GI fx GI Effects® Stool Profiles

The GI Effects® Advanced Interpretation

Digging Deeper

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GENOVA DIAGNOSTICS®

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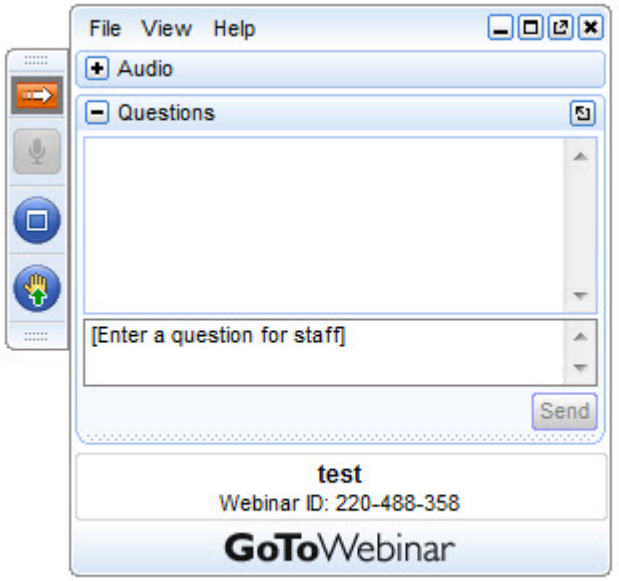
Technical Issues & Clinical Questions

- Please type any technical issue or clinical question into either the “Chat” or “Questions” boxes, making sure to send them to “Organizer” at any time during the webinar.

- We will be compiling your clinical questions and answering as many as we can the final 15 minutes of the webinar.



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Need More Resources?

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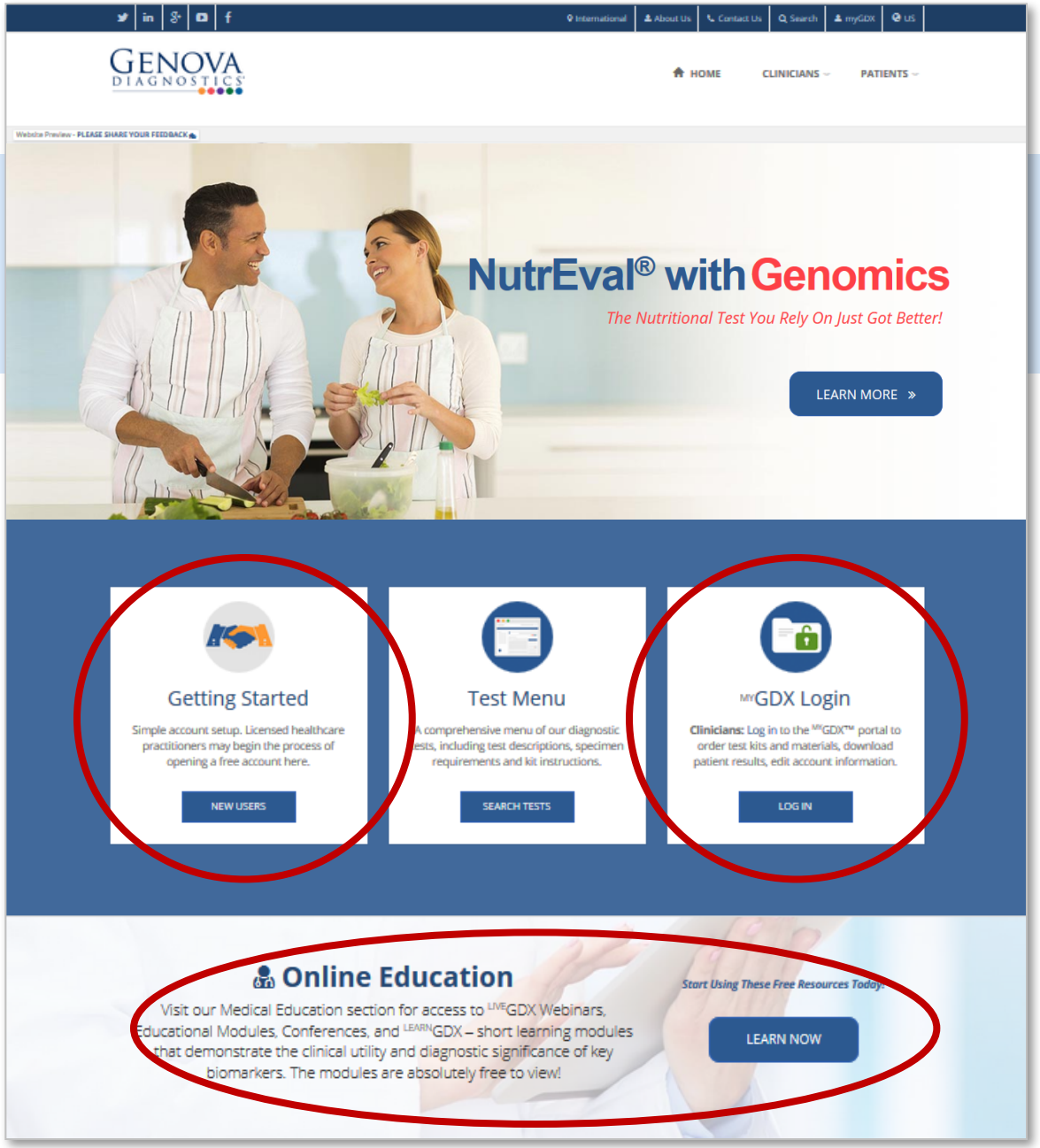

