
  - The use of immunosuppressive therapies or chemotherapy
  - Chronic physical or psychological stress
  - Extreme physical activity (workouts > 1.5 hours)
  - Recent appendectomy (< 3years) or tonsillectomy (< 20 years)
  - Administration of penicillamine or phenytoin may result in reversible sIgA deficiency
- sIgA levels may improve with the use of probiotics, *S. boulardii*, vitamin A, D, zinc, and glutamine. Moderate exercise and relaxation practices may also improve sIgA levels. Genetic IgA insufficiency does not respond to nutritional supports and will present with low serum IgA as well as low fecal sIgA.

Zonulin

Zonulin results reflect the levels of zonulin family proteins in the stool sample. The zonulin family proteins all share common functions with zonulin and have been clinically associated with increased inflammation in the gut mucosa and elsewhere in the body. Systemic hyperglycemia (metabolic syndrome, type II diabetes) can disrupt gut barrier functions and increase intestinal permeability; it is possible that other chronic inflammatory disorders may have similar effect.

- High levels of fecal zonulin have been associated with local inflammation and increased intestinal permeability. Increased intestinal permeability can occur due to:
  - Inflammation from active Celiac disease, inflammatory bowel disease, etc.
  - Food allergy, food sensitivity, some non-Celiac gluten sensitivity (HLA-DQ2/8-positive)
  - Irritation of the gut mucosa by pharmaceuticals or toxic exposures (chemicals, metals, mold mycotoxins)
  - Pathogens, parasites, or dysbiosis
  - Physical or psychological stress
- Reduction of inflammation and restoration of intestinal permeability may reduce zonulin levels, strengthen the gut barrier and improve gastrointestinal function. Consider:
  - Amino acid supports:
    - Glutamine
    - Taurine
    - Tryptophan
    - Bioactive whey proteins (lactoferrin, lactoperoxidase, immunoglobulins, etc.)

- Fat intake – consider a lower-fat diet and emphasize polyunsaturated omega-3 fats over saturated fats
- Flavonoids:
  - Quercetin, myricetin, and kaempferol
- Identify and avoid inflammatory foods and foods known to increase permeability:
  - Gliadin, a protein found in the gluten-containing grains wheat, rye and barley.
  - Chitosan, derived from crab or shrimp shells and is widely used in processed foods.
- Minerals such as calcium and zinc are essential for normal barrier function.
- Normalize intestinal motility.
- Phenolic compounds [such as tannins, epigallocatechin gallate (EGCG), chlorogenic acid (coffee), gymnemic acid (stevia)] to inhibit intestinal glucose transporters.
- Probiotics can increase the production of beneficial short chain fatty acids and also synthesize B vitamins and vitamin K. Support microbiome diversity with plant fibers.
- Vitamins A and D support tolerant, anti-inflammatory immune responses.

ß-glucuronidase

ß-glucuronidase is an enzyme produced by both the host and the gut microbiome. In the microbiome, the majority of the ß-glucuronidase genes are found in the bacteria of the Firmicutes phylum (Lactobacillus, Clostridium, Faecalibacterium, Eubacterium, Roseburia, Anaerostipes, etc.). Microbial ß-glucuronidase in the gut breaks apart conjugated phase II liver metabolites intended for excretion in the stool. Once deconjugated, these active metabolites can pass across the gut mucosa back into the host's circulation. ß-glucuronidase activity from both the host and bacteria can affect the levels of potentially active sex hormones, neurotransmitters, drug metabolites, plant polyphenol or isoflavone metabolites, and toxic metabolites from chemical exposures. Higher fecal ß-glucuronidase has been associated with an increased risk of colon cancer. Associations between fecal ß-glucuronidase levels and breast-cancer risk remains an active area of research.

- Increased fecal ß-glucuronidase has been associated with the consumption of red meats and high protein, low-carbohydrate diets in separate human studies. Higher intakes of vegetables and fruits have been shown to decrease serum ß-glucuronidase levels.
- Low levels of fecal ß-glucuronidase may occur during very low consumption of plant fibers (fruits, vegetables, pre-biotics). A gradual increase in dietary plant fibers may increase ß-glucuronidase levels. Some ß-glucuronidase activity is beneficial; plant phenols, flavonoids and isoflavones often require enzymatic modification before they are beneficial to the body.

Steatocrit

Steatocrit (fat stain) results indicate the body's ability to break down dietary fats for assimilation. Both dietary fats and fatty vitamins (A, D, E, K) require normal fat digestion. High levels of fat in the stool can indicate an insufficiency of bile from the liver or pancreatic lipase. Both bile and lipases are needed to break down dietary fats for absorption.

- The fat stain may show a false positive if the patient is using:
  - Rectal suppositories
  - Rectal creams and lubricants
  - Bismuth
  - Barium
  - Oily laxatives (mineral oil or castor oil)
- Bile insufficiency may occur due to removal of the gallbladder or blockage of the biliary ducts. Taurine insufficiency may also result in low bile levels.
  - Taurine may be insufficient in un-educated vegetarians, individuals with poor protein digestion, or individuals who do not efficiently synthesize cysteine or taurine due to inherited variations in their synthesis enzymes.
- Lipase levels are likely to be low if the pancreatic elastase level is also low (pancreatic insufficiency).

Anti-gliadin

Anti-gliadin IgA results may be used to monitor adherence to a gluten-free diet or to detect accidental gluten exposure. The protein gliadin is released from gluten during digestion. Gliadin binds with receptors in the gut mucosa which raises zonulin levels and increases intestinal permeability. Increased intestinal permeability can increase the risk of allergy, sensitivity and local or systemic inflammation.

- If results are high review potential sources of gluten exposure and eliminate
- If Celiac disease is suspected in an HLA-DQ2/8-positive individual consider confirmation testing with a **Celiac Panel**.
- ***NOTE:*** review fecal sIgA level. If sIgA is low then anti-gliadin IgA may be low as well despite gluten exposure. IgA insufficiency may be acquired or genetic. Genetic IgA insufficiency does not respond to nutritional supports, and will present with low serum IgA as well as low fecal sIgA.

Microbiome Mapping

Identification of Bacteria, Yeast, Parasites and Viral Pathogens

Several different methods may be used in clinical microbiology to identify various types of microbiome organisms. These methods include microscopy, antigen detection, molecular identification, and culture and sensitivities.

Microscopy, which was first used between 1665 and 1676, is by far the oldest technique. While still useful under certain circumstances, microscopy is rapidly being replaced by newer techniques.

Antigen detection techniques use polyclonal or monoclonal antibodies that bind to specific proteins in the target organism. Enzyme-linked immunosorbent assays (or ELISA) testing is familiar to most clinicians. After several steps of chemical manipulation, the bound antibody-enzyme complexes measure either the presence or absence (qualitative) or quantity (quantitative) of target protein in the sample to provide a "yes/no" qualitative or numerical quantitative result.

Molecular identification techniques include polymerase chain-reaction (PCR) and newer techniques such as matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS). PCR amplifies specific DNA segments called "probes" to identify specific bacteria. Validated PCR probes for pathogens and parasites are highly specific, and probes for commensal bacteria in the gut microbiome have been greatly refined since early experiments. While PCR identification can be highly specific, the probes work in a "lock and key" fashion so PCR will only identify the specific microbes for the available probes. Also, probes may not always identify down to species level, which is the reason that secondary methods such as culture and MALDI-TOF identification are essential tools for confirmation.

MALDI-TOF technology is extremely specific and different from PCR because it can analyze an unknown sample microbe and match it to a large library of known spectrographs. Because there is no microbe-specifc probe, MALDI-TOF identification can identify hundreds to thousands of different microbes, provided there is a matching pattern in the library.

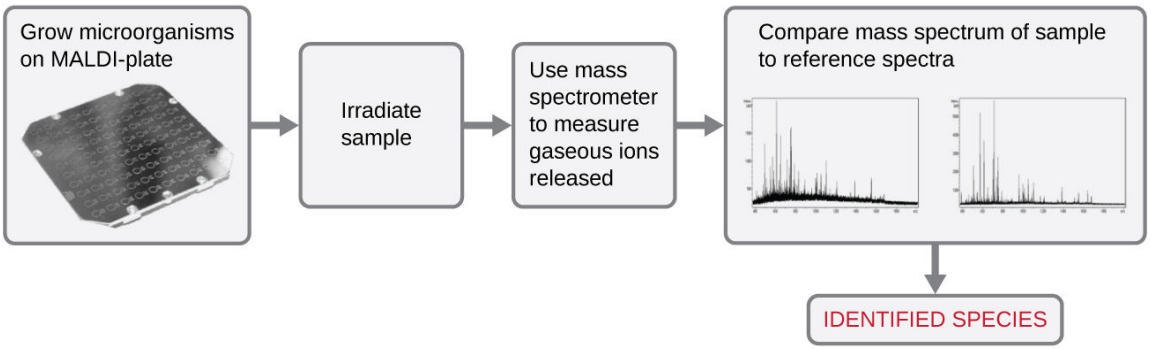
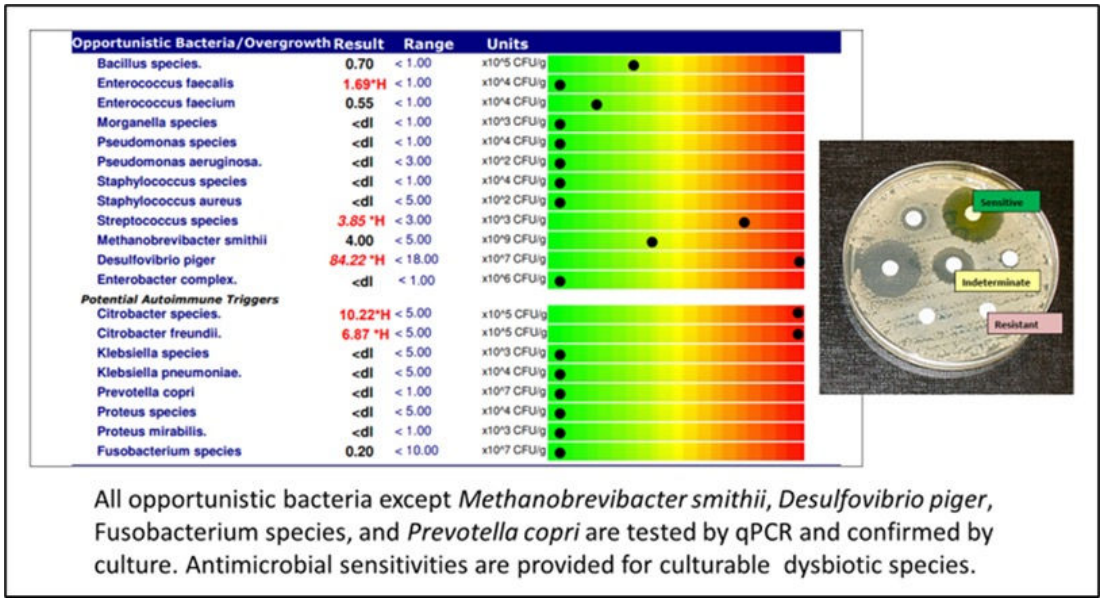


Image courtesy of OpenStax Microbiology (2016)

While the above techniques may be superior for microbe identification, none of them can predict which antimicrobial agent will kill specific undesirable bacteria. The identification of effective anti-microbial agents *requires* bacterial culture and sensitivities. Culture is the growth of organisms on specific culture media that provide all the nutrients the microorganism needs to grow. The evaluation of living organisms eliminates one of the confounding factors of molecular assessments, which cannot discriminate between living and dead bacteria, and may falsely report dead bacteria as part of the gastrointestinal microbiome. The culture and MALDI-TOF identification of living organisms allows for the spontaneous discovery of commensal and dysbiotic bacteria, and yeast undetectable by specific PCR probes.

Culture and Sensitivities

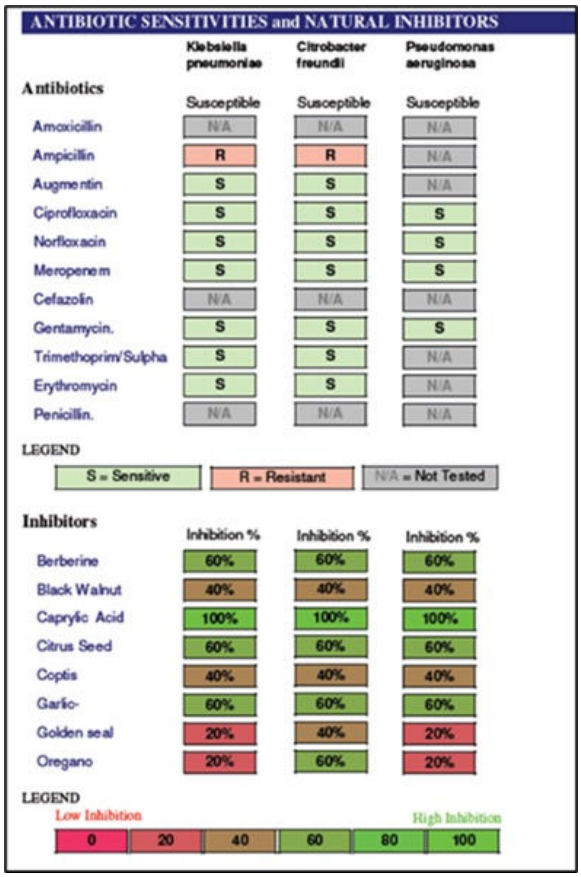
Culture is good for aerobic or facultative anaerobic bacteria that can tolerate oxygen exposure. Anaerobic bacteria are more difficult to culture because they require oxygen-free growth conditions; this has been problematic in the exploration of the gut microbiome as many resident bacteria are strict anaerobes. When antibiotics became part of standard medical treatment culture and sensitivities became commonplace to determine the best antibiotic treatment for a particular infection. Treating infections according to sensitivities decreases the incidence of anti-microbial resistance, which can develop with the blind overuse of either antibiotic drugs, herbal or nutraceutical antimicrobials. "Anti-microbial stewardship" is the judicious use of appropriate anti-microbials, avoiding overuse and blind treatment whenever possible. Anti-microbial stewardship is currently considered the "standard of care" in clinical practice.



Opportunistic Bacteria/Overgrowth	Result	Range	Units
Bacillus species.	0.70	< 1.00	x10^5 CFU/g
Enterococcus faecalis	1.69*H	< 1.00	x10^4 CFU/g
Enterococcus faecium	0.55	< 1.00	x10^4 CFU/g
Morganella species	<dl	< 1.00	x10^3 CFU/g
Pseudomonas species	<dl	< 1.00	x10^4 CFU/g
Pseudomonas aeruginosa.	<dl	< 3.00	x10^2 CFU/g
Staphylococcus species	<dl	< 1.00	x10^4 CFU/g
Staphylococcus aureus	<dl	< 5.00	x10^2 CFU/g
Streptococcus species	3.85 *H	< 3.00	x10^3 CFU/g
Methanobrevibacter smithii	4.00	< 5.00	x10^9 CFU/g
Desulfovibrio piger	84.22 *H	< 18.00	x10^7 CFU/g
Enterobacter complex.	<dl	< 1.00	x10^6 CFU/g
Potential Autoimmune Triggers			
Citrobacter species.	10.22*H	< 5.00	x10^5 CFU/g
Citrobacter freundii.	6.87 *H	< 5.00	x10^5 CFU/g
Klebsiella species	<dl	< 5.00	x10^3 CFU/g
Klebsiella pneumoniae.	<dl	< 5.00	x10^4 CFU/g
Prevotella copri	<dl	< 1.00	x10^7 CFU/g
Proteus species	<dl	< 5.00	x10^4 CFU/g
Proteus mirabilis.	<dl	< 1.00	x10^3 CFU/g
Fusobacterium species	0.20	< 10.00	x10^7 CFU/g

All opportunistic bacteria except *Methanobrevibacter smithii*, *Desulfovibrio piger*, Fusobacterium species, and *Prevotella copri* are tested by qPCR and confirmed by culture. Antimicrobial sensitivities are provided for culturable dysbiotic species.

Sensitivity to antimicrobial agents (pharmaceutical or natural) can only be performed on live cultures although there are a few obligate anaerobic bacteria that cannot survive in oxygen long enough for sensitivities to be performed. The presence of PCR "resistance genes" cannot be relied on for clinical treatment decisions, as there is no way to determine which bacteria, among the hundreds of species present, might actually possess (or be expressing) those genes.



A pathogenic or dysbiotic bacteria may be found in culture to resist to certain antimicrobial agents, as shown above. Resistant agents will not eliminate the bacteria or yeast. The use of use of resistant or indeterminant agents is discouraged – an indeterminate sensitivity indicates a partially-resistant bacterial population. Use of the indeterminant agent will kill the sensitive bacteria and create a completely anti-microbial resistant population of bacteria within the host (patient), which will then be even harder to eradicate.

