Introduction to the Comprehensive Stool Analysis

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Objectives

- Gain a general understanding of the purpose, testing methods, interpretation and limitations of the comprehensive stool analysis.
- Gain a general understanding of the purpose, testing methods, interpretation and limitations of the fatty acid analysis.
- Identify appropriate uses of the comprehensive stool analysis and the fatty acid analysis in the primary care/outpatient setting.

Conflicts of Interest

None

Scope of Chronic Gastrointestinal Symptoms

35.4M

Primary Care Visits

In 2019, diseases of the digestive system accounted for approximately 35.4M primary care office visits in the United States.

10-15%

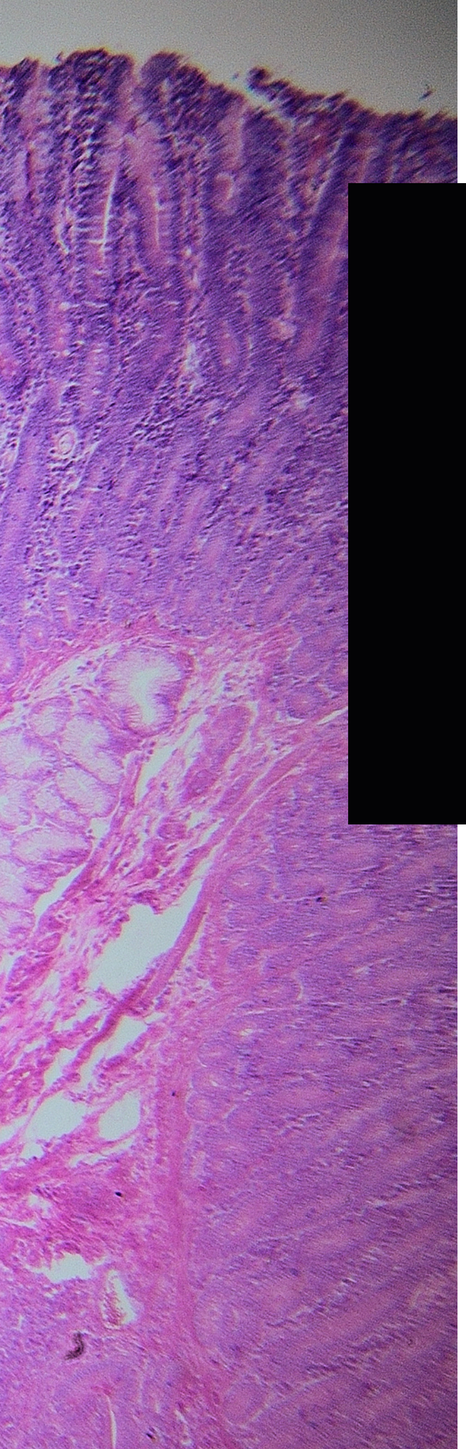
Prevalence

Irritable bowel syndrome affects approximately 10-15% of adults in the U.S., however only 5-7% have been formally diagnosed

$136b

Significant Cost

In 2015, healthcare expenditures for GI diseases totaled approx $135.9 billion surpassing costs associated with other common diseases



Why consider stool testing?

Exploring gut health is like opening a door to understanding the health of the entire system. By utilizing stool testing, we can help our patients understand their body in much greater detail and give them the opportunity to guide healthy changes in their life. Stool testing can provide our patients with additional answers that traditional workups cannot. It can help us build a stronger relationship with our patients and encourage shared decision-making.