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Learning Objectives

- Gain understanding of the complexity of the microbiome and its metabolic importance
- Recognize physiologic digestive and absorptive patterns and inflammation seen in specific clinical conditions
- Apply the GI Effects® Comprehensive Stool Profile in clinical practice

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Case Example

- 52 y/o female with bloating, excessive belching and flatulence
- Constipation
  - Uses a laxative three times per week to achieve a bowel movement
- Cerebellar ataxia
- Overweight
- Sedentary lifestyle
- WNL thyroid function
- Eats a standard American diet; sugar cravings & avoids fiber (aggravates symptoms)

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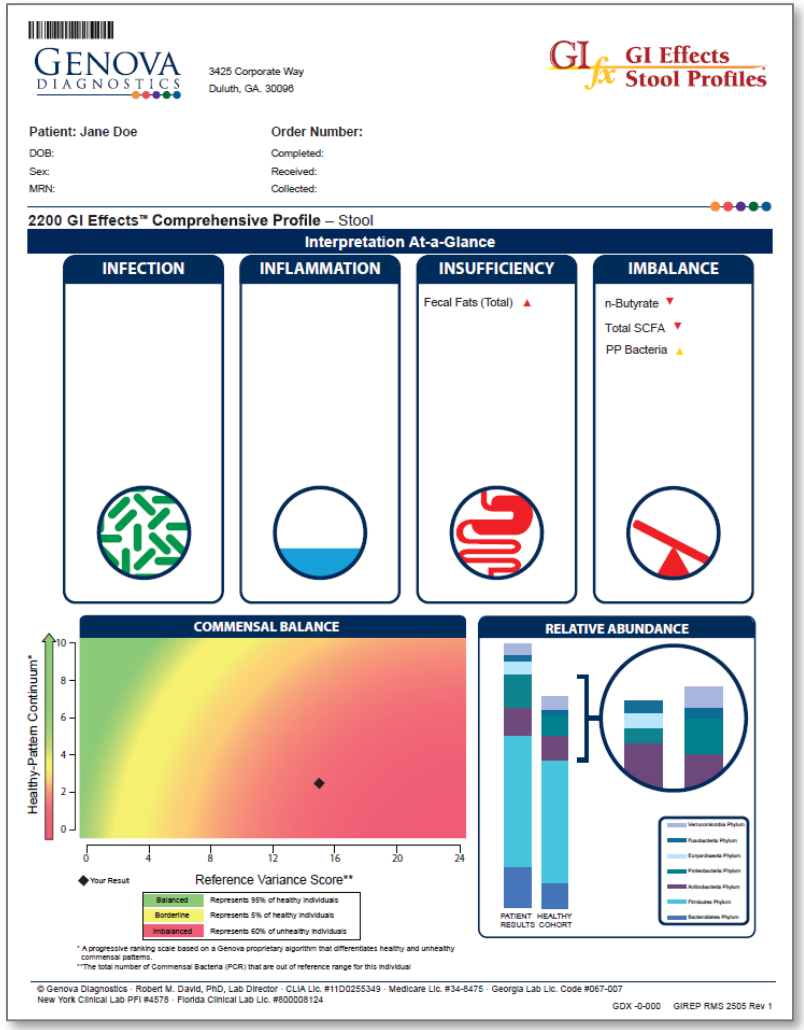
Interpretation-at-a-Glance: Developed using Evidence-based Rules and Weighted Algorithms