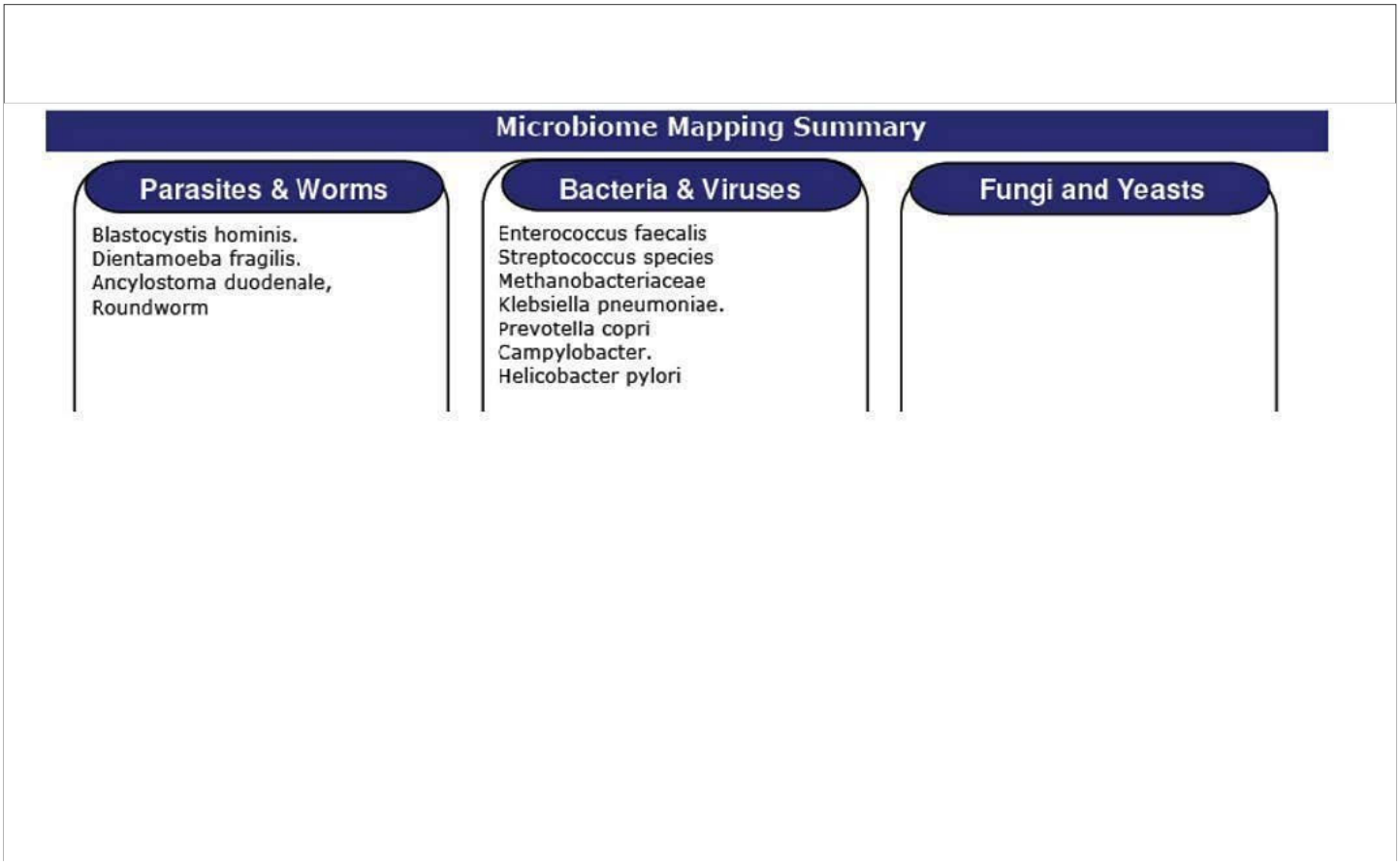
- If natural agents are used, then at least two of the sensitive natural anti-microbial agents (administered per the manufacturer's directions) may be used for at least 3-4 weeks.
- Not every bacterium may be cultured for sensitivities. Some bacteria are obligate anaerobes and cannot survive the exposure to oxygen necessary to complete sensitivities testing. Established pharmaceutical dosing agents have been included in the results interpretations for such bacteria. There are currently no human studies available to support the use of natural agents for these bacteria. The indiscriminate use of natural antimicrobials, without the guidance of culture and sensitivities, can only increase pathogen resistance.
  - Obligate anaerobic species include: Campylobacter, Clostridia, Arcobacter, Laribacter, *Helicobacterium pullorum*, *Listeria monocytogenes*, *Bacillus cereus* and *Bacillus anthracis*.
- Non-Candida yeasts are usually from the environment and are normally present in very low amounts in human stool. The use of azole antifungal agents is not indicated for such species and would lead to the development of anti-fungal resistance in these yeast species. Only natural anti-microbial sensitivities are provided in such cases.

The identification of pathogens, yeast and living dysbiotic organisms via microbial culture is vital for effective therapeutic intervention. The indiscriminate use of either antibiotic drugs or antimicrobial herbs breeds resistance in the yeasts, pathogens and dysbiotic bacteria that contribute to dysbiosis or digestive disorders as well as to local and systemic disease. **The GI Advanced Profile** improves clinical outcomes by providing live culture and sensitivities for both pharmaceutical and natural agents to minimize antimicrobial resistance and improve first-time therapeutic efficacy.

Microbiome Mapping Summary

The gastrointestinal microbiome is primarily composed of hundreds of different types of bacteria; smaller numbers of other microbes can be found in the gut microbiome including yeasts and bacteria-eating viruses (phages). The gut microbiome, in toto, may be considered an "accessory organ" that, when healthy, can assist the host with nutrient extraction and assimilation. A healthy, diverse microbiome will further support the host by synthesizing essential nutrients such as vitamin K and various B-vitamins, along with other nutritive compounds like short-chain fatty acids. Many of the compounds produced by the gut microbiome are essential for gut health and tolerant anti-inflammatory immune responses. These microbial compounds are signal molecules that "cross-talk" with other microbes or with the gut lining and underlying immune system. Clinicians requiring a more extensive "refresher" on the gastrointestinal microbiome than this guide can provide are referred to intechopen.com *"Gut Microbiome: A New Organ System in Body"* https://www.intechopen.com/chapters/69898.

Small amounts of yeast are normal inhabitants of the gut microbiome – although yeast overgrowth may result due to poor diet, dysbiosis, or inadequate immune responses. Some microbes found in the gut are pathogenic and are responsible for acute or chronic disease in the host. Parasites acquired from the environment may also be found in the gut microbiome. Commentaries on specific findings are included in individual patient results.

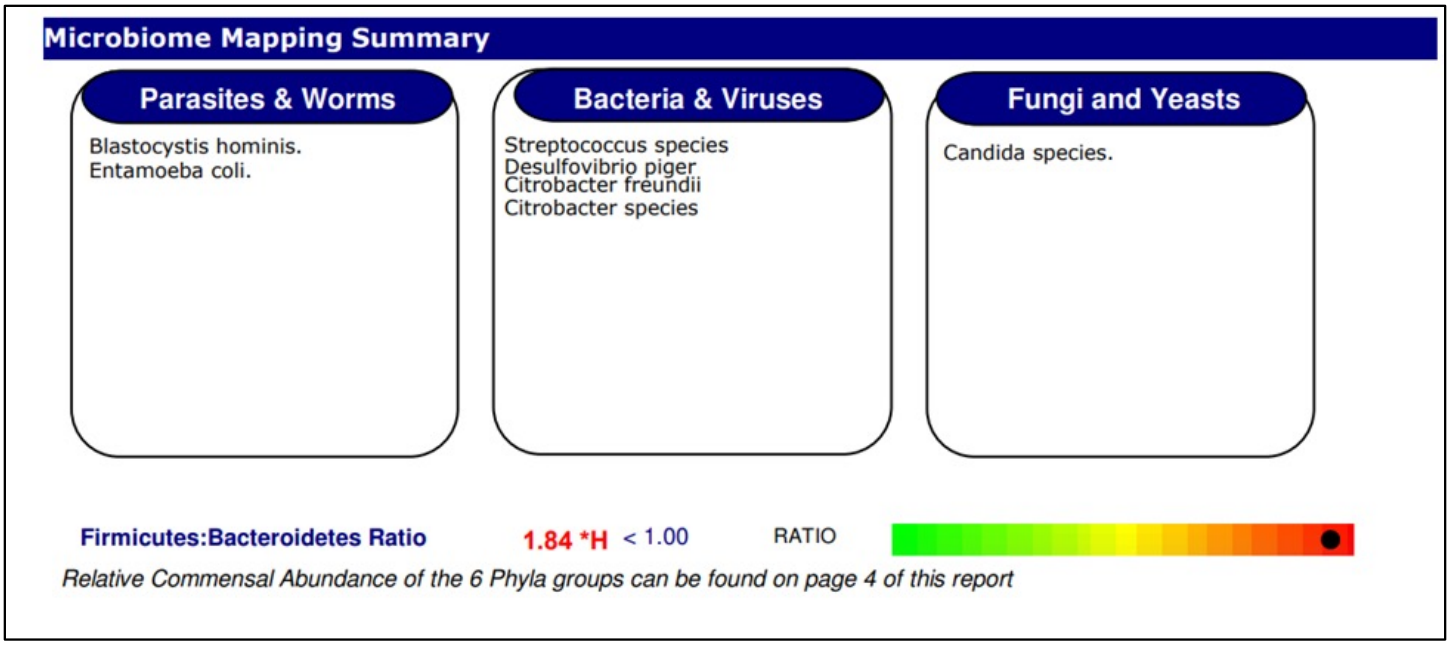


Firmicutes: Bacteroidetes Ratio

The dominant bacterial groups of the human microbiome are primarily found in five phyla; the Gram-positive Firmicutes and Actinobacteria, and the Gram-negative Bacteroidetes, Proteobacteria, and Verrucomicrobia. In most people at least 90% of the bacteria in their gut microbiomes belongs to either the Firmicutes or Bacteroidetes groups. Current evidence indicates that the Firmicutes:Bacteroidetes ratio is most closely associated with dietary choices (Western vs rural agrarian) and biological age. The two groups of bacteria perform different functions in the gut microbiome and both groups are intolerant of oxygen (obligate anaerobes). Diets high in animal proteins may increase the production of toxic metabolites, such as trimethylamine N-oxide (TMAO) by both Bacteroidetes and Firmicutes bacteria. High levels of TMAO in the gut have been associated with an increased risk of significant cardiovascular events and death.

While there are studies that show a shift in the Firmicutes:Bacteroides ratio in obese subjects, the studies were small and inconsistent in method. The early studies associating the Firmicutes:Bacteroidetes ratio as a consistent biomarker for obesity and its associated metabolic changes have not, unfortunately, been validated in recent larger studies such as the Human Microbiome Project. What newer studies have discovered is that Firmicutes and Bacteroides diversity is more strongly associated with diet and age. Several human population and twin studies have associated loss of gut microbiome diversity with increased frailty, dementia, etc. One study of European subjects demonstrated significant increases in the Firmicutes:Bacteroidetes ratio with increasing age (0-9 → 60-69 years). Other expected changes with increasing age may include:

- Increased Actinobacteria spp., Bacteroides spp., Proteobacteria



Bacteroidetes are thin-walled gram-negative bacteria. The outer layer of the cell wall contains lipopolysaccharide (LPS). Bacteroidetes ferment polysaccharides and other indigestible fibers; this fermentation produces beneficial short-chain fatty acids. Human studies indicate that two Bacteroidetes subgroups, Bacteroides and Prevotella, tend to dominate and individual human hosts tend to carry either Bacteroides or Prevotella microbiome based on dietary intakes. Prevotella carriage is most closely associated with a rural, agrarian Mediterranean-type diet, while Bacteroides carriage is associated with Paleo-type diets with higher protein and low-carbohydrates.

Firmicutes are thick-walled gram-positive bacteria. Diets high in soluble and insoluble plant fibers support the growth of beneficial Firmicutes bacteria in the gut microbiome. Firmicutes bacteria also produce butyrate and other beneficial short-chain fatty acids. There is evidence that psychological stress may decrease some of the Firmicutes bacteria groups in the microbiome. Loss of Firmicutes bacteria is also a common finding in chronic fatigue patients. Human studies comparing African children on agrarian diets to European children on Western diets demonstrated

a decrease in beneficial Firmicutes bacteria on the Western diets lower in fiber and higher in animal fat. Levels of butyrate and other short chain fatty acids produced by Firmicutes phylum members can be evaluated on 4U Health's **Advanced GI Profile**.

Parasites and Worms

Parasites are organisms that live on or inside a host organism. Human gastrointestinal parasites include protozoa and helminths. Protozoa parasites are single-celled organisms that can live and reproduce in humans. Transmission of gut protozoan parasites and worms typically involves the fecal-oral route, through contaminated food or water or direct contact with an infected person. Helminth parasites include worms and flukes. Populations at highest risk include rural residents, agricultural workers, those with lower socioeconomic status, recent immigrants, international travelers, children, immunocompromised individuals, recreational campers, etc.

Protozoa parasites

- Cryptosporidium species infections are caused by *C. parvum* or *C. hominis* and most often occur through water contamination. The parasite is resistant to chlorine water treatment and can survive outside the body for extended periods. Raw foods (unpasteurized milk, raw meat, etc.) can also be a source of infection. Cryptosporidium can cause an asymptomatic infection, mild diarrhea, or severe gastroenteritis with abdominal pain, malaise, nausea and fever. Therapeutic interventions may include:
  - Oral rehydration therapy
  - Adult pharmaceutical therapy (consult with pharmacist for pediatric dosing, nursing, pregnant):
    - Per CDC: Nitazoxanide 500mg BID x 3 days is approved for adults but may not work as well in immunocompromised individuals without extended treatment protocol of 7 days.
  - Antimotility agents may be used to help control diarrhea.
  - Probiotics
    - *Lactobacillus reuteri* or *L. acidophilus* may help reduce shedding of parasite eggs.
- ***Entamoeba histolytica*** infection is common in areas with poor sanitation; the infection is commonly acquired during international travel. The amoeba is transmitted through infected food or water. Not all infected individuals display symptoms, which begin 2-4 weeks after infection and include loose stools, abdominal pain, and cramping. Symptoms may progress to include bloody stools and fever. Very rarely, *E. histolytica* may invade the liver and form an abcess or migrate to other organs in the body, such as the lungs or brain. Interventions may include:
  - Oral rehydration and fever control
  - Adult pharmaceutical therapy (consult with pharmacist for pediatric dosing, nursing, pregnant):
    - Metronidazole 500 mg TID x 7-10 days followed by paromomycin 25 mg/kg/day in 3 divided doses x 7 days.
    - Tinidazole 2 gm QD x 3 days followed by paromomycin 25 mg/kg/day in 3 divided doses x 7 days.
    - Nitazoxanide 500 mg BID x 3 days followed by paromomycin 25 mg/kg/day in 3 divided doses x 7 days.
- ***Giardia intestinalis*** (aka ***G. lamblia*** or ***G. duodenalis***) is a protozoan flagellate found on contaminated surfaces, food or water. The transmission route is fecal-oral, and Giardia can spread easily from person to person. Transmission can also occur during recreational swimming or outdoor activities (hiking, camping, etc.). Giardia infections may be asymptomatic, or symptoms can develop 1-2 weeks after exposure. Symptoms can include loose stools and increasing fatigue. Some individuals may develop itchy skin and swelling of eyes and joints. Chronic or recurring Giardia can prevent absorption of fats and vitamins, over time weight loss may occur. Rarely, infections may progress to reactive arthritis, irritable bowel syndrome or recurrent diarrhea. In children, severe chronic infections may result in developmental delays. Shared strains of Giardia may infect humans, chinchillas, beavers, birds, opossum, and monkeys. Interventions may include:
  - Oral rehydration therapy
  - Dairy avoidance for 2-3 months after symptoms clear
  - Adult pharmaceutical therapy (consult with pharmacist for pediatric dosing, nursing, pregnant):

- Tinidazole 2 g x 1 dose
- Nitazoxanide 500 mg PO BID x 3 days
- Metronidazole 500 mg TID x 5-7 days
    - *Saccharomyces boulardii* may improve parasite elimination if used with metronidazole.

- Probiotics have been supportive in animal and in vitro studies:
    - *Lactobacillus johnsonii (LA1)*
    - *Lactobacillus casei MTCC 1423*

- ***Blastocystis hominis*** is a unicellular parasite related to algae and diatoms. Recent evidence indicates a high level of genetic diversity within the species – not all subtypes may cause symptoms, and asymptomatic *B. hominis* may not require treatment. Human studies indicate that *B. hominis* carriage may be higher in those handling livestock, over 50 years old, or travelling internationally. If symptomatic with loose, watery stools, diarrhea, abdominal pain, anal itching, weight loss, excess gas, or constipation, consider treatment once other causes for symptoms have been addressed and treated. Interventions may include:
    - Antibiotic therapies are controversial at present. Off-label dosing for several antibiotics are listed on the CDC website https://www.cdc.gov/parasites/blastocystis/health_professionals/index.html
    - *Sacharomyces boulardii* (as Reflor 250 mg BID) was as effective as metronidazole in a human study.
    - Aqueous extract of *Nigella sativa* 500 mg/kg/d (animal study).
- ***Dientamoeba fragilis*** is a common cause of parasitic infections in the large intestine. Transmission is fecal-oral, and risk of infection may be higher in areas of poor sanitation or during international travel. Not all infections are symptomatic with diarrhea, abdominal pain and sometimes anorexia, weight loss, nausea, and fatigue. Co-infection with pinworm (*Enterobius vermicularis*) can occur. Interventions may include:
    - Oral rehydration therapy
    - Adult pharmaceutical therapy with one of the following (consult with pharmacist for pediatric dosing, nursing, pregnant):
        - Iodoquinol 650 mg PO TID x 20 days
        - Paromomycin 25-35 mg/kg PO in 3 divided doses QD x 7 days
        - Metronidazole 500-750 mg PO TIC x 10 days
- ***Endolimax nana*** is currently considered commensal, not pathogenic, by the CDC, although current evidence indicates high genetic variability and occasional case reports describe symptoms such as diarrhea. Most infections are asymptomatic, although some early reports indicate that the amoeba may induce eosinophilia or perhaps be symptomatic in some individuals. *E. nana* may co-infect the large intestine with Blastocystis and is also transmitted via the fecal-oral route through contaminated food or water. Once all other causes of symptoms have been eliminated, if clinically indicated, intervention may be considered, as indicated by the available scientific literature. No treatment protocols are currently recommended by the CDC and overuse of antibiotics increases microbe and parasite drug resistance.
    - Probiotics may help decrease the parasite load.
    - Adult pharmaceutical therapy (consult with pharmacist for pediatric dosing, nursing, pregnant):
        - Metronidazole 250 mg 3 times a day for 10 days.
- ***Entamoeba coli*** is currently considered commensal, not pathogenic, by the CDC, although occasional case reports describe symptoms such as diarrhea. Most infections are asymptomatic. It is transmitted via the fecal-oral route through contaminated food or water. Once all other causes of symptoms have been eliminated, if clinically, intervention may be considered, as indicated by the available scientific literature. No treatment protocols are currently recommended by the CDC and overuse of antibiotics increases microbe and parasite drug resistance. Interventions may include:
    - Probiotics may decrease parasite load
    - Adult pharmaceutical therapy (consult with pharmacist for pediatric dosing, nursing, pregnant)
        - Diloxanide furoate 500 mg PO TID x 10 days
        - Metronidazole 400 mg PO TID