Ideal Candidates for Comprehensive Stool Testing

Chronic GI Symptoms

Diarrhea
Constipation
Gas, bloating
Reflux

GI-Specific Conditions

Irritable bowel syndrome
Inflammatory bowel disease
GERD

Systemic Symptoms

Chronic fatigue
Brain fog
Anxiety, depressive symptoms
Acne, eczema, rosacea

Metabolic Disorders

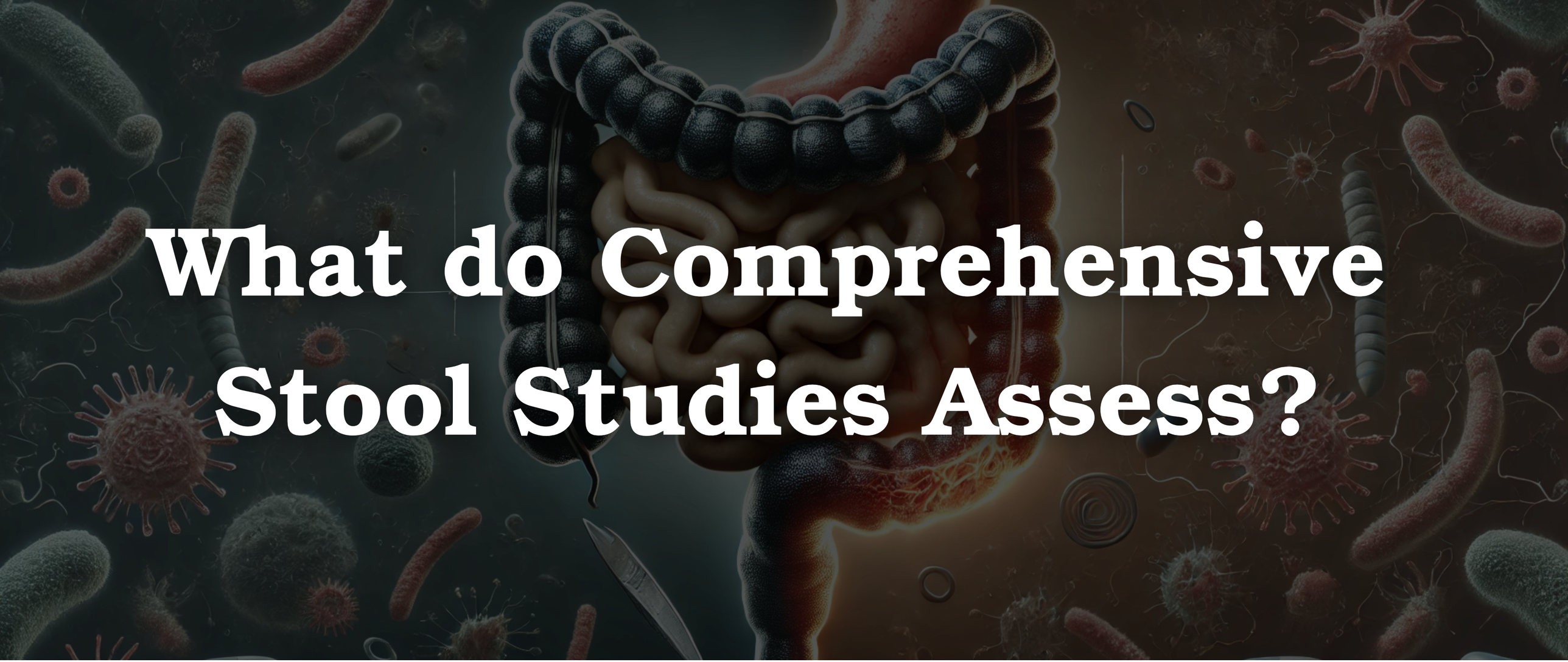
Insulin resistance
Metabolic syndrome
NAFLD

Lifestyle Factors

Frequent antibiotics
Regular alcohol use
NSAID use, PPI use
Stress, poor sleep/diet

Associated Conditions

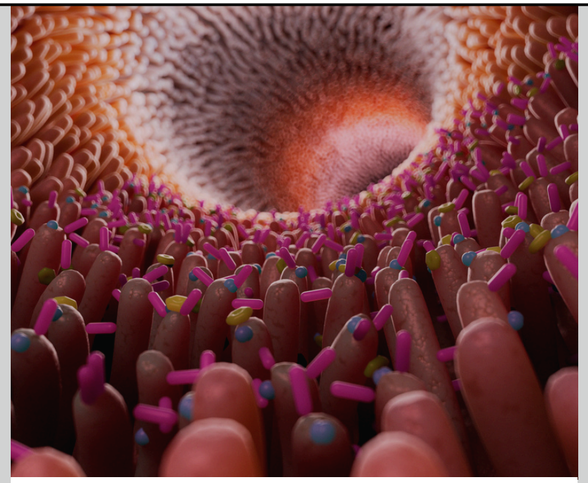
Autism spectrum disorder
Parkinson’s Disease
Fibromyalgia
Autoimmune conditions



What do Comprehensive Stool Studies Assess?