- Functional Pillars of Health
  - Infection
  - Inflammation
  - Insufficiency (Digestive)
  - Imbalance (Metabolic)
- Global Gut Health Markers
  - Commensal Balance
  - Relative Abundance



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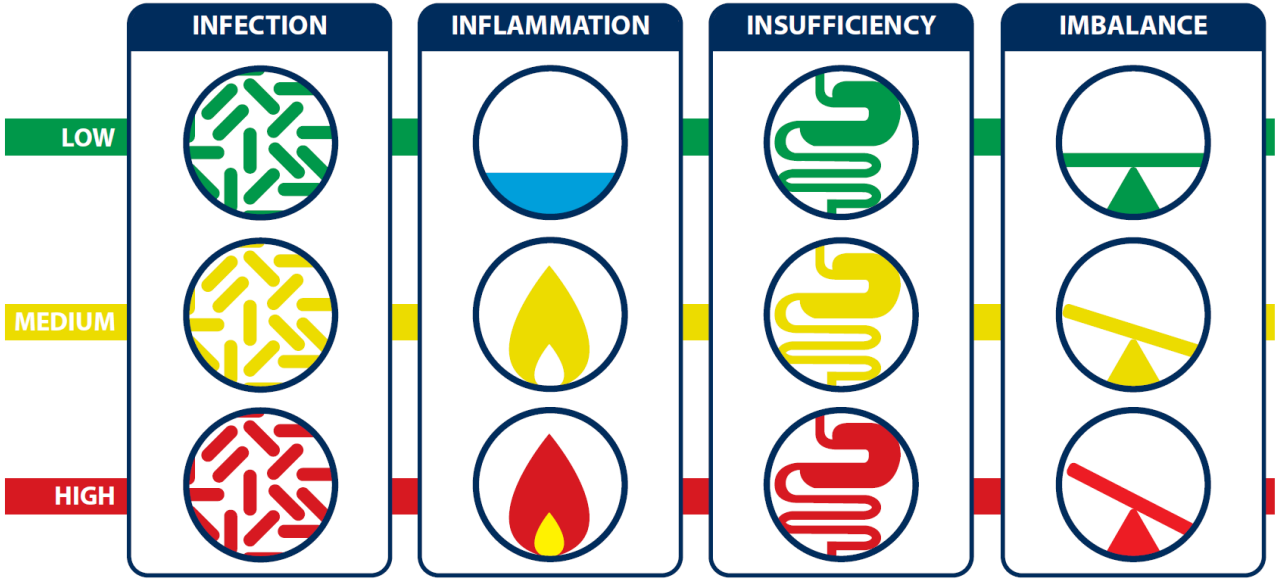
The Four Functional Pillars:

Provide immediate insight into clinically actionable findings in the areas of:

- Infection
- Inflammation
- Insufficiency
- Imbalance



Four Functional Pillars Biomarker Map

Infection Box	Inflammation Box	Insufficiency Box	Imbalance Box
any parasite present	Calprotectin	PE-1	n-Butyrate
any pathogen present	EPX	Total Fecal Fats	Total SCFA
	Fecal IgA	Total Protein Products	Beta-glucuronidase
			Lactobacillus
			Bifidobacterium
			E. coli
			any potential pathogen