- ***Pentatrichomonas hominis*** is currently considered commensal, not pathogenic, by the CDC. However, in recent studies individuals with gastric, colon, or small intestinal cancer have significantly higher carriage of this parasite. It is unclear at present if *P. hominis* contributes to the disease process, but other studies have associated this parasite with a decreased microbiome diversity in colon cancer patients. The parasite is transmitted via the fecal-oral route through contaminated food or water and resides in the large intestine of the host. The parasite has occasionally been associated with diarrhea in humans and some animal species. Symptoms may be more likely in immunocompromised individuals. *P. hominis* is found in humans, rats, mice, hamsters, dogs, cats, guinea pigs, ground squirrels, and some primates. Once all other causes of symptoms have been eliminated, if clinically indicated, intervention may be considered as indicated by the available scientific literature. No treatment protocols are currently recommended by the CDC and overuse of antibiotics increases microbe and parasite drug resistance.
  - Probiotics may decrease parasite load.
  - Metronidazole has been used to eradicate *P. hominis* in animals; no specific dosing protocols have been found in the scientific literature.
- **Enterocytozoon species** are intracellular parasites related to algae and fungi. Over 15 different species of Enterocytozoon may infect humans and infective spores can remain dormant in the environment for months. The human parasite species can be found in various animal reservoirs (pigs, dogs, cats, primates, rabbits, parrots). Transmission routes may include spore inhalation, fecal-oral, soil/water contamination of food, and donor organ contamination. Asymptomatic infection or self-limited watery diarrhea may occur with Enterocytozoon spp. infection in immunocompetent individuals. Immunocompromised individuals may present with varied symptoms of microsporidiosis, which occurs in immunocompromised individuals (HIV+, immunosuppressive medications, genetic immunodeficiency, etc.). Symptoms of microsporidiosis vary with different causal species and infection location (eyes, muscle, biliary system, small intestine) and infection may result in malabsorption of nutrients. In humans the most common GI infection are caused by *Enterocytozoon bieneusi* or *Encephalitozoon intestinalis* and can present with symptoms of chronic diarrhea, nausea, vomiting, malabsorption, and wasting. If Enterocytozoon escapes into circulation and disseminates throughout the body, then urinary, respiratory, musculoskeletal, neurologic and other systemic symptoms may be present. Interventions may include:
  - Optimize or initiate anti-retroviral therapy in HIV+ patients
  - Oral rehydration therapy, electrolyte and nutrient replacement
  - Initiate appropriate antimicrobial therapy for chronic diarrhea or immunocompromised patients
    - Treatment protocols vary based upon Enterocytozoon species and the presence of comorbid medical conditions. Treatment options and contraindications may be reviewed in the *Merck Manual of Medicine* https://www.merckmanuals.com/professional/infectious-diseases/intestinal-protozoa-and-microsporidia/microsporidiosis
  - Malabsorption of fats will be apparent on **4U Health's GI Advanced Profile**

Helminths

- ***Ancyclostoma duodenale*** is a soil-transmitted intestinal hookworm. Eggs are passed in stool, and developing larvae in soil penetrate the skin and migrate to the intestines where they remain and mature. *A. duodenale* may be able to infect a host through either oral ingestion or through breast milk. Infections may be asymptomatic, however, worm attachment to the intestine may result in nausea, abdominal pain, fecal occult blood, or anorexia. Heavy infestations may compete with the host for iron and protein resulting in anemia or protein malnutrition. Larval migration may occasionally result in urticaria or eosinophilic pneumonia. Interventions may include:
  - Pharmaceutical intervention with one of the following protocols. Adults and children are dosed the same (consult with pharmacist regarding dose for infants or children < 2 years old, pregnant, nursing):
    - Pyrantel pamoate 11 mg/kg (maximum 1 g/dose) PO QD x 3 days
    - Mebendazole 100 mg PO BID x 3 days or single 500 mg PO dose
  - Wash bedding in hot water during treatment to prevent reinfection

- Educate patients regarding hand-washing and skin-soil contacts

- ***Ascaris lumbricoides*** is a soil-transmitted roundworm parasite of the large intestine. Eggs are passed in stool, and developing larvae in soil penetrate the skin and migrate to the intestines where they remain and mature. Humans and pigs are the major hosts, and *A. lumbricoides* is the most common helminth infection worldwide. Infection risk is higher in areas of poor sanitation or in rural areas where pigs are kept, but transmission can also occur if eggs contaminate raw vegetables or fruits. Adult worms rarely cause symptoms in the host, but high worm burdens may contribute to host malnutrition, cause abdominal pain, or block the intestinal lumen. Migrating adult worms may occasionally block bile ducts, or infect the appendix. Rarely, worms may be expelled out of the nose or mouth. Interventions may include:
  - Pharmaceutical intervention with one of the following protocols. Adults and children are dosed the same. Consult with pharmacist regarding dose for infants or children < 2 years old.
    - Mebendazole 100 mg PO BID x 3 days or single 500 mg PO dose
    - Ivermectin 150-200 g/kg PO single dose empty stomach. Consult with pharmacist for dosing if children weigh < 15 kg
  - Wash bedding in hot water during treatment to prevent reinfection
  - Educate patients regarding hand-washing and skin-soil contacts
- ***Trichuris trichiura***, or whipworm, is a parasite of the large intestine. Eggs are passed in stool into the soil. Infection typically occurs from contaminated food or soil exposures. Infection is more common in the southern US or worldwide in areas with poor sanitation. Infections may be asymptomatic, but heavy *T. trichiura* burdens may cause abdominal pain, diarrhea, rectal prolapse, malnutrition or anemia in young children. Adult infections may present with watery, bloody stools, frequent painful bowel movements, and rectal prolapse. Interventions may include:
  - Adult pharmaceutical intervention (consult with pharmacist for pediatric dosing, nursing, pregnant):
    - Mebendazole 100 mg PO BID x 3 days is the only FDA-approved treatment
  - Wash bedding in hot water during treatment to prevent reinfection
  - Educate patients regarding hand-washing food handling, and skin-soil contacts
- **Strongyloides species** are soil-transmitted roundworm intestinal parasites. Larvae are passed in stool, and larvae deposited in soil penetrate the skin and migrate to the intestines where they remain and mature. Since the larvae hatch in the intestine, the host may be re-infected by larvae before they are expelled in the stool (auto-infection). Direct infection person-to-person may occasionally occur due to organ transplantation, in patient care facilities, or daycare centers. Infection may be more prevalent in those who travel to subtropical or tropical areas (southeast Asia, south Pacific regions, etc.) or in rural areas. Some primate strains may cause human infections. Most infections are asymptomatic, or present with vague symptoms such as abdominal pain and bloating, intermittent diarrhea and constipation, dry cough or skin rash. Rarely, symptoms may progress to include arthritis, kidney disorders, or cardiovascular problems. Infections may become life-threating in immunocompromised patients or those with leukemia or lymphoma. To prevent further spread, treatment is recommended for both symptomatic and asymptomatic individuals. Interventions may include:
  - Adult pharmaceutical therapy with one of the following (consult with pharmacist for pediatric dosing, nursing, pregnant):
    - Ivermectin 200 g/kg PO single dose x 1-2 days
    - Albendazole 400 mg PO BID x 7 days
    - Refer to CDC recomendations for disseminated infection, pregnancy and lactation contraindications https://www.cdc.gov/parasites/strongyloides/health_professionals/index.html#tx
  - Follow-up stool test in 2-4 weeks to confirm eradication of parasite
  - Educate patient to practice good hand hygiene, wear shoes outside, avoid direct contact with feces, have animal waste removed immediately after elimination, etc. to minimize reinfection risks
- ***Necator americanus*** is a soil-transmitted intestinal hookworm. Eggs are passed in stool, and developing larvae in soil penetrate the skin and migrate to the intestines where they remain and mature. *N. americanus* may be able

to infect a host through either oral ingestion or through breast milk. Infections may be asymptomatic, however, worm attachment to the intestine may result in nausea, abdominal pain, fecal occult blood, or anorexia. Heavy infestations may compete with the host for iron and protein resulting in anemia or protein malnutrition. Larval migration may occasionally result in urticaria or eosinophilic pneumonia. Interventions may include:

- Pharmaceutical intervention with one of the following protocols. Adults and children are dosed the same (consult with pharmacist regarding dose for infants or children < 2 years old, pregnant, nursing):
  - Pyrantel pamoate 11 mg/kg (maximum 1 g/dose) PO QD x 3 days
  - Mebendazole 100 mg PO BID x 3 days or single 500 mg PO dose
- Wash bedding in hot water during treatment to prevent reinfection
- Educate patients regarding hand-washing and skin-soil contacts

- ***Enterobius vermicularis*** is a pinworm parasite that infects the human large intestine and rectum. While the host sleeps, female pinworms migrate to the anus and deposit eggs on the surrounding skin; the egg deposits cause itching in the area. Eggs may transfer to fingers or may be ingested or inhaled from bedding, food, etc. Pinworms are commonly found in children, household members, institutionalized populations, daycare centers and caretakers. There are no known animal vectors for *E. vermicularis*. Pinworm eggs can be seen under a microscope but are rarely visualized in stool samples and the PCR testing used is more sensitive that traditional fecal diagnostic methods. Interventions may include:
  - Test all caretakers and family members for infection; treat all simultaneously if possible
  - Adult pharmaceutical therapy with one of the following (consult with pharmacist for dosing young children < 2 years old, pregnant or nursing):
    - Albendazole 400 mg PO single dose
    - Mebendazole 100 mg PO single dose; repeat in 3 weeks if parasite not eradicated
    - Pyrantel pamoate 11 mg/Kg (not to exceed 1 gm) PO single dose; repeat dose in two weeks per CDC
  - Wash all clothes and bedding in hot water, disinfect shared surfaces
  - Educate patient, caretakers and family about hand hygiene and parasite transmission
- ***Hymenolepis species*** are tapeworm parasites. There are two species that can infect humans; *Hymenolepis nana* (dwarf tapeworm) is the most common in humans. *Hymenolepis diminuta* (rat tapeworm) may occasionally infect humans as well. Eggs are passed in stool and survive 7-10 days in the environment. The eggs may be ingested by human hosts directly from contaminated food or water. This species can continue to reinfect the host during each reproductive cycle (autoinfection), and infections may last for years. Infections primarily occur in children, family and caretakers, institutionalized patients, or in areas of poor sanitation. Infections are usually asymptomatic, but a heavy parasite burden can result in symptoms of weakness, headache, anorexia, abdominal pain, and diarrhea. Children may also complain of anal itching, or disturbed sleep. Interventions may include:
  - Adult pharmaceutical therapy with one of the following (consult with pharmacist for pediatric dosing, nursing, pregnant):
    - Praziquantel 25 mg/Kg PO single dose
    - Nitazoxanide 500 mg PO BID x 3 days
  - Teach hand hygiene and safe food preparation
- ***Taenia species*** are parasitic human tapeworms. There are three different species, one in pigs and two in cattle, that infect humans when they eat undercooked meat. There are two different types of infections that can occur from a tapeworm infection. Intestinal tapeworms (taeniasis) occur when Taenia cysts are eaten in undercooked meats and are typically asymptomatic. Intestinal tapeworm symptoms include abdominal pain, anorexia, weight loss, and dyspepsia. Tapeworm segments may also be seen in stool. In contrast, *T. solium* (pork tapeworm) infections can result in cysticerosis, which can only occur if direct ingestion of tapeworm eggs occurs via the fecal-oral route or from egg-contaminated food. Cysticerosis happens when larval cysts embed in muscle, brain, or other tissues outside the gut. Larval cysts in the brain can cause adult-onset seizures spontaneously or during anti-helminth drug therapy. Interventions may include:

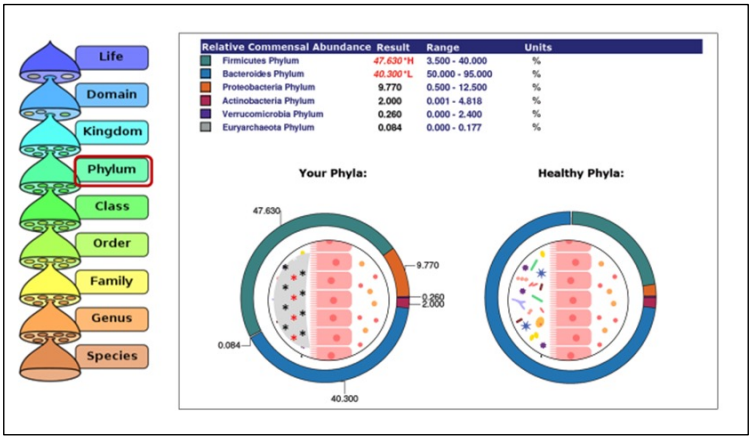
- Adult pharmaceutical therapy per the CDC (consult with pharmacist for young children < 2 years old, nursing, pregnant):
    - Praziquantel 5-10 mg/Kg PO single dose
    - Albendazole 400 mg QD x 3 days
- Have stool examined microscopically for Taenia eggs 1 month and 3 months after treatment to confirm eradication
- Educate patients about safe cooking practices
    - Use food thermometer for beef and pork
        - Thickest part of meat should register 145°F, then "rest" for at least 3 minutes prior to carving or consumption
        - Ground meats cook to 160°F prior to consumption

Opportunistic Bacteria/Overgrowth Results

Not all bacteria in the gut microbiome are created equal. Depending upon diet, genetics, manternal contribution, overall health, and environmental factors, a "core microbiome" is established within each individual, with more general microbiome patterns established within human populations with similar diet and lifestyle (cultural influences). The human gut microbiome typically contains 4-5 phyla of microbes: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucomicrobia. Most of the bacteria found in humans are from the Firmicutes or Bacteroidetes phyla; the bacteria within each phylum are subdivided further into groups based on genetic similarity. At the family and genus level, studies have identified signature genera and families that are significant for health or disease. Occasionally, within a small phylum or signature group individual bacterial species are considered relevant for health or disease. In small numbers some populations of bacteria associated with poor health may be considered "commensal" (species that neither harm nor help the host). Many bacteria can reside as commensals in the gut microbiome and may only be pathogenic in large numbers or if they escape the gut into surrounding tissues or into circulation (translocation) causing systemic infections. These bacteria are referred to as "opportunistic pathogens", and infections may be more likely in immunocompromised individuals. Of note, because infant immune systems are not fully developed, several groups of bacteria considered dysbiotic in older children or adults may be commonly found as non-pathogenic members of infant microbiomes. As infants are weaned, their gut microbiomes begin to resemble adult profiles over a few month's time. Most children's microbiomes are similar to adults by about two years of age.

Opportunistic Bacteria/Overgrowth	Result	Range	Units
Bacillus species.	0.70	< 1.00	x10^5 CFU/g
Enterococcus faecalis	1.69*H	< 1.00	x10^4 CFU/g
Enterococcus faecium	0.55	< 1.00	x10^4 CFU/g
Morganella species	<dl	< 1.00	x10^3 CFU/g
Pseudomonas species	<dl	< 1.00	x10^4 CFU/g
Pseudomonas aeruginosa.	<dl	< 3.00	x10^2 CFU/g
Staphylococcus species	<dl	< 1.00	x10^4 CFU/g
Staphylococcus aureus	<dl	< 5.00	x10^2 CFU/g
Streptococcus species	3.85 *H	< 3.00	x10^3 CFU/g
Methanobrevibacter smithii	4.00	< 5.00	x10^9 CFU/g
Desulfovibrio piger	84.22 *H	< 18.00	x10^7 CFU/g
Enterobacter complex.	<dl	< 1.00	x10^6 CFU/g
Potential Autoimmune Triggers			
Citrobacter species.	10.22*H	< 5.00	x10^5 CFU/g
Citrobacter freundii.	6.87 *H	< 5.00	x10^5 CFU/g
Klebsiella species	<dl	< 5.00	x10^3 CFU/g
Klebsiella pneumoniae.	<dl	< 5.00	x10^4 CFU/g
Prevotella copri	<dl	< 1.00	x10^7 CFU/g
Proteus species	<dl	< 5.00	x10^4 CFU/g
Proteus mirabilis.	<dl	< 1.00	x10^3 CFU/g
Fusobacterium species	0.20	< 10.00	x10^7 CFU/g

An imbalance in the microbiome that supports the growth of bacteria associated with poor health or disease is termed dysbiosis. A dysbiotic microbiome will have few health-associated species and a larger proportion of commensal bacterial groups or species associated with poor health or disease. The effect of diet, environment and lifestyle on the gut microbiome is reviewed in detail in the "Bacterial Diversity in the Gut Microbiome" section, Relative Commensal Abundance, on page 58.



GROUPING	BACTERIA	HEALTH ASSOCIATION	AUTOIMMUNE ASSOCIATION
Firmicutes * Bacillus species		Varies based on species.	N/A
Firmicutes * Bacillus	*Enterococcus faecalis*	Damages gut lining, disrupts gut barrier	N/A
Firmicutes * Bacillus	*Enterococcus faecium*	Damages gut lining, disrupts gut barrier	Ulcerative colitis
Proteobacteria * Morganella		Asthma	
Proteobacteria * Pseudomonas species		CFS	MS, RA, IBD, spondyloarthritis
Proteobacteria * Pseudomonas species	*Pseudomonas aeruginosa*	CFS	MS, RA, IBD, spondyloarthritis
Firmicutes * Staphylococcus species		Diarrhea	IBD, RA, spondyloarthritis
Firmicutes * Staphylococcus species	*Staphylococcus aureus*	Diarrhea, food poisoning, pseudomembranous colitis	IBD; Enterotoxins may trigger autoimmunity
Firmicutes * Streptococcus species		Pseudomembranous colitis, CRC, ASD, atherosclerosis	IBD, MS
Euryarchaeota	*Methanobrevibacter smithii*	Constipation, left-sided diverticulitis, IBS	IBD, MS
Thermodesulfobacteria	*Desulfovibrio piger*	Type II diabetes, Parkinson's disease	IBD
Proteobacteria * *Enterobacter cloacae* complex		GI disorders, atherosclerosis, CRC risk, liver disorders and cirrhosis	RA
Proteobacteria * Citrobacter species		CFS, IBS	Autoimmunity
Proteobacteria * Citrobacter species	*Citrobacter freundii*	Diarrhea, opportunistic pathogen, URI, UTI, neonatal meningitis	
Proteobacteria * Klebsiella species		IBD	IBD
Proteobacteria * Klebsiella species	*Klebsiella pneumoniae*	CFS, pyogenic liver abscess, CRC risk, reactive arthritis	IBD, ankylosing spondylitis, psoriatic arthritis
Bacteroidetes * Prevotella	*Prevotella copri*	Hypertension, insulin resistance	Rheumatoid arthritis
Proteobacteria * Proteus species		CFS, IBD	IBD
Proteobacteria * Proteus species	*Proteus mirablis*	Crohn's disease, RA	IBD, RA
Fusobacteria * Fusobacterium species		Appendicitis, esophageal, gastric and colorectal cancer (CRC), oral cavity disease, adverse pregnancy events, extraintestinal infections, CVD, Alzheimer's disease, pancreatic/breast cancers	IBD, RA

Bacillus species

- *Enterococcus faecalis*
- *Enterococcus faecium*

Bacillus species are common, spore-producing residents of the human gastrointestinal microbiome in the Firmicutes phylum. Many Bacillus species have beneficial effects and can provide pathogen exclusion, antioxidant, antimicrobial, and immuno-modulatory functions for the gut microbiome and host. Bacillus species currently used as probiotics include *B. coagulans* and *B. subtilis*. *B. coagulans* supplementation has been shown to decrease inflammation and improve the growth of commensal/beneficial Clostridia species and *Faecalibacterium prausnitzii* in elderly subjects. Some members of Bacillus species particularly *B. cereus, B. weihenstephanensis, B. anthracis,* and *B. thuringiensis* species are known to produce various enterotoxins. Other Bacillus species, such as *Acinobacter baumanii*, are opportunistic pathogens in immunocompromised individuals. The gut microbiome may serve as a reservoir for *A. baumanii* and other opportunisitic pathogens. The genus Enterococcus is a member of the Bacillus class.