1	2	3
Microbiome	Digestion & Absorption	Inflammation

Functions of the Intestinal Microbiome



Protection

Production of antimicrobial factors
Pathogen displacement
Immune system development

Structural

Barrier fortification
Short chain fatty acid generation

Metabolic

Vitamin synthesis
Metabolism of hormones and toxins
Production of neurotrasmitters

Disruptors

Stress
Medications
Toxins
Chronic disease
Early life factors

What can stool testing tell us about the intestinal microbiome ?

Microbiome assessment

PCR screens for bacteria (usually 20-30 species) and parasites (usually ~30)

Stool culture is used to identify additional microorganisms

¿ What is Dysbiosis

Dysbiosis

Disruption in the natural interaction of microorganisms

Lack of microbial diversity and/or balance

Digestion and Absorption

Markers of how efficiently food is able to be broken down and absorbed

Pancreatic Elastase

Low elastase suggests exocrine pancreatic insufficiency

Short Chain Fatty Acids Butyrate

Low levels indicate inadequate fiber intake, dysbiosis and/or chronic inflammation

Fecal Fat

Elevated fecal fat indicates malabsorption issues such as SIBO or gallbladder dysfunction

Digestion and Absorption

Products of Protein Breakdown

Putrefactive Short Chain Fatty Acids (PSCFA)

Putrefactive short chain fatty acids result from anaerobic fermentation of improperly broken down polypeptides and amino acids by gut flora.

PSCFAs Tested

1. Valerate
2. Isovalerate
3. Isobutyrate

Upstream Etiologies

Hypochlorhydria

Pancreatic exocrine dysfunction

Impaired absorption, such as with inflammation

Markers of Inflammation

1. Fecal Calprotectin
2. Secretory IgA
3. Lactoferrin
   Eosinophil Protein X
   Lysozyme

Beta-glucuronidase

Why is this Important to Test?

1. Toxins, including breakdown products of hormones, are conjugated with glucuronic acid in the liver for excretion in the stool

2. Conjugated substance travels to the bowel and is de-conjugated by beta-glucuronidase produced by dysbiotic bacteria

3. De-conjugated toxic substrate is then reabsorbed into circulation and can exert detrimental effects on health

Limitations

Information overload > Cumbersome > Accuracy

Strategies

Choose one area to start > Patient selection > Context

Results Overview

Functional Imbalance Scores

Key (< 2) : Low Need for Support (2-3) : Optional Need for Support (4-6) : Moderate Need for Support (7-10) : High Need for Support

	Need for Digestive Support	Need for Inflammation Modulation	Need for Microbiome Support	Need for Prebiotic Support	Need for Antimicrobial Support
	MALDIGESTION	INFLAMMATION	DYSBIOSIS	METABOLIC IMBALANCE	INFECTION
	0	1	4	5	0
Biomarkers	Products of Protein Breakdown ▽ Pancreatic Elastase ● Fecal Fats ●	Secretory IgA △ Calprotectin ● Eosinophil Protein X ● Occult Blood ●	Reference Variance △ Total Abundance △ IAD/Methane Score ● PP Bacteria/Yeast ●	Total SCFA's ▼ Beta-glucuronidase ▼ n-Butyrate Conc. ▽ SCFA (%) ●	Total Abundance △ Parasitic Infection ● Pathogenic Bacteria ● PP Bacteria/Yeast ●
Therapeutic Support Options	• Digestive Enzymes • Betaine HCl • Bile Salts • Apple Cider Vinegar • Mindful Eating Habits • Digestive Bitters	• Elimination Diet/ Food Sensitivity Testing • Mucosa Support: Slippery Elm, Althea, Aloe, DGL, etc. • Zinc Carnosine • L-Glutamine • Quercetin • Turmeric • Omega-3's • GI Referral (If Calpro is Elevated)	• Pre-/Probiotics • Increase Dietary Fiber Intake • Consider SIBO Testing • Increase Resistant Starches • Increase Fermented Foods • Meal Timing	• Pre-/Probiotics • Increased Dietary Fiber Intake • Increase Resistant Starches • Increase Fermented Foods • Calcium D-Glucarate (for high beta-glucuronidase)	• Antibiotics (if warranted) • Antimicrobial Herbal Therapy • Antiparasitic Herbal Therapy (if warranted) • *Saccharomyces boulardii*