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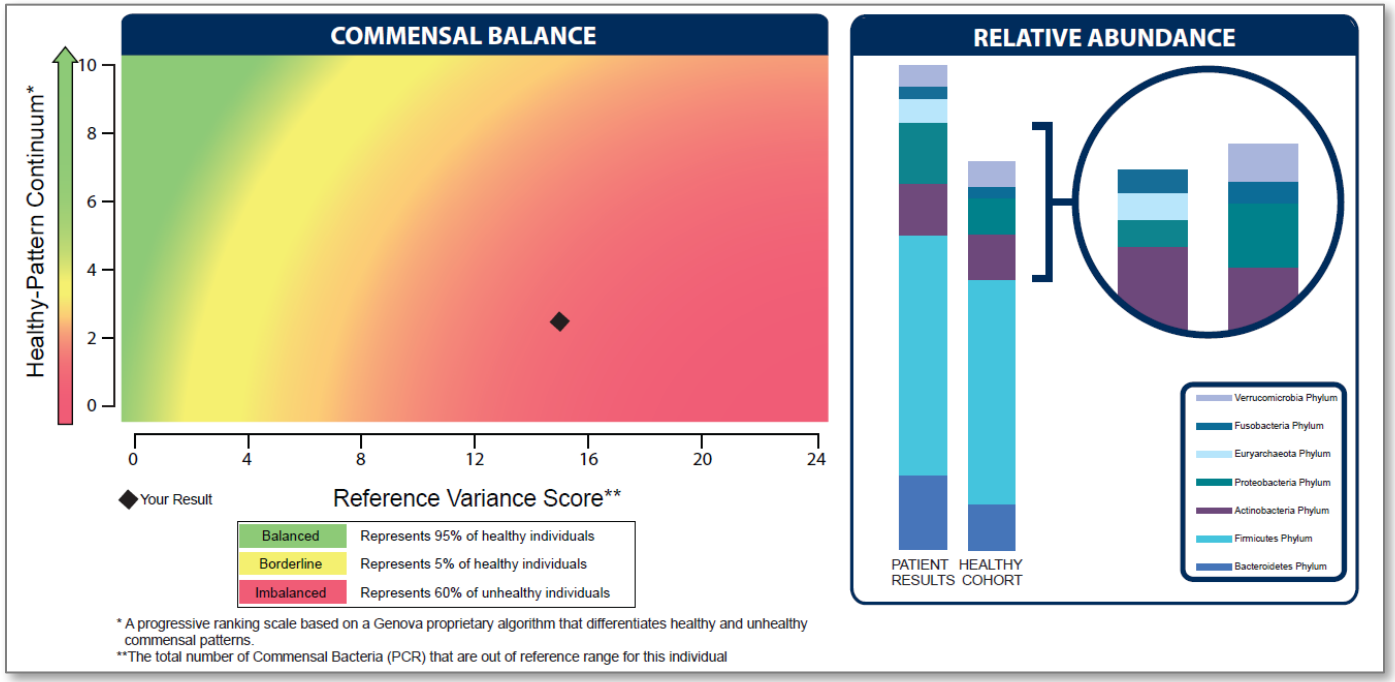
Commensal Balance and Relative Abundance Graphics Serve as General Markers of Gut Health



Commensal Balance (CB): Healthy-Pattern Continuum & Reference Variance Score
Relative Abundance (RA): Identifies the level of growth of select bacterial phyla

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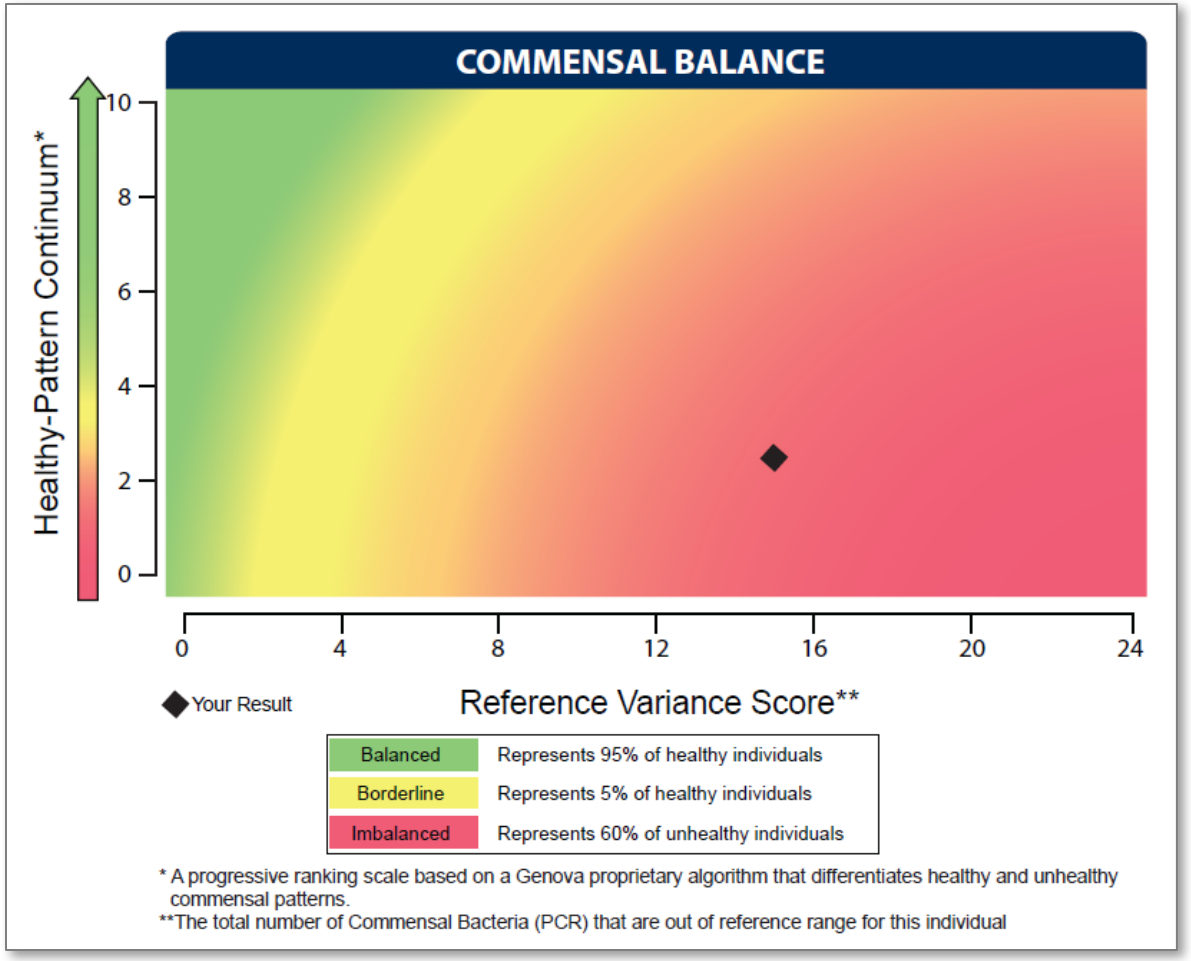
- The **Commensal Balance** is a composite of two measures:
  - 'Y-axis': The **Healthy-Pattern Continuum** (formerly known as the Diversity Association Index) is a progressive ranking scale which differentiates healthy and unhealthy commensal patterns
  - 'X-axis': The **Reference Variance Score** reflects the total number of an individual patient's commensal bacteria (PCR) results that are out of reference range