- *Enterococcus faecalis* may be considered a dysbiotic member of the class Bacillus. *E. faecalis* is often the first colonizing bacteria in infant guts. In infants *E. faecalis* has a protective role and is important in intestinal immune system development. In adults *E. faecalis* targets damaged gut mucosal tissues, where it can cause inflammation, prevent healing, disrupt mucosal barrier functions and increase permeability. Increased levels of *E. faecalis* have been associated with colorectal cancer, however it is not clear if *E. faecalis* contributes to cancer risk or is increased due to patient's dietary preferences or antibiotic use. Carriage is more common in individuals on a Western diet which may increase Enteroccocus species abundance..
- *Enterococcus faecium* is a member of the class Bacillus and strains are commonly found in the gut microbiome of healthy humans and animals. In a damaged gut, however, the bacteria has been associated with the development of ulcerative colitis (inflammatory bowel disease or IBD). Translocated *E. faecium* may cause urinary tract infections, endocarditis, or blood-borne bacterial infections. Hospital-associated strains are usually antibiotic-resistant, while gut microbiome strains are usually antibiotic-sensitive.

Morganella species

Morganella species are members of the Proteobacteria phylum. The best-known member of the group is *Morganella morganii*, a gut microbiome commensal that is commonly associated with urinary tract infections. Morganella is an opportunistic pathogen that may escape into the circulation through the hepatobiliary tract in the liver, or when it translocates through the mucosal gut barrier into surrounding tissues. The risk of translocation may be greater in immunocompromised individuals or those with low levels of secretory IgA . Morganella species may contribute to gastrointestinal inflammation. Higher levels of GI *M. morganii* have been found in non-obese asthmatics. Antibodies against *M. morganii* are higher in chronic fatigue patients than controls. Fecal secretory IgA is evaluated on **4U Health's Advanced GI Profiles**.

Pseudomonas species

- *Pseudomonas aeruginosa*

Pseudomonas species are members of the Proteobacteria phylum. Higher levels of Pseudomonas species have been associated with relapsing/remitting multiple sclerosis (MS), inflammatory bowel disease (IBD) and chronic rheumatic diseases (rheumatoid arthritis [RA], spondyloarthritis). Antibodies against Pseudomonas species are higher in chronic fatigue (CFS) patients than controls. Colonization with Pseudomonas species may occur after antibiotic use. Pseudomonas species may play a role in gluten sensitization in Celiac patients, which may be mitigated by some Lactobacillus species. Bifidobacterium probiotic *B. breve* may help reduce Pseudomonas populations in the gut microbiome.

- *Pseudomonas aeruginosa* is a common contaminant in food-processing facilities, and may be found in water, milk, meat, fruits, or vegetables. Higher levels of *P. aeruginosa* have been found in bottle-fed infants (compared to breast-fed). At high levels *P. aeruginosa* may act as a pathogen in the gastrointestinal tract and disrupt gut mucosal barrier functions, particularly when gut microbiome diversity has been lost. *P. aeruginosa* may be susceptible to inhibitory antimicrobial compounds secreted by Bacillus species bacteria.

Staphylococcus species

- *Staphylococcus aureus*

Staphylococcus species are members of the Firmicutes phylum. Some Staphylococcus species, and other Firmicutes members, can secrete enzymes that metabolize gluten proteins. Staphylococcus bacteria are typical early infant colonizers and may be transmitted via breast milk. Increased levels of Staphylococcus species have been associated with inflammatory bowel disease (IBD) and chronic rheumatic diseases (rheumatoid arthritis [RA], spondyloarthritis). Some Staphylococcus species can produce neurologically active trace amines (tryptamine, tyramine, and phenylethylamine) as well as dopamine and serotonin, from the amino acids released during protein digestion. Bifidobacterium probiotic *B. breve* may help reduce Staphylococcus populations in the gut microbiome.

- *Staphylococcus aureus* is a common opportunistic pathogen associated with many diseases in humans and animals. Immunocompromised or chronically inflamed individuals may be at greater risk of infection. The bacteria is oxygen-tolerant and may be found as a commensal in roughly 30% of human gut microbiomes and in 25% of nasal microbiomes. Chronic exposure to *S. aureus* enterotoxins may increase the risk of autoimmunity. *S. aureus* contamination of food produces toxins that cause food poisoning. In the gut, *S. aureus* has been associated with disruption of the gut barrier, antibiotic-assoicated diarrhea and inflammatory bowel disease (IBD). Gastrointestinal *S. aureus* infection may progress to life-threatening pseudomembranous colitis. Infant colonization with *S. aureus* has been associated with increased risk of allergy. *S. aureus* may be susceptible to inhibitory bacteriocins secreted by Bacillus species bacteria.

Streptococcus species

Streptococcus species are members of the Firmicutes phylum. Some Streptococcus species can secrete enzymes that metabolize gluten proteins. Streptococcus species are commonly found in breast milk, but Streptococcus species levels may be higher in bottle-fed infants. (Note: this test does not differentiate group B streptococcus). Streptococcus species levels may decline with increasing blood glucose dysregulation (metabolic syndrome, type II diabetes, etc.) and major depressive disorders (MDD). Streptococcus species levels may be higher in gastrectomy patients. Higher levels of Streptococcus species have been associated with gastrointestinal disorders such as inflammatory bowel disease (IBD), colorectal cancer (CRC) and pseudomembranous colitis. Increased levels of Streptococcus species have also been associated with systemic disorders such as autism spectrum disorder (ASD), relapsing/remitting multiple sclerosis (MS) and atherosclerosis. Commercial probiotic products may include Streptococcus species, such as *S. thermophiles.*

Methanobrevibacter smithii

M. smithii is a member of the phylum Euryarchaeota, in the superkingdom Archeota. Among the methane gas-producing bacteria, *M. smithi* is considered a primary colonizer in the human gut. Methane producers (methanogens) are found in older children and adults, but rarely isolated from children younger than 5 years old. Dietary factors may influence *M. smithii* levels; increasing insoluble fiber and decreasing simple carbohydrates may regulate levels and promote overall gut microbiome diversity. In the gastrointestinal lumen, the methanogen bacteria are dependent upon other gut microbiome bacteria that break down carbohydrates. The carbohydrate breakdown provides *M. smithii* with substrates it can metabolize (acetate, hydrogen, or methyl groups), producing methane gas in the gut lumen. The production of methane decreases hydrogen levels in the gut, which has health benefits for the host. Excess gastrointestinal hydrogen can significantly, increase gut lumen sensitivity in irritable bowel syndrome

(IBS), decrease gut transit times, increase intestinal muscle contractility, and may adversely affect populations of beneficial bacteria.

- **Increased abundance:** Human studies indicate that levels of *M. smithii* and other methanogens are inversely related to levels of fecal butyrate. Fecal butyrate levels are evaluated in **4U Health's Advanced GI Profile**. *M. smithii* tends to be found in association with Akkermansia species. Increased levels of *M. smithii* have been found in humans regularly exposed to methane gas. Increased levels of Methanobrevibacter species have been associated with multiple sclerosis (MS), constipation, left-sided diverticulitis, gastrointestinal cancer, obesity and irritable bowel syndrome (IBS).
- **Decreased abundance**: Decreased levels of *M. smithii* may occur with regular aspirin use. While other methanogens have increased in cases of inflammatory bowel disease (IBD), one study found that *M. smithii* levels decreased with active Crohn's disease; levels rebounded when the patients were in remission.

Desulfovibrio piger

Desulfovibrio piger is a member of the Thermodesulfobacteria phylum. *D. piger* is the most common of the sulfate-reducing bacteria that may be found in about 50% of human microbiomes. Higher levels of *D. piger* have been associated with inflammatory bowel diseases (IBD) and type II diabetes. Like *Methanobrevibacter smithii*, Desulfovibrio species can consume hydrogen gas produced by bacterial fermentation in the gut microbiome while they reduce dietary sulfates and sulfites from food additives or preservatives in breads, meats, dried fruits and wines. Increasing levels of fermentable dietary carbohydrates (pectins, ß-glucans, ß-fructans, inulins, oligosaccharides, resistant starches, etc.) may help decrease levels of *D. piger* and other Desulfovibrio species.

Enterobacter cloacae complex

Enterobacter species are members of the Proteobacteria phylum. Enterobacter species may colonize the gut from fecal-oral transmission, soil, water, or raw plant foods. The bacteria are also natural members of the gut microbiome. Six different species (*E. cloacae, E. asburiae, E. hormaechei, E. kobei, E. ludwigii* and *E. nimipressuralis*) are so genetically similar that the term "complex" is used to refer to them as a group.

Some strains of Enterobacter may have beneficial effects for the host. In the mouse gut *E. ludwiggi* promoted immunotolerance during chemically induced colon inflammation. Some Enterobacter species such as *E. aerogenes, E. hormaechei, E. cloacae, E. asburiae, E. kobei,* and *E. ludwigii* are considered opportunistic pathogens if they escape the GI tract and are multi-drug resistant. Other Enterobacter strains may form biofilms and secrete toxins that can cause gastroenteritis. Infection is most likely in immunocompromised individuals or those with chronic inflammatory conditions such as vascular disease, diabetes, or kidney disease.

- **Increased abundance:** Increased numbers of Enterobacter species have been seen with some gastrointestinal disorders, atherosclerosis, colorectal cancer risk, liver disorders such as fatty liver (NAFLD or MAFLD) and cirrhosis. Melatonin has been shown to increase the growth of *E. aerogenes* in culture.
- **Decreased abundance:** Some studies report a decrease in Enterobacter species in rheumatoid arthritis patients.

Citrobacter species

- *Citrobacter freundii* is the Citrobacter species most commonly associated with diarrheal illness. Some *C. freundii* strains may carry virulence factors that can cause food poisoning. This species can metabolize citrate, a common ingredient in many nutraceutical products. *C. freundii* has been associated with both UTI and upper respiratory infections (URI). *C. freundii* may be able to disrupt both the gut mucosal barrier and the bloodbrain barrier under certain circumstances if it translocates. If it translocates out of the gut lumen into the circulation *C. freundii* is an opportunistic infectious pathogen that can be difficult to eradicate due to its multi-drug resistance. It has been found in joint synovial fluid and transplant organs and is a rare cause of neonatal meningitis. While loss

of gut barrier functions and chronic infections are both risk factors for the development of autoimmunity, recent molecular human gut microbiome studies of rheumatic autoimmune conditions fail to support early associations with autoimmune conditions.

Citrobacter species are glucose-fermenting members of the Proteobacteria phylum. Citrobacter species' enzymes metabolize mannitol to gas, but can also metabolize tryptophan to indole. Some species are able to ferment lactose. Citrobacter species *C. freundii, C. koseri,* and *C. braakii* are commonly associated with urinary tract infections (UTI). Citrobacter species may be found in food or water and may also be passed via the fecal-oral route.

- **Increased abundance**: Higher levels of Citrobacter have been associated with bloating and irritable bowel syndrome (IBS). High levels of antibodies against some Citrobacter species have been found in chronic fatigue (CFS) patients. Use of some biologic immune suppressants (secukinumab, etc.) may increase Citrobacter levels in the microbiome. Animal studies indicate that probiotics and quercitin (30 mg/kg) may mitigate some of the host symptoms associated with Citrobacter species carriage.
- **Decreased abundance:** One human study found lower levels of Citrobacter in rheumatoid arthritis patients.

Klebsiella species

- *Klebsiella pneumoniae*

Klebsiella species are members of the Proteobacteria phylum. Klebisella is a common early colonizer of infant microbiomes. Higher levels of Klebsiella species have been associated with bloating, irritable bowel syndrome (IBS) and gastroenteritis. Klebisella species have also been associated with obesity in Chinese adolescents. One human study found lower levels of Klebisella species in rheumatoid arthritis patients. Klebsiella species may be susceptible to inhibitory bacteriocins secreted by Bacillus species bacteria. Klebsiella species can break down both simple and complex carbohydrates; low-carbohydrate diets may be considered as a therapeutic intervention.

- *Klebsiella pneumoniae* is commonly isolated from patients with gastrointestinal disorders. It may colonize mucosal tissues in both the oral cavity and the gastrointestinal tract and it releases a toxin that promotes chronic inflammation. Circulating antibody levels against *K. pneumoniae* are higher in chronic fatigue (CFS) patients. Recurrent infection with *K. pneumoniae* is considered a predisposing factor in the development of ankylosing spondylitis, reactive arthritis, psoriatic arthritis, Crohn's disease and ulcerative colitis. *K. pneumoniae* is also a cause of pyogenic liver abscess which can increase the risk of colorectal cancer. *Klebsiella pneumoniae* carriage is associated with higher ß-glucuronidase levels. ß-glucuronidase activity from both the host and bacteria can affect the detoxification efficiency and levels of potentially active sex hormones, neurotransmitters, drug metabolites, plant polyphenol or isoflavone metabolites, and toxic metabolites from chemical exposures. Both *K. pneumoniae* toxin production and higher fecal ß-glucuronidase has been associated with an increased risk of colorectal cancer (CRC). ß-glucuronidase activity can be assessed with **4U Health's Advanced GI Profile**.

Prevotella copri

Prevotella copri is a member of the Bacteroidetes phylum. It has been associated with hypertension and insulin resistance and it may influence the synthesis of host branch-chain amino acids during glucose-insulin dysregulation. *P. copri* has also been associated with the development of rheumatoid arthritis (RA); human studies indicate that Prevotella levels increased (and Bacteroides species decreased) in at-risk individuals with pre-clinical RA and those with newly diagnosed RA. *Prevotella copri* has enzymes that can break down complex polysaccharides typically associated with a high-fiber diet. However, other environmental influences may affect *P. copri* status. One small study of obese adolesents found that *P. copri* was positively associated with physical exercise and protein intake, while coffee consumption was associated with increased Prevotella numbers in a large study of Chinese adults.

Proteus species

- *Proteus mirablis*

Proteus species are members of the Proteobacteria phylum commonly found in soil, water and the human GI tract. They are considered opportunistic pathogens found in wounds, urine and post-surgical infections. Infection is more likely in immunocompromised individuals. Proteus species in the gut microbiome have been associated with appendicitis, and with several chronic inflammatory disorders including rheumatoid arthritis (RA), inflammatory bowel disease (IBD), and chronic fatigue syndrome (CFS). Proteus species levels in the microbiome may increase when food additives such as polysorbate 80 and carboxymethylcellulose are included in the diet.

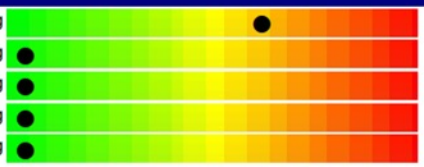
- *Proteus mirablis* induces inflammatory responses when it adheres to mucosal surfaces in the body (animal studies) and is commonly isolated from patients with Crohn's disease. *P. mirablis* can secrete an enzyme that breaks down host antibacterial antibody defenses and it is most commonly associated with urinary tract infections. Rats are natural hosts for *P. mirablis*, and higher levels are associated with increased obesity. Other animals can also host *P. mirablis*, with documented transfer between dogs and humans.

Fusobacterium species

Fusobacterium species are members of the Fusobacteria phylum found in the oral cavity (*F. nucleatum*) and intestinal tract. In the gut Fusobacterium species have been associated with appendicitis, inflammatory bowel disease (IBD), esophageal, gastric and colorectal cancer (CRC). As opportunistic pathogens elsewhere in the body, one Fusobacterium species, *F. nucleatum*, has been associated with oral cavity disease, adverse pregnancy events, respiratory infections, throat infection complications, cardiovascular disease, rheumatoid arthritis, Alzheimer's disease, and cancers of the pancreas, and breast. Smoking or comorbid type II diabetes may increase *F. nucleatum* levels and periodontal disease risk in the mouth.

Fungi and Yeast

Fungi & Yeast	Result	Range	Units
Candida species.	5.58 *H	< 5.00	x10^3 CFU/g
Candida albicans.	<dl	< 5.00	x10^2 CFU/g
Geotrichum species.	<dl	< 3.00	x10^2 CFU/g
Saccharomyces cerevisiae.	<dl	< 3.00	x10^3 CFU/g
Rhodotorula species.	<dl	< 1.00	x10^3 CFU/g



The kingdom Fungi includes multiple phyla which include yeasts and mushrooms. Over 50 yeasts may be found in the gastrointestinal microbiome, some of which have been associated with human disease. Yeasts found in the gut may be part of the normal gut microbiome or may be temporary residents from soil, environment, or dietary sources. Fungi, including yeasts, can be significant sources of allergens or inflammatory antigens, and cross-reactivity between different types of fungi can occur. In contrast, some fungi are beneficial members of the gut microbiome and assist in the degradation of dietary fiber while others help detoxify dietary toxins. The best defense against yeast overgrowth is the restoration of bacterial diversity in the gut (or other) microbiome and the restoration of normal immune functions and immunotolerance (see "Restoration of the Gut Microbiome" section). Yeasts are effective as opportunistic pathogens because they can thrive under the inflammatory conditions (such as increased oxidative or nitrosative stress) that cause tissue destruction and eliminate beneficial bacteria from the gastrointestinal microbiome.