Methodology: GC-FID, Automated Chemistry, EIA	Result	QUINTILE DISTRIBUTION 1st 2nd 3rd 4th 5th	Reference Range
Digestion and Absorption			
Pancreatic Elastase 1 †	>500	100 200	>200 mcg/g
Products of Protein Breakdown (Total*) (Valerate, Isobutyrate, Isovalerate)	2.2		1.8-9.9 micromol/g
Fecal Fat (Total*)	20.1		3.2-38.6 mg/g
Triglycerides	1.0		0.3-2.8 mg/g
Long-Chain Fatty Acids	14.3		1.2-29.1 mg/g
Cholesterol	1.1		0.4-4.8 mg/g
Phospholipids	3.7		0.2-6.9 mg/g
Inflammation and Immunology			
Calprotectin †	<17	50 120	<=50 mcg/g
Eosinophil Protein X (EPX)†	<DL	0.5 2.7	<=2.7 mcg/g
Fecal secretory IgA	1,398	680 2040	<=2,040 mcg/mL
Gut Microbiome Metabolites			
Metabolic			
Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	22.3 L		>=23.3 micromol/g
n-Butyrate Concentration	3.9		>=3.6 micromol/g
n-Butyrate %	17.5		11.8-33.3 %
Acetate %	61.6		48.1-69.2 %
Propionate %	20.8		<=29.3 %
Beta-glucuronidase	186 L		368-6,266 U/g

Methodology: DNA by qPCR

Gastrointestinal Microbiome (PCR)

Commensal Bacteria (PCR)	Result CFU/g stool	QUINTILE DISTRIBUTION (1st 2nd 3rd 4th 5th)	Reference Range CFU/g stool
Bacteroidetes Phylum			
Bacteroides uniformis	9.7**E7**		<=9.5**E8**
Phocaeicola vulgatus	3.2**E8**		<=8.3**E8**
Barnesiella spp.	3.5**E7**		3.0**E6** -2.9**E8**
Odoribacter spp.	1.3**E7**		<=9.5**E7**
Prevotella spp.	4.4**E8**		6.6**E7** -3.8**E9**
Firmicutes Phylum			
Anaerotruncus colihominis/massiliensis	9.4**E6**		<=2.0**E7**
Butyrivibrio crossotus	<DL		<=3.3**E7**
Clostridium spp.	2.1**E6**		<=1.5**E7**
Coprococcus eutactus	<DL		<=1.2**E8**
Faecalibacterium prausnitzii	3.4**E8**		1.1**E6** -1.1**E9**
Lactobacillus spp.	3.4**E4**		<=1.6**E6**
Pseudoflavonifractor spp.	5.1**E6**		1.3**E4** -2.9**E7**
Roseburia spp.	1.8**E8**		3.6**E5** -4.6**E8**
Ruminococcus bromii	7.3**E8**		<=1.5**E9**
Veillonella spp.	3.3**E4**		<=4.1**E6**
Actinobacteria Phylum			
Bifidobacterium spp.	1.2**E8**		4.6**E5** -2.6**E8**
Bifidobacterium longum subsp. longum	3.4**E7**		<=1.3**E8**
Collinsella aerofaciens	4.2**E7**		<=1.3**E8**
Proteobacteria Phylum			
Desulfovibrio piger	<DL		<=5.4**E7**
Escherichia coli	6.7**E5**		<=7.5**E6**
Oxalobacter formigenes	4.0**E6**		<=1.1**E7**
Euryarchaeota Phylum			
Methanobrevibacter smithii	<DL		<=2.0**E7**
Fusobacteria Phylum			
Fusobacterium spp.	<DL		<=1.8**E5**
Verrucomicrobia Phylum			
Akkermansia muciniphila	<DL L		>=8.5**E3**

The gray-shaded portion of a quintile reporting bar represents the proportion of the reference population with results below detection limit.