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Commensal Balance

- Intersection of two variables – The Healthy Pattern Continuum and the Reference Variance Score - is denoted by black diamond against a color-coded gradient (green, yellow and red)
- The position of the patient's result against this background provides an *At-a-Glance* comparison of the patient's current commensal findings against those seen in healthy and diseased cohorts
  - Green suggests **balanced** commensal health status
    - Represents 95% of individuals in the healthy cohort
  - Yellow suggests **borderline** commensal health status
  - Red suggests **imbalanced** commensal health status

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Relative Abundance

Identifies the level of growth of each measured bacterial phyla

- Significantly lower total Relative Abundance may represent an imbalance due to low beneficial commensal organisms
- Significantly higher total RA may represent an imbalance due to microbial overgrowth

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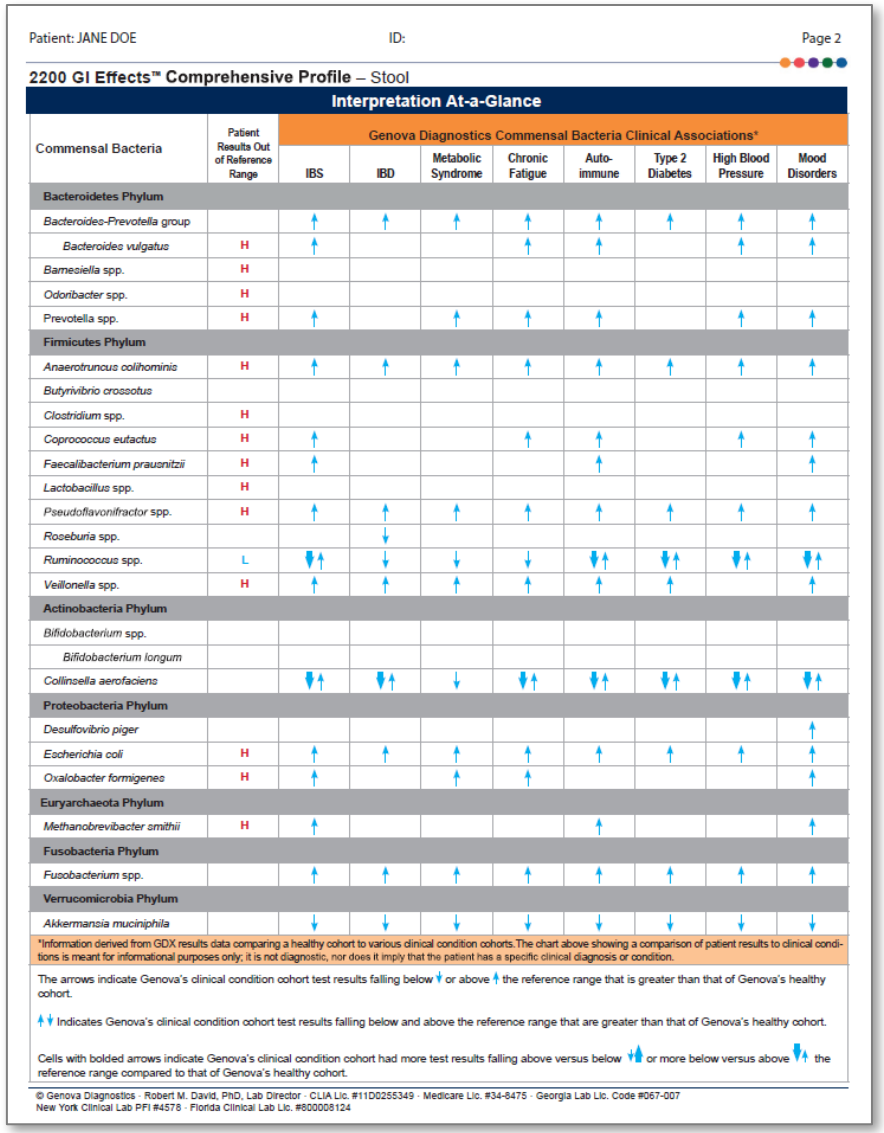
Commensal and Biomarker Clinical Association Charts