Some individuals may become sensitized or allergic to fungal proteins and some fungal proteins can cross-react with other members of the kingdom Fungi. **4U Health tests** for many of these cross-reactive antigens as either IgE allergy or IgG sensitivities.

NOTE: With the advent of genomic classification of microbes, the yeasts of the kingdom Fungi have been reclassified and renamed. Since many of these names have not yet been finalized, this document will use established names and note newer names in parentheses for clarity.

Candida species

Candida species are members of the Ascomycetes (Ascomycota) phylum of the kingdom Fungi. Several Candida species can cause disease in humans, although about 80% of infections are attributed to *C. albicans*. Candida species induce cell damage and inflammation when they attach to and interact with mucosal epithelial cells. Immunocompromised individuals (whether due to genetics, immunosuppressive medications, malnutrition, or toxic exposures) may be more susceptible to Candida species infections. Candida species dominate the oral microbiome. Candida populations increase when the host diet is higher in carbohydrates and decrease when diets are higher in protein and fatty acids.

- Nutritional status, exposure to toxic chemicals or mold mycotoxins can be evaluated using **4U Health's Organic Acids Profile**, **Environmental Pollutants Profile**, and **Mycotoxin Profile**.

Candida species levels may increase when intestinal disease is present, when there is a loss of diversity in the gut microbiome, and after antibiotic use. Immune responses to other types of yeast may cross-react with Candida species, resulting in IgE allergy or sensitization via IgG or IgA. Several probiotic species have been shown to inhibit *Candida albicans* and other Candida species in the gut microbiome, including *Lactobacillus johnsonii, Lactobacillus reuteri, Bacteroides thetaiotaomicron, Bacillus subtilis* variants, *Bifidobacterium adolescentis*, etc. Recent evidence also indicates that most Candida species found in the gut originate in the oral cavity and suggests that improved oral hygiene may decrease the presence of Candida species in the gut microbiome.

- *Candida albicans* in small amounts is considered commensal in the gut and vaginal microbiomes and on other mucous membranes. In the gastrointestinal tract, higher levels of *C. albicans* have been associated with Candida infections of the mouth, gums, esophagus, and intestines. Overgrowth in the vaginal microbiome can result in vulvovaginal infections. Elsewhere in the body *C. albicans* is an opportunistic infection that can travel in the

bloodstream to infect distant tissues in the eye, bone, and brain. In mice, *C. albicans* in the gut can trigger either food antigen sensitization or systemic autoimmune reactions, however, this type of reactivity has yet to be proven in humans.

Geotrichum species

Geotrichum species are members of the Ascomycetes (Ascomycota) phylum of the kingdom Fungi that may be found in dermal, oral, intestinal, urinary, or respiratory microbiomes. Geotrichum species may contaminate milk, fruits and vegetables. One species, *Geotrichum candidum* is used in cheese-making and animal studies show no inflammatory effects upon ingestion. Without live culture confirmation, it is possible that PCR testing may reflect *G. candidum* proteins found in dairy foods, not live Geotrichum. While rare, Geotrichum species may occasionally act as an opportunistic pathogen, particularly in immunocompromised individuals. Oral geotrichosis has been associated with comorbid diabetes mellitus; the clinical appearance is similar to oral Candida infections.

Sacharomyces cerevisiae

Sacharomyces cerevisiae is a member of the Ascomycetes (Ascomycota) phylum of the kingdom Fungi. *S. cervevisiae* typically enters the gut microbiome from the diet (bread, beer, wine, food additives, etc.) or as the nutritional supplement Brewer's yeast. S. cerevisiae in processed foods is likely heat-killed and may not represent live yeast.

Some dietary strains of *S. cerevisiae* can act as opportunistic pathogens in immunocompromised individuals. If *S. cerevisiae* infection is suspected, **4U Health's GI Advanced Profile** can confirm the presence of living yeast via culture and provide pharmaceutical and nutraceutical antimicrobial recommendations.

- Reactivity to *S. cerevisiae* antigens can be assessed using **4U Health allergy and sensitivity panels**
  - **158 Food/Components IgE panel** (as *S. cerevisiae*)
  - **IgG Food Sensitivity panels** (as Baker's/Brewer's yeast)

Rhodotorula species

Rhodotorula species are members of the Basidiomycetes (Basidomycota) phylum of the kingdom Fungi. Rhodotorula species are common in the environment and may be carried on the skin (feet), it may enter the gut microbiome in fresh or seawater, soil, dairy products, sausages, seafoods, fruit or fruit juice. Rhodotorula can exist on the surfaces of foods or in the environment (showers, toothbrush, etc.). The growing colonies will appear pinkish-orange due to carotenoid synthesis by the yeast. Rhodotorula is considered an opportunistic pathogen after antiobiotic use or in immunocompromised patients, and is commonly found in hospital settings. Studies in immunocompetent animals indicate that some Rhodotorula strains may initially stimulate immune responses but then ultimately improve the diversity of beneficial Firmicutes bacteria in the microbiome.

Bacterial Pathogens

Bacterial pathogens are typically acquired from the environment, often via the fecal-oral route. In addition to antibiotic therapy (when indicated), good hand hygiene, rehydration therapy and symptomatic treatment may be used to support the patient and minimize the spread of infection.

- Hand hygiene can be reviewed on the CDC website https://www.cdc.gov/handwashing/when-how-handwashing.html
- While severe dehydration, vomiting or diarrhea may make intravenous fluids or hospital support necessary, oral rehydration is usually sufficient. Rehydrate patients with water, diluted juices, commercial electrolyte drinks, broths, soups, etc. at a rate of 200 mL/kg/24 hours. Dairy products should be avoided because there may be a transient post-infection loss of dairy-digestive enzymes. Caffeine and alcohol may increase intestinal motility and should be avoided.
- Patients may be allowed soft, easily digestible foods as tolerated. Foods such as applesauce, bananas, rice, potatoes, soups, crackers, toast, etc. as allowed in the patient's normal diet, may be provided.

Bacterial Pathogens	Result	Range	Units
Aeromonas hydrophila.	<dl	< 1.00	x10^3 CFU/g
Campylobacter species.	<dl	< 1.00	x10^3 CFU/g
C. difficile, Toxin A	<dl	< 1.00	x10^3 CFU/g
C. difficile, Toxin B	<dl	< 1.00	x10^3 CFU/g
Enterohemorrhagic E. coli	<dl	< 1.00	x10^3 CFU/g
Enteroinvasive E. coli/Shigella	<dl	< 1.00	x10^3 CFU/g
Enterotoxigenic E. coli LT/ST	<dl	< 1.00	x10^3 CFU/g
Shiga-like Toxin E. coli stx1	<dl	< 1.00	x10^3 CFU/g
Shiga-like Toxin E. coli stx2	<dl	< 1.00	x10^3 CFU/g
Salmonella species.	<dl	< 1.00	x10^4 CFU/g
Vibrio species.	<dl	< 1.00	x10^5 CFU/g
Yersinia species.	<dl	< 1.00	x10^5 CFU/g
Helicobacter pylori	<dl	< 1.0	x10^3 CFU/g

Aeromonas hydrophilia

Aeromonas hydrophilia is a member of the Proteobacteria phylum and naturally inhabits aquatic environments, but may also enter the gastrointestinal system via soil, fruits, vegetables or contaminated processed foods. In the gut *A. hydrophilia* is considered the most virulent of the pathogenic Aeromonas species and typically presents in an immunocompetent host with self-limiting acute watery diarrhea. The risk of progression to a chronic or more severe cholera-like diarrheal illness may be higher in immunocompromised individuals. Post-infection reactive arthritis may occur in some individuals. Elsewhere in the body Aeromonas has been associated with a variety of tissue infections and it has also been found in wound infections. Occasionally the liver or biliary tract may be infected by Aeromonas bacteria, such infections are more likely with preexisting liver/biliary tract disease. Aeromonas species may be resistant to many types of antibiotics. Loss of gut microbiome diversity may increase the risk of symptomatic disease; antimicrobial compounds produced by some Bacillus species may help inhibit Aeromonas colonization. Immunocompromised patients or those with severe diarrhea may require antibiotics and intravenous rehydration. Therapeutic interventions include:

- Adult Antibiotic therapy with one of the following (if clinically indicated); consult with pharmacist if pregnant, nursing, pediatric:
  - Cefixime 400 mg PO QD or 200 mg BID x 5 days
  - Ciprofloxacin 500 mg PO BID x 5-7 days
  - Trimethoprim 160 mg/Sulfamethoxazole 800 mg PO BID x 5 days
- Rehydration and probiotics to minimize symptoms in cases of self-limited diarrhea. Immunocompromised patients or those with severe diarrhea may require antibiotics and intravenous rehydration.

Campylobacter species

Campylobacter species are members of the Proteobacteria phylum and can cause symptomatic illness even when present at low levels. In the gastrointestinal tract Campylobacter infections present with acute, watery (often bloody) diarrhea with cramping, fever, nausea, headache, and muscle pain. Campylobacter infections are more common in children (< 4 years old) or in the elderly (> 75 years old), and in immunocompromised individuals. Campylobacter species may be transmitted because of poor sanitation, by contact with farm animals or pets, or due to consumption of unpasteurized dairy products, undercooked poultry or meat. In patients with inflammatory bowel disease, Campylobacter infection may exacerbate gut inflammation and may progress to appendicitis. Post-infection sequelae may include post-infection irritable bowel syndrome, Guillain-Barré syndrome, and reactive arthritis. Elsewhere in the body, Campylobacter species can cause serious local or systemic infections. Antibiotics are not routinely recommended for immunocompetent patients, but may be beneficial for children, immunocompromised patients, or long-lasting infections (> 7 days). Interventions include:

- Antidiarrheal medications are contraindicated due to bacterial toxin production.
- Adult antibiotic therapy (if clinically indicated) with one of the following (consult with pharmacist if patient is pregnant, nursing, pediatric):
  - Erythromycin 500 mg PO BID x 10 days
  - Azithromycin 500 mg PO QD x 3 days
  - Ciprofloxacin and other fluoroquinolone antibiotics may be ineffective due to high levels of Campylobacter resistance worldwide.
- Electrolyte replacement and rehydration.
- Symptomatic treatments for fevers, muscle aches, or headache.

C. difficile Toxin A
C. difficile Toxin B (*Clostridium difficile* [*Clostridioides difficile*])

Clostridium difficile is a member of the Firmicutes phylum and may be found as a commensal member of the gut microbiome because *C. difficile* cannot cause disease without producing toxins, and not all *Clostridium difficile* strains produce toxins. Both toxin A or toxin B can cause symptoms; the toxins are are proinflammatory and cause gastrointestinal tissue destruction.

C. difficile is spread via the fecal-oral route. Asymptomatic carriage can occur with high levels of microbiome diversity (healthy gut) or in young children. *C. difficile* can be difficult to eradicate because it produces resistant spores that can repopulate the gastrointestinal tract after treatment. While toxin production typically begins after antibiotic use, evidence indicates that prior disruption of the gut microbiome, including loss of the protective mucus layer at the gut mucosal surface, and increased numbers of dysbiotic microbes such as fungi, *Escherichia coli* and *Pseudomonas aeruginosa*, may predispose individuals for *C. difficile* infection. Additional risk factors for *C. difficile* infection include increased age, kidney disease, and impaired antibody responses (immunocompromised). Toxins induce symptoms of watery diarrhea with fever and malaise, which may progress pseudomembranous colitis, a life-threatening condition with symptoms of bloody diarrhea, severe abdominal pain, and fever. Approximately 20% of *C. difficile* infections will spontaneously resolve 2-3 days after antibiotic use is discontinued. Interventions include:

- Discontinuation of any antibiotic use; this may be the only treatment necessary for mild symptoms (no fever, no abdominal pain, normal white blood cell count).
- Monitor all patients closely for progression to toxic megacolon (elevated white blood cells, fever, abdominal pain/tenderness/distension, diarrhea, low urine output, increased respiratory rate, fever, low blood pressure, vomiting)
- Anti-diarrheal medications are contraindicated due to bacterial toxin production.
- Adult antibiotic therapy (as clinically indicated) with one of the following (consult with pharmacist if patient is pregnant, breast-feeding, pediatric):
  - Metronidazole 500 mg PO TID for 10-14 days for mild/moderate infection (normal white blood cells, no

megacolon, no fever)
    - Vancomycin 125 mg PO QID for 10-14 days or Fidaxomycin 200 mg PO BID x 10 days for more severe infections
- Co-administration of *Saccharomyces boulardii* and *Lactobacillus rhamnosus* during antibiotic therapy may reduce the risk of infection or relapse.

Enterohemorrhagic *E. coli*

Enterohemorrhagic *Escherichia coli* 0157:H7 (EHEC) is a member of the Proteobacteria phylum. Infection symptoms may vary from asymptomatic to severe and include bloody diarrhea and abdominal pain 3-4 days after exposure; there is no associated fever. EHEC can be acquired directly via the fecal-oral route from infectious people or animals (cattle) and from contaminated water or food (beef, dairy, produce, etc.). If *E. coli* 0157:H7 toxin enters the bloodstream it may progress to hemolytic uremic syndrome (HUS) which occurs primarily in children. HUS progression is most likely when bloody diarrhea occurs; symptoms include altered kidney function, low platelet count and red blood cell destruction. Therapeutic interventions include:

- Anti-diarrheal medications are contraindicated due to the presence of bacterial toxins
- Antibiotic therapy is contraindicated, particularly in children, as it increases the risk of hemolytic uremic syndrome
- Oral rehydration therapy
- Hand hygiene to prevent spreading the infection

Enteroinvasive *E. coli*/Shigella

Enteroinvasive *Escherichia coli* (Shigella) is a member of the Proteobacteria phylum. Shigella infections typically present with profuse, watery or bloody diarrhea with small amounts of stool, fever, abdominal pain, fatigue, and occasionally, vomiting. The infection will last 3-7 days in an immunocompetent host. *E. coli*/Shigella may be transmitted via the fecal/oral route (international travel or food contamination) or through sexual activity; men having sex with men are at higher risk for Shigella infection. *E. coli* bacteria can survive in fresh water and swimming in, or drinking, contaminated water is another way to acquire the infection. If *E.coli*/Shigella toxin enters the bloodstream it may progress to hemolytic uremic syndrome (HUS) which occurs primarily in children. HUS progression is most likely when bloody diarrhea occurs; symptoms include altered kidney function, low platelet count and red blood cell destruction. Therapeutic interventions include:

- Anti-diarrheal medications are contraindicated due to the presence of bacterial toxins.
- Antibiotic therapy with one of the following (consult with pharmacist for pregnant, nursing, pediatric patients) if indicated or if patient is immunocompromised. Antibiotic therapy reduces time of illness by about 48 hours. Antibiotic resistance is increasing in *E. coli*/Shigella
    - Trimethorprim/sulfamethoxazole 160/800 mg PO BID x 3 days
    - Ciprofloxacin 500 mg PO BID x 3 days
    - Levofloxacin 500 mg PO QD x 3 days
- Oral rehydration therapy
- Symptomatic treatment for fever
- Hand hygiene to prevent spreading the infection

Enterotoxigenic *E. coli* LT/ST (ETEC)

Enterotoxigenic *Escherichia coli* LT/ST is a member or the Proteobacteria phylum. *E. coli* LT/ST infections typically present with profuse, watery or bloody diarrhea with abdominal cramping and occasionally fever, nausea, vomiting, chills loss of appetite, headache, muscle aches and bloating. The infection lasts about seven days in an immunocompetent host. Severe cases are more likely in immunocompromised individuals and may be similar to cholera, presenting with "rice-water" stools and dehydration lasting about seven days. *E. coli* LT/ST may be

transmitted via the fecal/oral route (international travel or food contamination) or through sexual activity; men having sex with men are at higher risk for *E.coli* infections. *E. coli* bacteria can survive in fresh water and swimming in, or drinking, contaminated water is another way to acquire the infection. Therapeutic interventions include:

- Anti-diarrheal medications are contraindicated due to the presence of bacterial toxins.
- Antibiotic therapy with one of the following (consult with pharmacist for pregnant, nursing, pediatric patients) if indicated or if patient is immunocompromised. Antibiotic therapy reduces time of illness by about 48 hours. Antibiotic resistance is increasing in *E. coli*/Shigella
  - Azithromycin as 1 gram PO single dose or 500 mg QD x 3 days
  - Ciprofloxacin 500 mg PO BID x 3 days
  - Levofloxacin 500 mg PO QD x 3 days
- Oral rehydration therapy
- Symptomatic treatment for headache, fever, muscle aches, etc.
- Hand hygiene to prevent spreading infection

Shiga-like Toxin *E.coli* stx1
Shiga-like Toxin *E.coli* stx2

Shiga-like toxin *Escherichia coli* stx 1 is a member of the Proteobacteria phylum. The *E. coli* stx1 toxin is very similar to Shigella toxin. A single *E. coli* strain may carry both stx1 and stx2 toxins. The *E. coli* stx1 toxin is more virulent than *E.coli* stx2 or Shigella toxin. Stx1/stx2 symptoms include severe abdominal cramps, bloody diarrhea, vomiting and fever < 101°F/38.5°C. *E.coli* stx1/stx2 infection may be acquired from contaminated salad greens, unpasteurized or raw dairy products, unpasteurized apple cider, undercooked meats or contaminated water. Handling ruminant animals such as cattle, goats, sheep, deer, elk, etc. may also be a contamination vector. Depending upon the *E.coli* strain symptoms may last 1-6 days. If Shiga-like toxin enters the bloodstream it may progress to hemolytic uremic syndrome (HUS) which occurs primarily in children. HUS progression is most likely when bloody diarrhea occurs; symptoms include altered kidney function, low platelet count and red blood cell destruction. Therapeutic interventions include:

- Antibiotics and antidiarrheal medications are contraindicated because they increase the risk of progression to hemolytic uremic syndrome
- Rehydration therapy
- Symptomatic treatment of fever

Salmonella species

Salmonella species are members or the Proteobacteria phylum. Infection is more likely in international travellers, the elderly, and children < 5 years old. Salmonella infections account for about 25% of all infectious diarrhea cases and can present two different ways:

- Typhoidal Salmonella presents with a debilitating high fever and headache, and can progress to rash with liver and spleen enlargement, colon inflammation, septic arthritis, cardiovascular inflammation or pneumonia. There may or may not be gastrointestinal symptoms. Chronic, asymptomatic typhoidal infection may occur in some individuals; such infections, over time, may increase the risk of inflammatory bowel disease (IBD) or certain cancers. Asymptomatic typhoidal Salmonella patients can become carriers and infect others. Typhoidal salmonella is more common in areas with poor sanitation.
- Non-typhoidal Salmonella presents with diarrhea, fever, nausea, vomiting and abdominal cramps. Occasionally, symptoms do not present until weeks after the typical 1-6 day post-exposure window, and occasionally symptoms persist for weeks instead of resolving as expected 3-7 days. Fevers usually resolve within the first 3 days of symptoms. Antibiotics are contraindicated for immunocompetent non-typhoidal Salmonella patients because use may increase asymptomatic Salmonella carriage up to a year.