Commensal results and reference range values are displayed in a computer version of scientific notation, where the capital letter "E" indicates the exponent value (e.g., 7.3E6 equates to 7.3 x 10^6 or 7,300,000).

The methodology for the PCR Commensal Bacteria has been updated to qPCR. The reference ranges have been updated accordingly.

Methodology: Culture/MALDI-TOF MS, Automated and Manual Biochemical Methods, Vitek® 2 System Microbial identification and Antibiotic susceptibility

Gastrointestinal Microbiome (Culture)

Human microflora is influenced by environmental factors and the competitive ecosystem of the organisms in the GI tract. Pathogenic significance should be based upon clinical symptoms.

Microbiology Legend

NG	NP	PP	P
No Growth	Non-Pathogen	Potential Pathogen	Pathogen

Additional Bacteria

Non-Pathogen: Organisms that fall under this category are those that constitute normal, commensal flora, or have not been recognized as etiological agents of disease.
Potential Pathogen: Organisms that fall under this category are considered potential or opportunistic pathogens when present in heavy growth.
Pathogen: The organisms that fall under this category have a well-recognized mechanism of pathogenicity in clinical literature and are considered significant regardless of the quantity that appears in the culture.

Bacteriology (Culture)

			1+	2+	3+	4+
Lactobacillus spp.		NG				
Escherichia coli	4+	NP				◆
Bifidobacterium (Anaerobic Culture)	2+	NP		◆		

Additional Bacteria

			1+	2+	3+	4+
Salmonella spp.		NG				
Shigella spp.		NG				
Enterococcus casseliflavus	4+	NP				◆
Bacillus species	1+	NP	◆			

Mycology (Culture)

			1+	2+	3+	4+
Yeast, not Candida albicans	1+	NP	◆			

Parasitology

PCR Parasitology - Protozoa

Methodologies: DNA by PCR

Organism	Result	Units		Expected Result
Blastocystis spp.	<2.14e2	femtograms/microliter C&S stool	Not Detected	Not Detected
Cryptosporidium parvum/hominis	<1.76e2	genome copies/microliter C&S stool	Not Detected	Not Detected
Cyclospora cayetanensis	<2.65e2	genome copies/microliter C&S stool	Not Detected	Not Detected
Dientamoeba fragilis	<1.84e2	genome copies/microliter C&S stool	Not Detected	Not Detected
Entamoeba histolytica	<9.64e1	genome copies/microliter C&S stool	Not Detected	Not Detected
Giardia	<1.36e1	genome copies/microliter C&S stool	Not Detected	Not Detected

Additional Results

Methodology: Fecal Immunochemical Testing (FIT)

	Result	Expected Value
Fecal Occult Blood◆	Negative	Negative
Color††	Brown	

Parasitology

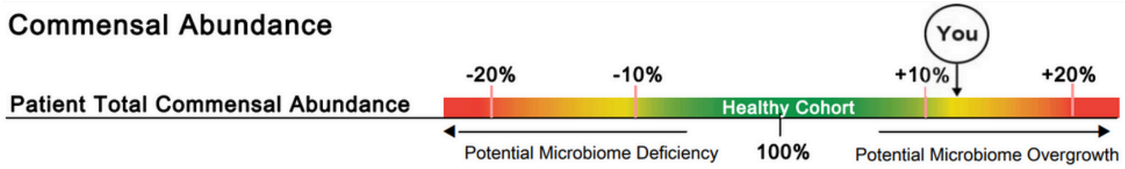
Microscopic O&P Results

Microscopic O&P is capable of detecting all described gastrointestinal parasites. The organisms listed in the box represent those commonly found in microscopic stool analysis. Should an organism be detected that is not included in the list below, it will be reported in the Additional Results section. These results were obtained using wet preparation(s) and trichrome stained smear. For an extensive reference of all potentially detectable organisms, please visit www.gdx.net/product/gi-effects-comprehensive-stool-test