Patient: JANE DOE ID: Page 2

2200 GI Effects™ Comprehensive Profile – Stool

Interpretation At-a-Glance

Commensal Bacteria	Patient Results Out of Reference Range	Genova Diagnostics Commensal Bacteria Clinical Associations*							
		IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
Bacteroidetes Phylum									
Bacteroides-Prevotella group		↑	↑	↑	↑	↑	↑	↑	↑
Bacteroides vulgatus	H	↑			↑	↑		↑	↑
Barnesiella spp.	H								
Odoribacter spp.	H								
Prevotella spp.	H	↑		↑	↑	↑		↑	↑
Firmicutes Phylum									
Anaerotruncus colihominis	H	↑	↑	↑	↑	↑	↑	↑	↑
Butyrivibrio crossotus									
Clostridium spp.	H								
Coprococcus eutactus	H	↑			↑	↑		↑	↑
Faecalibacterium prausnitzii	H	↑				↑			↑
Lactobacillus spp.	H								
Pseudoflavonifractor spp.	H	↑	↑	↑	↑	↑	↑	↑	↑
Roseburia spp.			↓						
Ruminococcus spp.	L	↓↑	↓	↓	↓	↓↑	↓↑	↓↑	↓↑
Veillonella spp.	H	↑	↑	↑	↑	↑	↑		↑
Actinobacteria Phylum									
Bifidobacterium spp.									
Bifidobacterium longum									
Collinsella aerofaciens		↓↑	↓↑	↓	↓↑	↓↑	↓↑	↓↑	↓↑
Proteobacteria Phylum									
Desulfovibrio piger									↑
Escherichia coli	H	↑	↑	↑	↑	↑	↑	↑	↑
Oxalobacter formigenes	H	↑		↑	↑				↑
Euryarchaeota Phylum									
Methanobrevibacter smithii	H	↑				↑			↑
Fusobacteria Phylum									
Fusobacterium spp.		↑	↑	↑	↑	↑	↑	↑	↑
Verrucomicrobia Phylum									
Akkermansia muciniphila		↓	↓	↓	↓	↓	↓	↓	↓