Salmonella is a common bacterial contaminant on eggs, meat, dairy products, shellfish and produce, processed foods and pet foods may also contain Salmonella. Animal vectors include handling of young poultry (chicks, ducklings, etc.), kittens, hedgehogs, or reptiles. Therapeutic interventions may include:

- Antibiotic therapy as clinically indicated for immunocompromised or relapsing patients with one of the following (consult with pharmacist if patient is pregnant, nursing, pediatric). NOTE: Immunocompromised patients require antibiotic regimen for 14 days.
  - Levofloxacin 500 mg PO QD x 7 days
  - Ciprofloxacin 500 mg PO BID x 7 days
  - Azithromycin 500 mg PO QD x 7 days
  - Trimethorprim/sulfamethoxazole 160/800 mg PO BID x 7 days
- Rehydration therapy
- Good hand hygiene
- *In vitro* (test tube) studies indicate that methanol extracts of neem (*Azadirachta indica*) or garlic (*Allium sativa*) may have some efficacy as antimicrobial agents against Salmonella. Without human studies they may be considered as adjunctive treatments only.

Vibrio species

Vibrio species are members of the Proteobacteria phylum. Several Vibrio species may cause human disease. *Vibrio cholera* causes cholera which may present with either mild diarrheal illness or an acute infection with profuse watery diarrhea and vomiting that can lead to circulatory collapse and shock. Severe cholera can be fatal. Cholera is spread through contaminated food or water. International travel and consumption of raw or undercooked seafood or other contaminated foods or water spread the infection, which is rarely encountered in the United States. Worse outcomes for Vibrio cholera infection are predicted by comorbid chronic medical conditions, lack of stomach acid, or blood type O.

Vibriosis may be caused by over 20 different Vibrio species; these Vibrio species are the most likely cause of infection in the United States. All Vibrio species live in brackish water and can infest seafood. Vibriosis may present as a gastrointestinal infection or a localized wound infection:

- Eating raw or undercooked seafood, such as oysters, can result in infection. Vibriosis symptoms include watery diarrhea, typically with abdominal cramping, nausea, vomiting and fever. Antibiotic therapy in immunocompetent patients is not recommended for gastrointestinal vibriosis.
- Wounds exposed to seawater may also develop Vibrio species infections; prompt antibiotic therapy is indicated for such cases. If Vibrio species escape into the bloodstream symptoms of fever, chills, blistering skin lesions, and hypotension. The risk of blood-borne Vibrio infection is greater in those with comorbid liver disease or other chronic medical conditions. Unless the Vibrio bacteria co-infected the gastrointestinal system, extraintestinal Vibrio infections cannot be detected by this gastrointestinal test.

Therapeutic interventions for cholera or gastrointestinal vibriosis (if clinically indicated for an immunocompromised patient) may include:

- Antibiotic therapy with one of the following (consult with pharmacist if patient is pregnant, nursing, pediatric):
  - Doxycycline 100 mg PO BID x 3-5 days
  - Azithromycin as either 1 gram PO single dose or 500 mg PO QD x 3 days
- Rehydration therapy
- Good hand hygiene
- *In vitro* test tube studies indicate that fresh lime juice (*Citrus aurantofolia*) or an ethanol extract of wood apple (*Limonia acidissima*) may have antimicrobial activity against *Vibrio cholera*. Without human studies they may be considered as adjunctive treatments only.

Yersinia species

Yersinia species are members of the Proteobacteria phylum. When Yersinia is identified in the stool sample, it is cultured out and the species identified is mentioned in the results report for that patient. Yersinia species are associated with a variety of illnesses; two species *Y. enterocolitica* and *Y. pseudotuberculosis* can cause gastrointestinal yersinosis. Once in the body gastrointestinal Yersina species invade gut mucosal cells and colonize the gut-associated lymphoid tissues; from there Yersinia may spread to other organs in the body. In the gut Yersinosis may present two different ways:

- Acute Yersinosis presents with diarrhea (may be bloody in children), abdominal pain, nausea vomiting and fever. Symptoms may last 12-22 days and pain may localize to the right lower quadrant of the abdomen. If Yersina progresses to a blood-borne infection mortality may approach 50% in immunocompromised, iron-overloaded, or pediatric patients. After gastrointestinal symptoms resolve, the bacteria may shed in stool for over a month.
- Yersinia pseudoappendicitis presents with right lower quadrant pain, fever, vomiting, and diarrhea with an increased white blood cell count.

Extra-intestinal Yersinia species infections may present as sore throat, inflammation of the heart muscle, dermatitis, erythema nodosum, or swelling of abdominal lymph glands, such infections will not be detected by this test unless there is gastrointestinal Yersinia co-infection. Immune responses to Yersinia antigens may cause reactive arthritis. Individuals positive for HLA-B27 may be at increased risk for arthritis; symptoms typically begin 1-2 weeks after GI symptoms and can last for several months. Pigs and raw or undercooked pork products may be the most common source of Yersinia infection, however, since gastrointestinal Yersinia species can survive outside the host and are transmitted via the fecal-oral , other food or water contamination may carry Yersinia species as well. Yersinia may also be transmitted during blood transfusion or occasionally via contact with household pets. Excess dietary iron may promote Yersinia species growth. Antibiotic therapy is not recommended for immunocompetent patients, but may be considered for immunocompromised patients or for severe or prolonged symptoms. Therapeutic interventions for gastrointestinal Yersinia species may include:

- Antibiotic therapy with one of the following (consult with pharmacist if patient is pregnant, nursing, pediatric):
  - Trimethoprim-sulfamethoxazole 160/800 mg PO BID x 5 days
  - Ciprofloxacin 500 mg PO BID x 5 days
- Rehydration therapy

Helicobacter pylori and *H. pylori* Virulence Factors

Helicobacter pylori is a member of the Campylobacterota phylum. It is an acid-tolerant bacteria found in the stomach in about 50% of people around the world. *H. pylori* becomes pathogenic in approximately 15% of individuals and is commonly associated with gastric ulcers. Once ulcers are present *H. pylori* must be eradicated to prevent further damage to gastric tissues. Pre-disposing factors include comorbid disease, psychological stress, or other lifestyle choices that alter the acidity in the stomach. Various mutations in the *H. pylori* bacteria's genetics can increase the risk of severe disease or increase the risk of gastric cancer.

H.pylori Virulence Factor, babA	Not Detected	H.pylori Virulence Factor, cagA	Not Detected
H.pylori Virulence Factor, dupA	Not Detected	H.pylori Virulence Factor, iceA	Not Detected
H.pylori Virulence Factor, oipA	Not Detected	H.pylori Virulence Factor, vacA	Not Detected
H.pylori Virulence Factor, virB	Not Detected	H.pylori Virulence Factor, virD	Not Detected

The distribution of these genetic *H. pylori* "virulence factors" varies widely around the world in different human populations. While individual human studies associate increased disease or cancer risk with each of the virulence factors, there is equal impact on disease risk from host genetics and environmental factors. It is reasonable, based

upon current evidence, to assume that the greater the number of virulence factors present, the greater the increased risk of disease or cancer might be.

If virulence factors are present, or the treatment of *H. pylori* is otherwise clinically indicated by the presence of gastric ulcers, therapeutic interventions may include:

- Antibiotic and proton pump inhibitor (PPI) combined therapy with either
  - Triple regimen (FDA approved)
    - PPI PO at standard or double dose BID x 14 days
    - Clarythromycin 500 mg PO BID x 14 days
    - Amoxicillin 1 gram or Metronidazole 500 mg PO TID x 14 days
  - Bismuth quadruple regimen x 10-14 days
    - PPI PO standard dose BID
    - Bismuth subsalicylate PO 300 mg QID
    - Tetracycline 500 mg PO QID
    - Metronidazole 250 mg QID

There are also some natural agents that may be used to support the restoration of normal gastric mucosal function and/or the eradication of pathogenic *H. pylori*. These agents may work best as adjunctive agents used with conventional therapies. Natural therapeutic interventions have been shown superior to medications such as omeprazole or cimetidine and may include:

- Repair of gastric mucosal damage (ulcers, NSAID use, stress, etc.)
  - Avoid known irritants such as NSAIDs (aspirin, indomethacin, ibuprofen, naproxen), psychological stress, etc.
    - Food allergy or sensitivity may increase mucosal inflammation throughout the GI tract. Evaluate IgE allergy with **4U Health IgE Food Panels**. Evaluate IgG food sensitivity with **4U Health's Food Sensitivity Panels**.
  - Ingested mycotoxins can disrupt mucosal barrier functions. Mold contamination is common in processed foods, grains, and dried foods.
    - If mold exposure is suspected, consider **4U Health's Mycotoxin Profile**.
  - Consider herbal demulcents and anti-inflammatories
    - *Camellia sinensis* – tea, green or black, can be effective in healing gastric lesions. Animal studies indicate that black tea theaflavins may be particularly beneficial in repairing the effects of NSAID use.
    - Curcumin has antioxidant and anti-inflammatory effects that may help repair the gut mucosal epithelium.
    - Flavonoids
      - Quercetin and other flavonoids have anti-inflammatory and antioxidant properties.
      - Catechin, epicatechin, and rutin may decrease the risk of gastric cancers.
    - *Glycyrrhiza glabra* or deglycyrrhizinated licorice (DGL) 20-30 minutes before meals.
    - *Vaccinium myrtillus* (European blueberry) contains anthocyanoside comounds with anti-ulcer activity.
- Vitamins may help regulate the immune system and restore the gut mucosa
  - Carotenoids (vitamin A precursors) have potent antioxidant effects.
  - Vitamin C supplementation supports normal gastric digestion. *H. pylori* infections can inhibit the secretion of vitamin C into the stomach lumen.
  - *H. pylori* infections impair normal B-12 assimilation from the gut. Evaluate B-12 levels and supplement as indicated.
  - Vitamin D may suppress inflammatory cytokines induced by pathogenic *H. pylori*.

Viral Pathogens

Viral pathogens are often responsible for acute infections gastroenteritis. In developed countries acute viral diarrhea is usually self-limited, but the risk of adverse outcomes increase if there is preexisting malnutrition, immunosuppression or other comorbid illness. Good hand hygiene, rehydration therapy and symptomatic treatment may be used to support the patient and minimize the spread of infection.

- Hand hygiene can be reviewed on the CDC website https://www.cdc.gov/handwashing/when-how-handwashing.html
- While severe dehydration, vomiting or diarrhea may make intravenous fluids or hospital support necessary, oral rehydration is usually sufficient. Rehydrate patients with water, diluted juices, commercial electrolyte drinks, broths, soups, etc. at a rate of 200 mL/kg/24 hours. Dairy products should be avoided because there may be a transient post-infection loss of dairy-digestive enzymes. Caffeine and alcohol may increase intestinal motility and should be avoided.
- Patients may be allowed soft, easily digestible foods as tolerated. Foods such as applesauce, bananas, rice, potatoes, soups, crackers, toast, etc. as allowed in the patient's normal diet, may be provided.
- Zinc may reduce severity of illness in zinc-deficient individuals. The World Health Organization recommends zinc 10 mg QD for infants < 6 months old or 20 mg QD for children > 6 months for the duration of symptoms.
- *Lactobacillus casei* GG and *Saccharomyces boulardii* may provide some relief with symptoms of watery (but not bloody) diarrhea.

Viral Pathogens	Result	Range	Units
Adenovirus 40/41	Not Detected		
Norovirus GI/II	Not Detected		
Rotavirus A	Not Detected		
Sapovirus (I,II,IV,V)	Not Detected		
Astrovirus (hAstro)	Not Detected		

Adenovirus 40/41

Adenovirus is a common cause of infant gastroenteritis. Adenovirus typically presents with fever and vomiting which progresses to diarrhea and abdominal pain lasting 5-8 days. The illness may occasionally include respiratory symptoms. Viral transmission can occur through the fecal-oral route or as aerosolized droplets from respiratory infection. Asymptomatic gut carriage is also possible, the virus shed in stool can infect others. Antibiotics are contraindicated for viral infections. Therapeutic interventions may include:

- Rehydration therapy
- Symptomatic treatment for fever, etc.
- Zinc as clinically indicated for the duration of symptoms
- Prevent spread using 1:5 bleach dilution or other viricides on contaminated objects

Norovirus G I/II

Norovirus G typically presents with an acute onset of vomiting accompanied by watery (non-bloody) diarrhea and abdominal cramping. The illness usually lasts 12-48 hours and may occasionally include fever, headache, muscle aches, and fatigue. Viral transmission occurs via the fecal-oral route and may be passed on contaminated objects, contaminated food or water, and in aerosolized vomit. Norovirus may also be found in waters used for recreation (swimming, etc.). The virus is usually shed prior to the onset of symptoms. Antibiotics are contraindicated for viral infections. Therapeutic interventions may include:

- Rehydration therapy
- Symptomatic treatment for fever, etc.
- Zinc as clinically indicated for the duration of symptoms

- Good hand hygiene
- Prevent spread using 1:5 bleach dilution or other viricides on contaminated objects
- *Lactobacillus casei* GG and *Saccharomyces boulardii* may provide some symptomatic relief

Rotavirus A

Rotavirus A typically presents with non-bloody watery diarrhea, vomiting, abdominal cramping, loss of appetite, and low-grade fever. Symptoms usually last about 2 days and may be severe for infants or young children. The virus is usually transmitted via the fecal-oral route or on contaminated objects, food or water. The virus may shed prior to symptom onset, and may continue to shed after the symptoms have resolved. Antibiotics are contraindicated for viral infections. Therapeutic interventions may include:

- Rehydration therapy
- Anti-emetic medications may be used in children > 6 months old as clinically indicated
- Symptomatic treatment for fever, etc.
- Zinc as clinically indicated for the duration of symptoms
- Good hand hygiene
- Prevent spread using 1:5 bleach dilution or other viricides on contaminated objects
- *Lactobacillus casei* GG and *Saccharomyces boulardii* may provide some symptomatic relief

Sapovirus (I, II, IV, V)

Sapoviruses typically presents with diarrhea and vomiting 1-4 days after exposure. Symptoms may also include nausea, abdominal cramping, chills, headaches, muscle aches or fatigue. Symptoms are usually self-limiting in immunocompetent individuals and resolve within seven days. Infants and immunocompromised individuals may experience longer symptom duration or more severe illness. The virus may be shed in stool for 1-4 weeks after the illness begins. Sapoviruses may be transmitted in contaminated food or water, or on contaminated surfaces, via the fecal-oral route. Sapoviruses have also been found in animals such as pigs and dogs, although transmission from animal vectors has not been confirmed. Antibiotics are contraindicated for viral infections. Therapeutic interventions may include:

- Rehydration therapy
- Symptomatic treatment for fever, etc.
- Zinc as clinically indicated for the duration of symptoms
- Good hand hygiene
- Prevent spread using 1:5 bleach dilution or other viricides on contaminated objects
- *Lactobacillus casei* GG and *Saccharomyces boulardii* may provide some symptomatic relief

Astrovirus (hAstro)

Astroviruses can infect humans, mammals (including domestic animals), and birds. Increasing evidence suggests that some Astroviruses may jump between species; this test result is for human-associated Astrovirus strains. Astroviruses typically present with mild, watery diarrhea that may be associated with vomiting, loss of appetite, abdominal pain, and fever. The virus is very common in children and is transmitted via the fecal-oral route directly or through contaminated food or water. Astrovirus may shed for about two weeks after symptoms resolve. The Astrovirus capsid (shell) can act as an enterotoxin that disrupts the gut mucosal barrier. In older or immunocompromised individuals Astrovirus infection may be prolonged and lead to wasting symptoms due to its effects on the gut mucosal barrier. In premature infants the virus has been associated with necrotizing colitis. If an Astrovirus infection enters the bloodstream encephalitis or meningitis may occur. Antibiotics are contraindicated for viral infections. Therapeutic interventions may include:

- Rehydration therapy

- Symptomatic treatment for fever, etc.
- Good hand hygiene
- Prevent spread using 1:5 bleach dilution or other viricides on contaminated objects
- *Lactobacillus casei* GG and *Saccharomyces boulardii* may provide some symptomatic relief

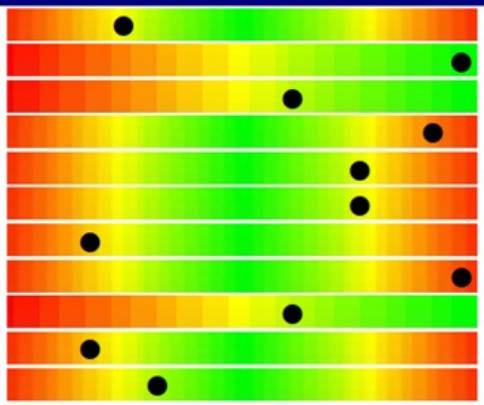
Normal Bacterial GUT Flora

The human gastrointestinal microbiome is a vital component of human health. Many types of bacteria have co-evolved with (and within) humans and are now considered essential partners in the health of their host. Clinicians requiring a "refresher" on the gastrointestinal microbiome are referred to intechopen.com "Gut Microbiome: A New Organ System in Body" https://www.intechopen.com/chapters/69898 .