Genus/species	Result
Nematodes - roundworms	
Ancylostoma/Necator (Hookworm)	Not Detected
Ascaris lumbricoides	Not Detected
Capillaria philippinensis	Not Detected
Enterobius vermicularis	Not Detected
Strongyloides stercoralis	Not Detected
Trichuris trichiura	Not Detected
Cestodes - tapeworms	
Diphyllobothrium latum	Not Detected
Dipylidium caninum	Not Detected
Hymenolepis diminuta	Not Detected
Hymenolepis nana	Not Detected
Taenia spp.	Not Detected
Trematodes - flukes	
Clonorchis/Opisthorchis spp.	Not Detected
Fasciola spp./ *Fasciolopsis buski*	Not Detected
Heterophyes/Metagonimus	Not Detected
Paragonimus spp.	Not Detected
Schistosoma spp.	Not Detected
Protozoa	
Balantidium coli	Not Detected
Blastocystis spp.	Not Detected
Chilomastix mesnili	Not Detected
Cryptosporidium spp.	Not Detected
Cyclospora cayetanensis	Not Detected
Dientamoeba fragilis	Not Detected
Entamoeba coli	Not Detected
Entamoeba histolytica/dispar	Not Detected
Entamoeba hartmanii	Not Detected
Entamoeba polecki	Not Detected
Endolimax nana	Not Detected
Giardia	Not Detected
Iodamoeba buetschlii	Not Detected
Cystoisospora spp.	Not Detected
Trichomonads (e.g. *Pentatrichomonas*)	Not Detected
Additional Findings	
White Blood Cells	Not Detected
Charcot-Leyden Crystals	Not Detected
Other Infectious Findings	

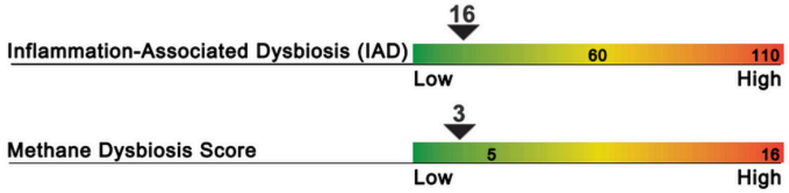
Commensal Microbiome Analysis

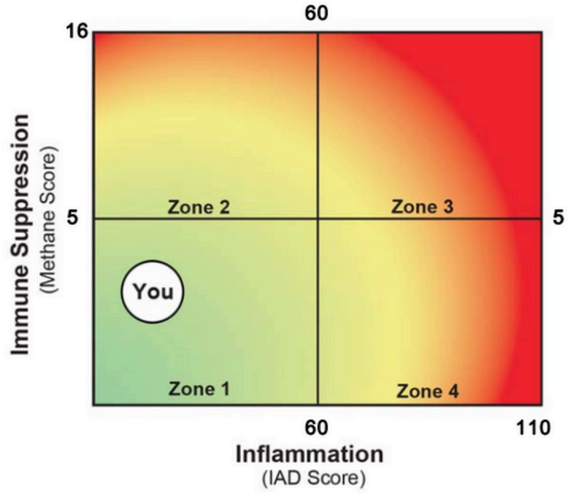
Commensal Abundance



Total Commensal Abundance: The total commensal abundance is a sum-total of the reported commensal bacteria compared to a healthy cohort. Low levels of commensal bacteria are often observed after antimicrobial therapy, or in diets lacking fiber and/or prebiotic-rich foods and may indicate the need for microbiome support. Conversely, higher total commensal abundance may indicate potential bacteria overgrowth or probiotic supplementation.

Dysbiosis Patterns





Dysbiosis Patterns: [redacted] data analysis has led to the development of unique dysbiosis patterns, related to key physiologic disruptions, such as immunosuppression and inflammation. These patterns may represent dysbiotic changes that could pose clinical significance. Please see [redacted] published literature for more details: https://rdcu.be/bRhzv

Zone 1: The commensal profile in this zone does not align with profiles associated with intestinal inflammation or immunosuppression. If inflammatory biomarkers are present, other causes need to be excluded, such as infection, food allergy, or more serious pathology.