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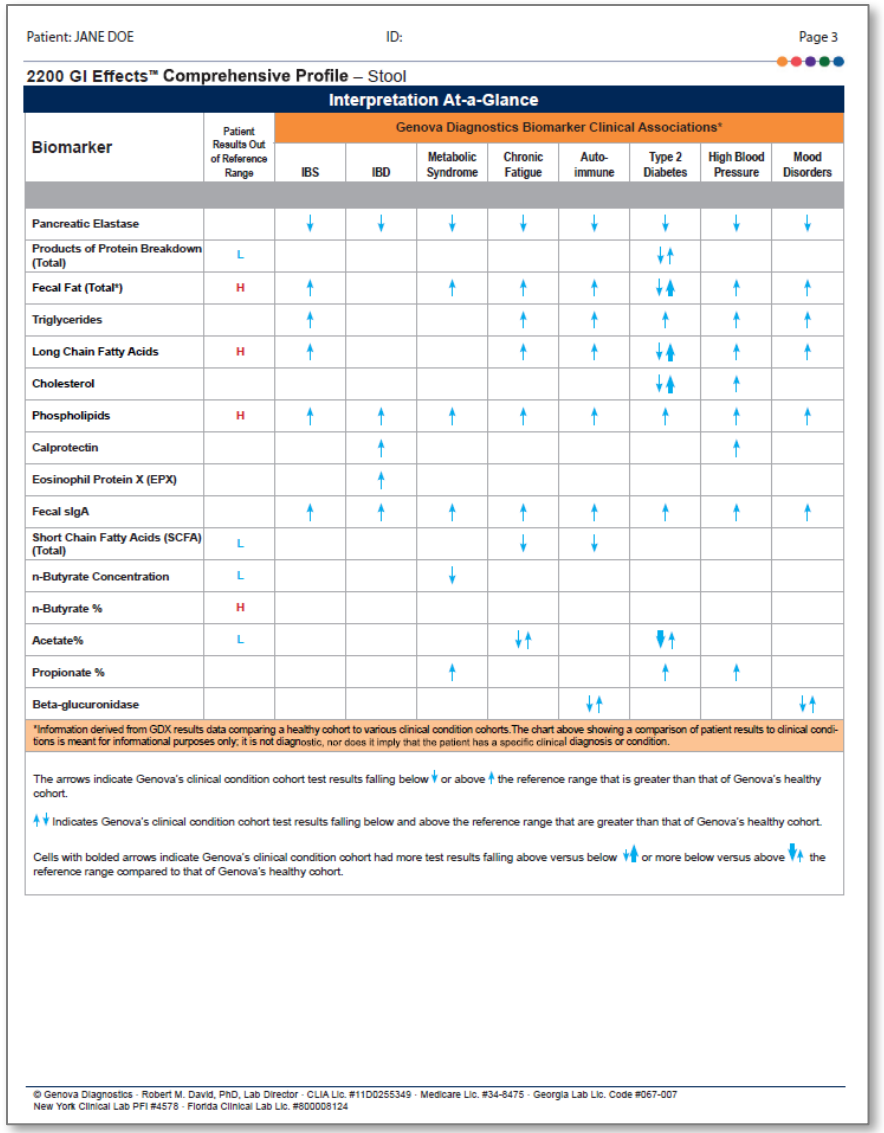

Patient: JANE DOE ID: Page 3

2200 GI Effects™ Comprehensive Profile – Stool

Interpretation At-a-Glance

Biomarker	Patient Results Out of Reference Range	Genova Diagnostics Biomarker Clinical Associations*							
		IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
Pancreatic Elastase		↓	↓	↓	↓	↓	↓	↓	↓
Products of Protein Breakdown (Total)	L						↓↑		
Fecal Fat (Total¹)	H	↑		↑	↑	↑	↓↑	↑	↑
Triglycerides		↑			↑	↑	↑	↑	↑
Long Chain Fatty Acids	H	↑			↑	↑	↓↑	↑	↑
Cholesterol							↓↑	↑	
Phospholipids	H	↑	↑	↑	↑	↑	↑	↑	↑
Calprotectin			↑					↑	
Eosinophil Protein X (EPX)			↑						
Fecal sIgA		↑	↑	↑	↑	↑	↑	↑	↑
Short Chain Fatty Acids (SCFA) (Total)	L				↓	↓			
n-Butyrate Concentration	L			↓					
n-Butyrate %	H								
Acetate%	L				↓↑		↓↑		
Propionate %				↑			↑	↑	
Beta-glucuronidase						↓↑			↓↑