As discussed in the "The Ecology of the Gastrointestinal Microbiome" section, various groups of bacteria with shared food preferences and shared metabolic functions work together in "guilds" that may ferment certain fibers, synthesize essential B vitamins that nourish the host, detoxify environmental toxins, sequester toxic metals in their cell walls, or produce short-chain fatty acids or other metabolites essential for cross-talk with the host immune system. Members of these guilds may be considered "keystone species" essential for the health of the microbial ecosystem, and by extension, the health of the host. Many beneficial bacteria are oxygen-intolerant (obligate anaerobes), unlike dysbiotic species that may be more oxygen-tolerant (facultative aerobes). Increased oxygen in the gut lumen typically occurs when the gut mucosal cells and underlying tissues experience oxidative (ROS) or nitrosative stress.

A highly diverse, well-nourished gut microbiome supports tolerant anti-inflammatory immune system signaling that benefits the host and minimizes the risk of developing allergies or food sensitivities. Poor diet, unhealthy lifestyle, antibiotic use, toxic exposures and psychological or physical stress can all disrupt the populations in the gut microbiome; certain groups of bacteria may die off, only to be replaced by dysbiotic or commensal species with different functions. Often the dysbiotic replacement bacteria or yeast produce different metabolites that are pro-inflammatory and adversely affect host health and gut barrier functions. Multiple human studies have identified dysbiosis or loss of microbiome bacterial diversity as contributing factors in gastrointestinal and systemic inflammatory disorders. So, in addition to identifying dysbiotic and pathogenic species, it is essential that beneficial members of the microbiome be assessed as well.

Normal Bacterial GUT Flora	Result	Range	Units
Bacteroides fragilis	35.0	1.6 - 250.0	x10^9 CFU/g
Bifidobacterium species	254.1	> 6.7	x10^7 CFU/g
Bifidobacterium longum	6.1	> 5.2	x10^6 CFU/g
Enterococcus species	2144*H	1.9 - 2000.0	x10^5 CFU/g
Escherichia species	3705.0	3.7 - 3800.0	x10^6 CFU/g
Lactobacillus species	6102.0	8.6 - 6200.0	x10^5 CFU/g
Lactobacillus Rhamnosus	25.0	8.3 - 885.0	x10^4 CFU/g
Clostridium species	*99.0* *H	5.0 - 50.0	x10^6 CFU/g
Oxalobacter formigenes	17.00	> 15.00	x10^7 CFU/g
Akkermansia muciniphila	*<dl* *L	1.00 - 50.00	x10^3 CFU/g
Faecalibacterium prausnitzii	1084.9	200.0 - 3500.0	x10^3 CFU/g



Bacteroides fragilis

Bacteroides fragilis is a member of the Bacteroidetes phylum. It is an obligate anaerobe that is found in both the gut lumen and the mucin layer coating the gut mucosal lining. *B. fragilis* supports host health by synthesizing vitamin K. *B. fragilis* is one of several bacteria that can modulate inflammatory signals from *E. coli* bacteria and promote the production of T-regulatory cells and anti-inflammatory interleukin-10 (IL-10) signals that promote immunotolerance. While most strains of *B. fragilis* are considered beneficial, several toxigenic strains can secrete enterotoxins that disrupt the gut mucosa and intestinal barrier, especially if the gut mucus layer is lost. These toxigenic strains have been associated with inflammatory bowel disease (IBD) and colorectal cancer. Animal studies indicate that high-fat, low-fiber diets may support the growth of toxigenic *B. fragilis* strains.

- *Increased abundance:* Higher levels of *B. fragilis* have been associated with childhood, pediatric functional constipation, pediatric Celiac disease, active Crohn's disease and Crohn's-related intestinal strictures.

Omnivorous diets, animal proteins (meat) and higher-protein ketogenic diets promote *B. fragilis* growth. Antibiotic use may contribute to overgrowth; *B. fragilis* is resistant to several commonly used antibiotics such as penicillin, clindamycin, cefoxitin, and moxifloxacin. Animal and *in vitro* (test-tube) studies indicate that *B. fragilis, E. coli* and *B. longum* levels increased when they were co-incubated with an infective strain of Blastocystis.

- *Decreased abundance: Bacteroides fragilis* levels decrease in subjects on vegan/vegetarian diets, low-fat whole-grain diets, and in breast-fed infants. High dietary increase of whey proteins and pea proteins decreased Bacteroides species in human studies.

Bifidobacterium species

Bifidobacterium species, or Bifidobacteria, are members of the Actinobacteria phylum. The bacteria are obligate anaerobes and are important for host health because they help maintain the gut mucosal barrier, produce immunotolerant short chain fatty acids, aid enterohepatic bile acid recycling, and inhibit the growth of detrimental microbiome members such as *Clostridium perfringens*. Some strains of Bifidobacteria can break down arabinose, ribose, lactose, etc., resistant starches and indigestible plant fibers. *B. breve* supplementation has significantly improved colonoscopy scores for ulcerative colitis patients; it has been shown to inhibit Rotavirus replication and decrease the risk of necrotizing colitis in premature infants.

- *Increased abundance:* Beneficial Bifidobacteria increases can occur for adults ingesting lactulose or infants on milk diets (breast, cow, goat vs soy) had higher levels of Bifidobacterium species. A study in elderly patients showed increased levels Bifidobacteria, and decreasing levels of inflammatory factors, with probiotic Bifidobacterium supplementation.
- *Decreased abundance:* Some members of the genus are diminished by the use of antibiotics and it may take a year after the antibiotics are finished for Bifidobacteria populations to recover. Bifidobacteria may also decline when gastrointestinal disorders, obesity or metabolic diseases (such as type II diabetes) are present, or with increasing age. Bifidobacterium species levels may also decline on vegan or low FODMAPs diets.

Bifidobacterium longum

Bifidobacterium longum is a member of the Actinobacteria phylum. *B. longum* is acid-tolerant and able to survive passage through the stomach into the intestinal tract and may be more resistant to antibiotics than other species of Bifidobacterium. Some strains of *B. longum* may protect the gut mucosal cells from carcinogen-induced DNA damage (animal study). Like other beneficial Bifidobacteria, the presence of *B. longum* promotes anti-inflammatory, tolerant immune responses and helps with bile acid recirculation. When administered with prebiotics (inulin, oligofructose), *B. longum* has been shown to improve symptoms in Crohn's disease patients. Supplemented strains of *B. longum* and *B. breve* have been shown to decrease the risk of atopic disease in infants. The presence of *B. longum* in the gut microbiome has been shown to improve the growth and survival of other Bifidobacterium species.

- *Increased abundance:* Breast-fed infants tend to have higher levels of *B. longum*, because the bacterium can break down arabinose, gut mucus, glycans, and other polysaccharides. Probiotic supplementation and diets high in plant fiber may increase *B. longum* levels.
- *Decreased abundance: B. longum* levels may decrease in patients with obesity, insulin dysregulation, lipid dysregulation, and inflammation. These health-related decreases may be moderated by increased dietary plant fiber. While present in up to 90% of gut microbiomes, *B. longum* levels tend to decline with increasing age. *B. longum* and other Bifidobacterium species may also decline with antibiotic use or when gastrointestinal disorders, obesity or metabolic diseases (such as type II diabetes) are present. Bifidobacteria levels may also decline on vegan or low FODMAPs diets.

Enterococcus species

Enterococcus species (Enterococci) are members of the Bacillus class in the Firmicutes phylum, and the species commonly found in human guts include *E. faecalis* and *E. faecium*. *E. faecalis* is often the first colonizing bacteria

in infant guts. In infants *E. faecalis* has a protective role and is important in intestinal immune system development. Enterococci contribute to gut homeostasis where they improve nutrient extraction from food, aid the development of mucosal immunity, and secrete antimicrobial compounds that inhibit pathogens and dysbiotic bacteria. Dietary preferences can affect Enterococcus species abundance, levels tend to be higher on typical Western diets and lower in vegetarians. Enterococcus species are found in insect and mammal guts, and strains may be shared between humans, dogs and pigs. If they escape into the circulation Enterococcus species may cause meningitis, heart inflammation, or blood infections, which may be fatal. There are genetic differences between the strains causing blood-borne infections and the bacteria typically found in the gut microbiome, which tend to be more antibiotic-sensitive and less virulent. Over time, as gut function and microbiome diversity is restored, the gut-specific Enterococcus species can outcompete the more virulent antibiotic-resistant strains acquired from medical facilities.

- *Increased abundance:* Enterococcus species are resistant to many commonly used antibiotics including ampicillin, penicillin, imipenem, and vancomycin, and levels may be increased when other beneficial bacteria are eradicated by antibiotic use. Enterococcus species have been shown to increase in patients with inflammatory bowel disease (IBD), rheumatic arthritis (RA), and spondyloarthritis. Increased levels of *E. faecalis* have been associated with colorectal cancer, however it is not clear if *E. faecalis* contributes to cancer risk or is increased due to patient's dietary preferences or antibiotic use. Western diets may increase Enterococcus species. Some Enterococci may contaminate poultry and dairy products while others are used in the fermentation or preservation of meats and cheeses.
- *Decreased abundance:* Vegans/vegetarians may have lower levels of Enterococcus species.

Escherichia species

Escherichia species are members of the Proteobacteria; most can contribute to the health of the host and the gut microbiome by decreasing oxygen levels in the gut lumen, synthesis of vitamin K, and pathogen resistance. Escherichia species are early colonizers of the infant gut and are resistant to both stomach acid and bile. Colonization from the environment is ubiquitous and ongoing. Antibiotic use promotes rapid development of antibiotic resistance in Escherichia species and diet plays a role in Escherichia colonization. *E. coli* is one of the few Escherichia that can break down lactose. Different Escherichia species can ferment or use different types of simple sugars (glucose, arabinose, mannose, galactose, amino-sugars, etc.) available from the diet, bacterial carbohydrate metabolism, or in the mucus layer lining the gut mucosal barrier. A low-carbohydrate diet may be considered as a therapeutic intervention.

- *Increased abundance:* Levels of Escherichia have been shown to increase when the gut microbiome is disrupted. In human studies Escherichia species were more abundant in subjects with chronic kidney disease, atherosclerosis, non-alcoholic steatohepatitis (NASH), rheumatoid arthritis (RA) and Crohn's disease. Bottle-fed infants may have higher levels of Escherichia species. Gastroenteritis patients have been shown to have higher levels of *E. coli*. Some *E. coli* strains secrete histamine or colibactin, a pro-carcinogenic compound; the colibactin strains are highly associated with inflammatory bowel disease (IBD) and colorectal cancer.
- *Decreased abundance:* Antibiotic use, very low carbohydrate diets or an anaerobic (oxygen-free) gut lumen may decrease Escherichia species levels. Probiotic use may decrease levels of Escherichia species and antimicrobial compounds produced by Bacillus species may inhibit Escherichia growth. Escherichia levels may be lower in patients with major depressive disorder.

Lactobacillus species

(NOTE: taxonomic split into multiple genera as of March 2020: Lactobacillus, Lacticaseibacillus, Ligilactobacillus, Limosilactobacillus, Levilactobacillus, Lentilactobacillus)

Lactobacillus species are facultative anaerobes in the Firmicutes phylum that may be found in the oral and gastrointestinal microbiomes. Lactobacillus has been found in human breast milk and year-old infant microbiomes.

Lactobacillus population levels have been inversely associated with intestinal permeability. Lactobacillus species are commonly found in probiotic blends and are used in the fermentation of foods such as yogurt, cheese, kefir, sauerkraut, etc. species such as *L. acidophilus, L. plantarum,* and *L. paracasei* can metabolize complex prebiotic carbohydrates. Inulin is the preferred prebiotic for such Lactobacillus species. *L. rueteri* strains have been shown to break down tryptophan into metabolites that promote immunotolerance via the aryl hydrocarbon receptor (AhR); this may help protect the host against inflammatory AhR stimulation by toxic chemicals. *L. reuteri* may also promote gut mucosal barrier functions and prevent Candida species adhesion to the gut mucosa. Many Lactobacillus species can break down oxalate in stool. *In vitro* (test tube) studies demonstrate that many Lactobacillus species exert a protective effect against colorectal cancer growth via antioxidant and antitumor exopolysaccharides secreted into bacterial biofilm. Several Lactobacillus species, including *L.casei,* can secrete histamine; *L. casei* persistence in the microbiome may require the use of dairy products. Some Lactobacillus species can break down gluten, while others may sequester toxic metals in their cell walls. *L. acidophilus* has been shown to inhibit the growth of *E.coli* and other dysbiotic or pathogenic bacteria.

- *Increased abundance*: *L. acidophilus* levels may be higher in bottle-fed infants, in the presence of fermentable carbohydrates such as glucose, or when gastrointestinal disease (including IBD), autism spectrum disorder, spondyloarthritis, or high blood pressure is present. It is also commonly found in probiotic blends.
- *Decreased abundance*: Opioid or alcohol use may decrease Lactobacillus levels and levels may be lower in patients with cirrhosis, type I diabetes, multiple sclerosis, HIV, irritable bowel syndrome (IBS) or colorectal cancer. Heavy salt use may deplete Lactobacillus levels in the gut.

Lactobacillus rhamnosus (2020 taxonomy: *Lacticaseibacillus rhamnosus*)

Lactobacillus rhamnosus is a facultative anaerobe in the Firmicutes phylum that may be found in the oral and gastrointestinal microbiomes. As a member of the *Lactobacillus casei* group, *L. rhamnosus* is also commonly found in human breast milk, dairy ferments, and probiotic blends. During gut inflammation *L. rhamnosus* has been shown to improve Treg mitochondrial and immunosuppressive functions (animal study). Limited human studies show that *L. rhamnosus* may have some beneficial effects on weight control in women and children. *In vitro* (test tube) studies demonstrate that *L. rhamnosus* has a protective effect against colorectal cancer growth via antioxidant and antitumor exopolysaccharides secreted into its bacterial biofilm.

- *Increased abundance: L. rhamnosus GG* is one of the most common probiotics in use; probiotic use has been shown to increase levels in the gut microbiome.
- *Decreased abundance*: Opioid or alcohol use may decrease *L. rhamnosus* and other Lactobacillus levels and may be lower in patients with cirrhosis, type I diabetes, multiple sclerosis, HIV, irritable bowel syndrome (IBS) or colorectal cancer. Heavy salt use or low-FODMAPs diet may deplete Lactobacillus levels in the gut. Some strains may require dairy products for growth.

Clostridium species

Clostridium species (Clostridia) are members of the Firmicutes phylum and most are essential members of a diverse, stable, gastrointestinal microbiome. Clostridia colonize the human gut in the first month of life and maintain gut homeostasis through direct interaction with gut mucosal cells, and the production of essential host nutrients such as vitamins B12 and K. Clostridia stimulate receptors on colon cells that initiate tight mucosal cell junctions and proper gut barrier function. Clostridia promote gut health and host immunotolerance through the production of butyrate, a key "cross-talk" molecule and nutrition source for colon mucosal cells. Butyrate has been shown to have anti-cancer and anti-inflammatory effects. Clostridium species may also help regulate the level of free neurotransmitters in the gut lumen that regulate motility and water resorption in the colon.

Of approximately 100 species, only a few Clostridia: *C. difficile, C. tetani,* and *C. perfrigens,* are pathogenic to humans. Both *C. difficile* and *C. perfingens* can cause gastrointestinal disease. Only toxin-producing strains of *C. difficle* can cause disease; *C. difficle* toxins A and B are tested for in the Bacterial Pathogens section of the patient's

results report. *C. perfringens* is a common cause of food poisoning from improperly stored gravy, poultry or meats – symptoms typically begin 8-16 hours after consumption and patients present with diffuse abdominal pain, watery non-bloody diarrhea, and vomiting. *C. perfringens* gastroenteritis is self-limiting in most individuals, and antibiotics are not indicated. Human studies indicate that fruit/vegetable intakes may inhibit the growth of some pathogenic Clostridium species.

- *Increased abundance*: Increased levels of some Clostridium species have been associated with bloating, colon inflammation (animal study), type I diabetes, and some types of autism spectrum disorder (ASD) which is associated with decreased levels of Bifidobacteria. Antibiotic use may reduce the abundance of other gut microbiome bacteria, resulting in a relative increase in antibiotic-resistant Clostridia species, Clostridia and other resistant groups of bacteria.
- *Decreased abundance:* Decreased levels of Clostridia may be seen with recent vancomycin use, in type II diabetics, in untreated HIV patients, and when oxygen levels in the gut lumen are increased (as in infants and children < 2 years old). Lower levels of some Clostridum species have also been associated with inflammatory bowel disease; a Crohn's disease study also noted associated declines in *Faecalibacterium prausnitzii* and Bifidobacterium species abundance.

Oxalobacter formigenes

Oxalobacter formigenes is a member of the Proteobacteria phylum that metabolizes oxalic acid (oxalate) obtained from dietary plants and host metabolism. Significant dietary sources include coffee, chocolate, rhubarb, spinach, beets, legumes and nuts; lesser amounts are found in other fruits and vegetables. High levels of oxalic acid can increase the risk kidney stone formation and there is clear evidence that gut microbiome colonization by *O. formigenes* decreases that risk. Not all human gut microbiomes host Oxalobacter species, their presence is typically associated with high levels of microbiome species diversity. Antiobiotic use, high fat diets, or high levels of calcium supplementation may have a negative impact on Oxalobacter colonization. Individuals at risk for calcium oxalate kidney stone formation may benefit from diets containing moderate oxalate (250 mg) and lower calcium (400 mg) levels.

Akkermansia muciniphila

Akkermansia muciniphila is a member of the Verrucomicrobia phylum. Most Verrucomicrobia are found in water, where they degrade aquatic plant polysaccharides. While it may be found in breast-feeding infants, *A. muciniphila* typically enters the gut microbiome when breast-feeding ends and solid foods are the primary diet for infants. In the gut *A. muciniphila* breaks down mucins produced by the gut mucosal cells; the metabolized mucin glycans are used by Firmicutes phylum Roseburia species and other beneficial members of the gut microbiome. *A. muciniphila* is found in the human gut microbiome and is associated with healthy diverse microbiomes; these microbiomes decrease hydrogen sulfide gas in the gut lumen, support gut barrier functions and immunotolerance. Diverse microbiomes are also associated with decreased risk of metabolic disorders and obesity. Supplementation with *A. muciniphila* improved insulin sensitivity and reduced plasma total cholesterol in patients with metabolic syndrome. The provision of fucoidan polysaccharides from brown seaweeds may improve Akkermansia levels.