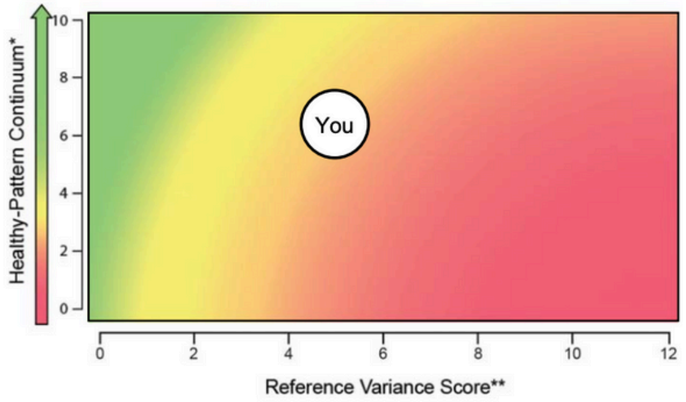
Zone 2: This pattern of bacteria is associated with impaired intestinal barrier function (low fecal sIgA and EPX). Patients in this zone have higher rates of opportunistic infections (e.g. *Blastocystis spp.* & *Dientamoeba fragilis*) as well as fecal fat malabsorption. Commensal abundance is higher in this group suggesting potential bacterial overgrowth.

Zone 3: Patients in this zone may have more inflammation compared to those in zone 4. However, commensal abundance is usually higher making use of antimicrobial therapy relatively safer. Patients in this zone may have higher rates of pathogenic infections.

Zone 4: This commensal profile is associated with increased intestinal inflammation. IBD patients are more likely to have this pattern of bacteria. Commensal abundance is lower in this zone; therefore, antibiotic use for GI potential pathogens should be used with caution. In addition to standard treatment for intestinal inflammation, modulation of the commensal gut profile is encouraged.

Commensal Microbiome Analysis

Commensal Balance

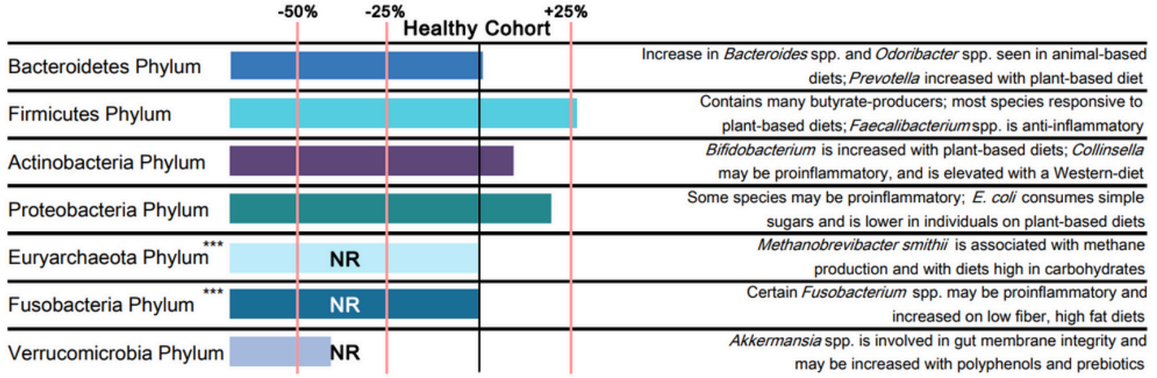


Balanced	Represents 95% of healthy individuals
Borderline	Represents 5% of healthy individuals
Imbalanced	Represents 60% of unhealthy individuals

*A progressive ranking scale based on [redacted] proprietary algorithm that differentiates healthy and unhealthy commensal patterns.

**The total number of commensal bacteria (qPCR) that are out of balance for this individual on a scale of 0 to >12.

Relative Commensal Abundance



Relative Abundance: The relative abundance compares the quantity of each of 7 major bacterial phyla to a healthy cohort. This can indicate broader variances in the patient's gut microbiome profile. Certain interventions may promote or limit individual phyla when clinically appropriate. Please refer to [redacted] Stool Testing Support Guide for more information on modulation of commensal bacteria through diet & nutrient interventions. ***Approximately 70% of the healthy cohort had below detectable levels of *Methanobrevibacter smithii.* Approximately 90% of the healthy cohort had below detectable levels of *Fusobacterium spp.*

Physician Notes/Recommendations

Resources

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