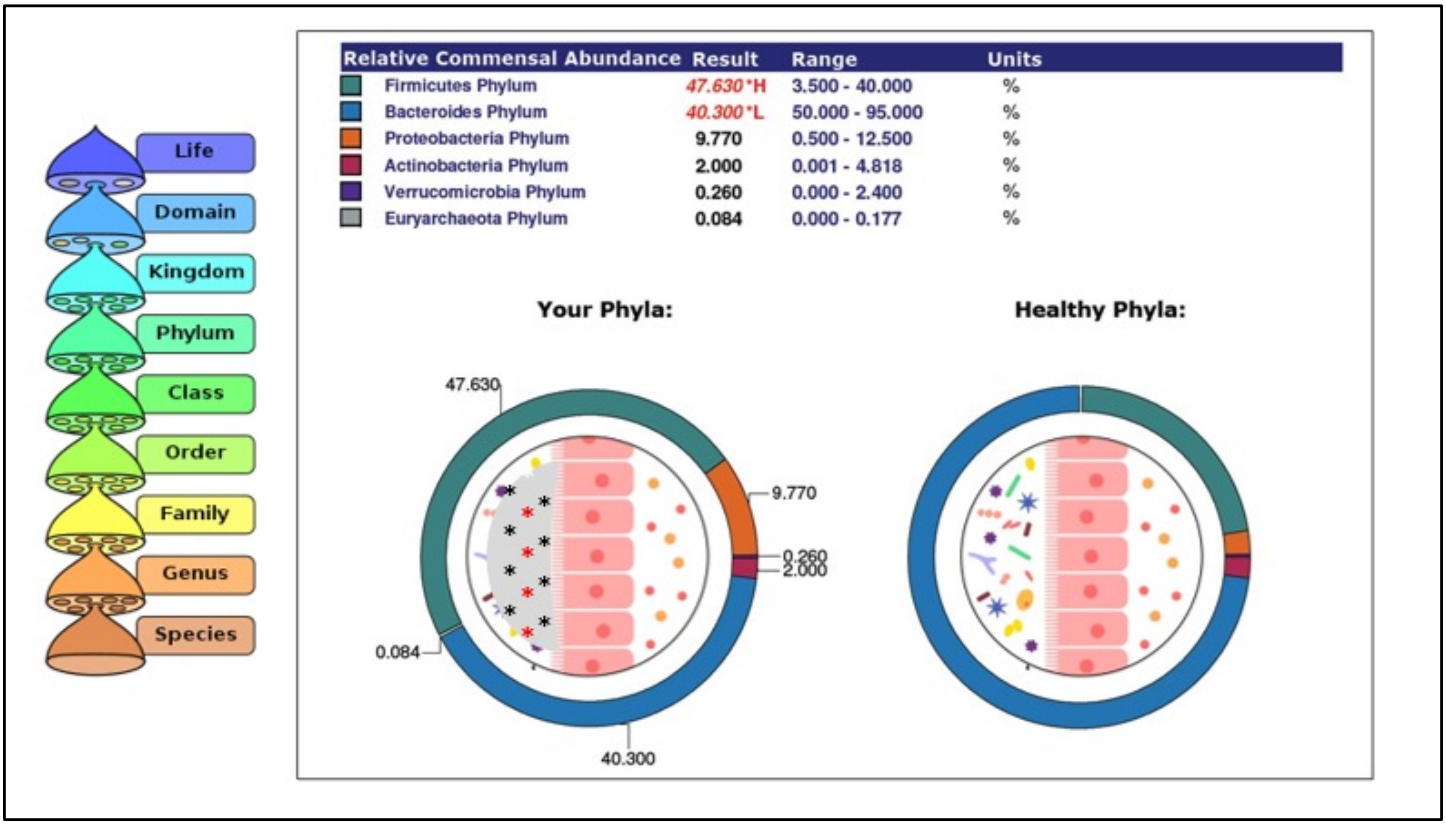
- *Increased abundance: A. muciniphila* levels may be higher in patients with relapsing/remitting multiple sclerosis, Parkinson's disease, REM sleep disorders, colorectal cancer, or in those with higher levels of dietary saturated fats. Metformin use in type II diabetics may encourage *A. muciniphilia* growth (animal studies).
- *Decreased abundance: A. muciniphila* levels may be lower in patients with psoriasis, early type II diabetes or metabolic syndrome, obesity, inflammatory bowel disease, or chronic inflammation. Alcohol use may decrease abundance.

Faecalibacterium prausnitzii

Faecalibacterium prausnitzii is a butyrate-producing member of the Firmicutes phylum. *F. prausnitzii* metabolism is vitamin B2-dependent and the bacteria prefers a gut pH between 5.7-6.7. The bacteria has been shown to increase the expression of anti-inflammatory interleukin-10 (IL-10) in the GI tract. *F. prausnitzii* requires the SCFA acetate for butyrate production and may need to interact with Bifidobateria species for optimal butyrate production. Short chain fatty acids and fecal occult blood are measured on 4U Health's **GI Advanced Profiles**.
F. prausnitzii growth may be supported through the inclusion of dietary fibers found in soy meal, coconut, guar seeds, artichoke, banana, asparagus, etc. Prebiotic fibers (pectin, inulin) and *Bacillus coagulans* probiotics have been shown to increase *F. prausnitzii* abundance in human studies.

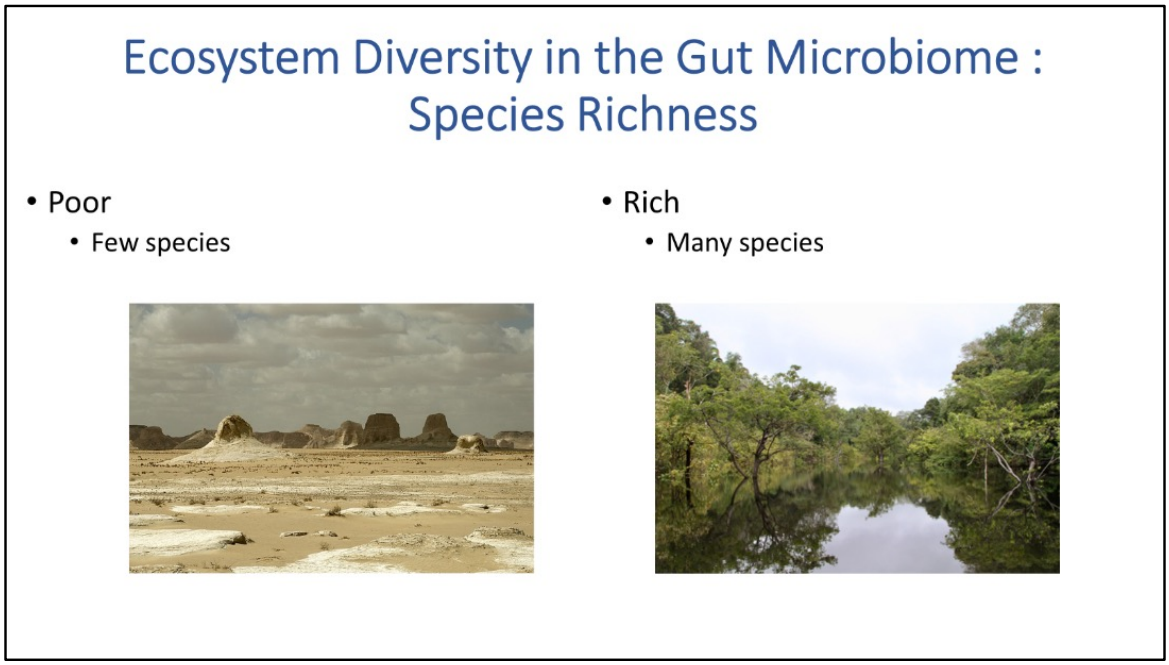
- Increased abundance: Levels of *F. prausnitizii* may be higher in vegan/vegetarians and with inflammation. Abundance may be increased in obese individuals because some *F. prausnitzii* strains can use simple sugars (glucose, fructose, etc.). *F. prausnitzii* abundance may be lower with alcohol use, in patients with leaky guts, increased bile salts, insulin resistance, inflammatory bowel disease (IBD) or other GI disorders, gastrointestinal bleeding (occult blood), cystic fibrosis, fraility, high blood pressure, rheumatoid arthritis and other rheumatic diseases, or attention deficit hyperactivity disorder (ADHD).
- *Decreased abundance*: Decreased *F. prausnitzii* abundance has been associated with an increased severity of COVID-19 symptoms in hospitalized patients. Use of the emulsifier polysorbate 80 or the preservative sodium sulfite inhibits *F. prausnitzii* growth in culture, and some strains may also be sensitive to saccharin and/or carrageenan food additives.

Relative Commensal Abundance

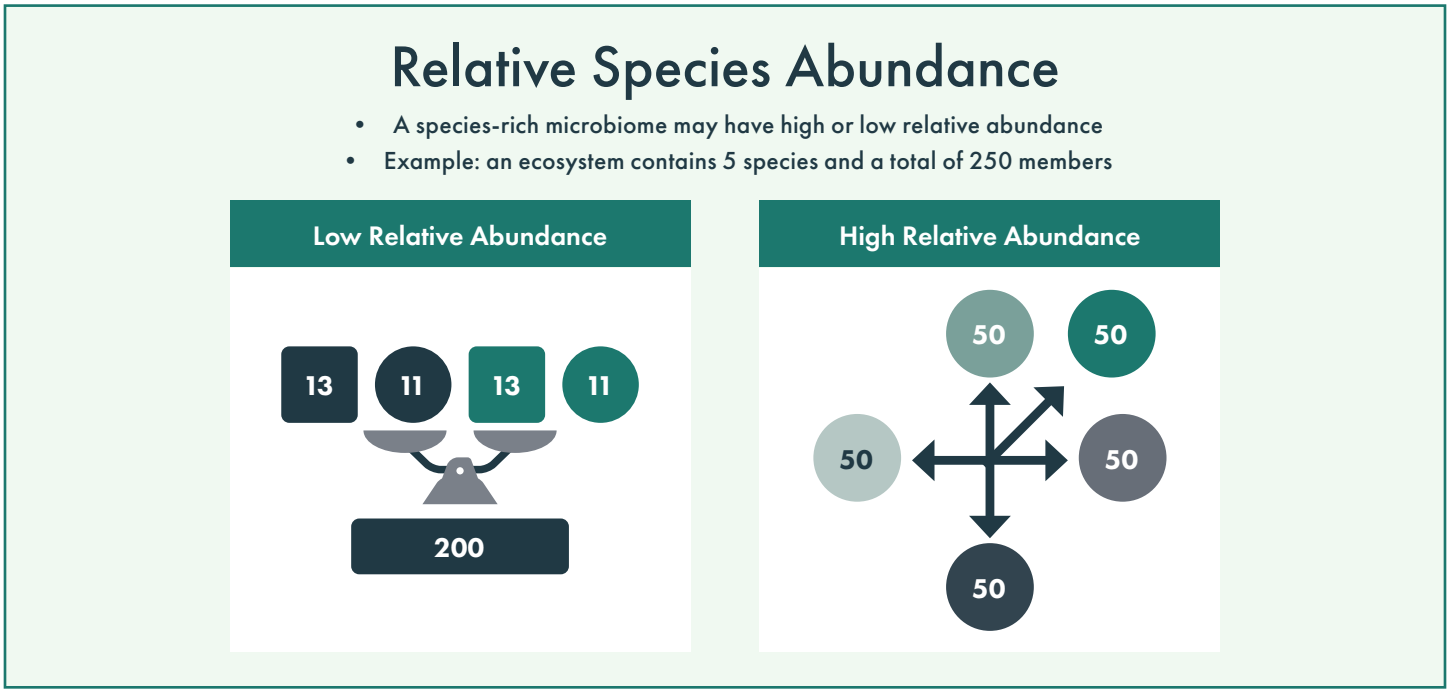


The health of an individual's gut microbiome is reflected by the diversity of bacterial species and functions represented in the bacterial population. The actual species present in any individual will reflect maternal contributions, lifestyle and diet. As noted previously, a healthy microbiome contains approximately 1,000-1,500 different types of bacteria along with a variety of different types of fungus, viruses, parasites, and bacteriophages. Estimating the relative abundance of populations within the microbiome is one method used by scientists and researchers to determine the bacterial diversity found in an ecosystem such as the gut microbiome.

Diversity is measured by comparing the number of species within the ecosystem (richness) *and* the number of individuals within each species (relative abundance).



Rich or poor, any ecosystem may have high or low relative species abundance (see image below). When relative species abundance is low, the loss of one or two keystone species is significant and may make ecosystem recovery difficult. In the gut microbiome, keystone species may be important because they degrade fiber or other compounds into usable substrates for other bacteria or create cross-talk molecules that create host tolerance in the GI tract. By providing usable resources to other bacteria, keystone species would be important guild members and needed to establish the complex trophic interactions vital to microbiome diversity. Review the "The Ecology of the Gastrointestinal Microbiome" section for more information about guilds and trophic layers.



The Relative Commensal Abundance estimate provided in the **GI Profiles** is based upon the normal ratios presented in **4U Health's GI Profiles** are from the available human research on a Dutch cohort of 1135 normal weight and obese individuals on varied diets (Zernakova, 2016). Additional study of urban and rural populations around the world indicates that there is a "universal core" microbiome (Piquer-Esteban, 2021). The majority of bacterial groups are non-motile obligate anaerobes. While some Firmicutes bacteria are motile and spore-forming, the traits are not common in the universal core microbiome. The study also noted that diet and lifestyle greatly influenced the relative abundance of the different bacteria in the core microbiome:

- Urban city dwellers had higher levels of Bacteroidetes
- "Peri-urban" dwellers from an industrialized shanty town supported Firmicutes phylum members such as Faecalibacterium species and Actinobacteria phylum bacteria
- Rural populations and non-Western populations with high vegetable consumption better supported Firmicutes phylum members such as Clostridia and Prevotella species

Gut Microbiome Restoration

Restoration of normal digestive functions, and elimination or correction of diet and lifestyle factors that may induce dysbiosis can help improve bacterial diversity in the gut microbiome; higher rates of bacterial diversity are associated with host health and immune tolerance. When using herbs or supplements always follow the manufacturer's recommended dosing. Interventions may include:

- Eliminate detected pathogens or parasites using established antimicrobial stewardship protocols; culture for sensitivities when available, CDC-recommended treatments when the pathogen cannot be cultured.
- Correct dental or oral problems inhibiting normal chewing, and re-establish good chewing hygiene such as attention on chewing and swallowing, counting chews or seconds until food is swallowed, etc.
- Correction of comorbid digestive disorders:
  - Consider digestive enzymes, hydrochloric acid or herbal "bitters" as clinically indicated to improve digestion
  - Normalize motility
    - Iberogast ® is an herbal blend that has been shown to regulate intestinal motility in human studies; it may also have some anti-inflammatory activity
    - Peppermint oil acts as a smooth muscle relaxant and may also regulate the enteric nervous system
    - Hypermotility (diarrhea) may improve with the use of herbal interventions such as tannins (Quercus spp.), flavonoids or cinnamon, and soluble fiber supplements such as psyllium or acacia
    - Hypomotility (constipation) may improve with the use of herbal interventions such as aloe, senna, turkey rhubarb (*Rheum palmatum*), etc.
    - Increase insoluble fiber to 30 grams/day. Taper up and increase fluid intakes to compensate; fibers to consider include inulin, psyllium, acacia, flaxseed, chia seed, etc.
- Replace mucus layer of gut mucosa
  - Provide polysaccharides such as brown seaweeds, *Althaea officinalis, Linum usitatissimum, Malva neglecta, Malva sylvestris,* and *Matricaria chamomilla*
  - Amino acids threonine, serine, proline and cysteine are required to promote mucin synthesis and improve intestinal barrier function
- Resolve inflammation
  - Identify and eliminate food allergy or sensitivity
    - Evaluate true food or mold allergy with **4U Health's IgE Allergy Panels**
    - Evaluate non-IgE food sensitivity with **4U Health's IgG Food Panels**
  - Glutamine modulates the production of inflammatory cytokines
  - Herbal anti-inflammatories may have local and systemic effects. Consider curcumin (*Curcuma longa*), ginger (*Zingiber officinalis*), frankincense (*Boswellia serrata*), cat's claw (*Uncaria tomentosa*), etc.
- Restoration of "rest and digest" parasympathetic signaling:
  - Mindfulness, stress reduction programs, etc.
  - Leisurely, distraction-free meals (no phone, TV, podcast, etc.)
  - Thorough chewing, attention to and appreciation of food
- Diet
  - Adjust diet macronutrient ratios (protein, fat, carbohydrate) per the Guide interpretive paragraphs to support beneficial bacteria and starve dysbiotic bacteria
    - Human studies indicate that either a Mediterranean diet or a moderate-protein, plant-rich Paleo diet can maintain gut microbiome diversity
  - Inclusion of fruits and vegetables (fresh, frozen, or canned)
  - Decrease or eliminate trans-fats, simple carbohydrates, colorants, additives and other food-processing additives
- Improve fiber tolerance via texture modification to decrease bloating/gas

- Plant fibers in fruits and vegetables can be modified by cooking, grinding, or purée
- Consider gradual increase in plant fiber length and complexity over weeks to months:
  - Cooked purées →cooked/raw purées and cooked chopped (salsa consistency) → cooked/raw purées and cooked/raw chopped → cooked/raw purées and cooked/raw chopped and cooked chunked (cubes or floret-sized) → cooked/raw purées and cooked/raw chopped and cooked/raw chunks or sticks
- Once fruits and vegetables are tolerated, consider a similar texture modification program for legumes (if desired)

- Improving metabolic health
  - Normalize weight
  - Improve glucose/insulin signaling
    - **4U Health's Organic Acids Profile** provides an excellent overview of metabolic health and the patient's metabolic use of dietary carbohydrates, fats, and protiens
  - Exercise: 150 minutes of moderate exercise activity weekly
  - Decrease chronic inflammation with antioxidant foods/supplements and/or glutathione
- Improve SCFA status and immune tolerance
  - Butyrate supplements may be beneficial short-term until the microbiome regains its diversity and SCFA synthesis by the gut microbiome is restored; most butyrate producers are found in the Firmicutes phylum
- Consider additional nutritional supports based on the stool chemistry results from **4U Health's Advanced GI Profile** results

In Conclusion

The **GI profiles** offered by 4U Health provide a thorough functional overview of the microbiome, immune status and digestive capacity of the gastrointestinal tract. When combined with patient history and a thorough physical examination the **GI Profiles** provide clinicians with additional insights to guide therapeutic interventions and promote patient health.

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