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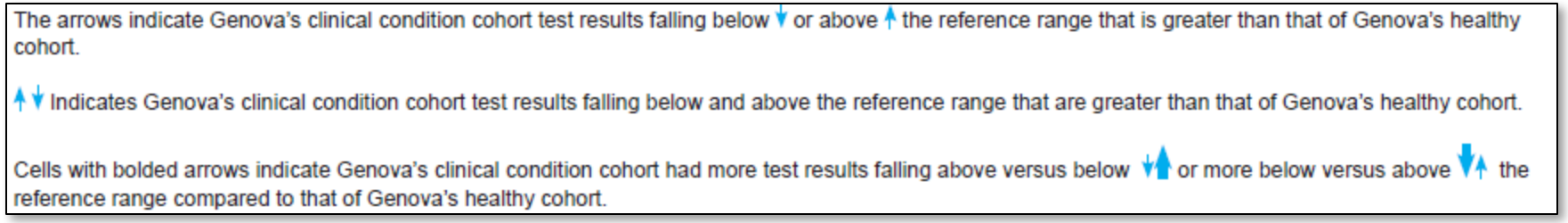
Commensal and Biomarker Clinical Association Charts

- Based on Genova Diagnostics GI Effects test-results database; the Commensal and Biomarker Clinical Association charts were developed
  - Based on self-reported clinical conditions (IBS, IBD, Metabolic Syndrome, Chronic Fatigue, Autoimmune dysfunction, Type 2 Diabetes, High Blood Pressure, and Mood Disorders)
- Differences between the healthy cohort and individuals with clinical conditions are denoted by the arrows in the Clinical Association charts



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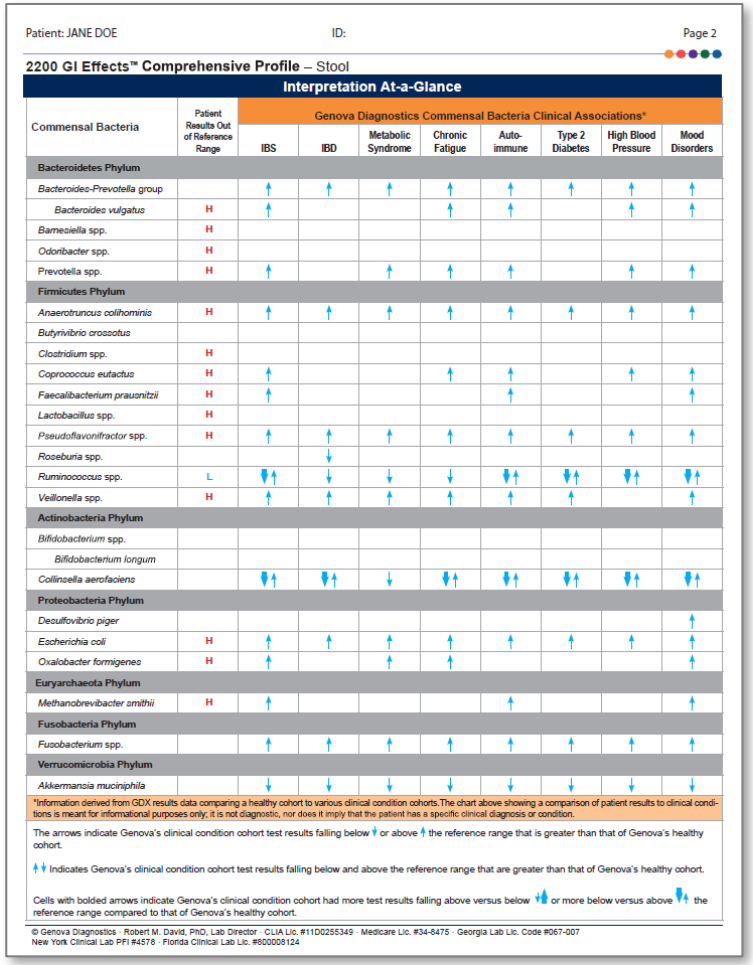
Commensal Bacteria Clinical Association Chart



Patient: JANE DOE ID: Page 2

2200 GI Effects™ Comprehensive Profile – Stool

Interpretation At-a-Glance

Commensal Bacteria	Patient Results Out of Reference Range	Genova Diagnostics Commensal Bacteria Clinical Associations*							
		IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
Bacteroidetes Phylum									
Bacteroides-Prevotella group		↑	↑	↑	↑	↑	↑	↑	↑
Bacteroides vulgatus	H	↑			↑	↑		↑	↑
Barnesiella spp.	H								
Odoribacter spp.	H								
Prevotella spp.	H	↑		↑	↑	↑		↑	↑
Firmicutes Phylum									
Anaerotruncus colihominis	H	↑	↑	↑	↑	↑	↑	↑	↑
Butyrivibrio crossotus									
Clostridium spp.	H								
Coprococcus eutactus	H	↑			↑	↑		↑	↑
Faecalibacterium prausnitzii	H	↑				↑			↑
Lactobacillus spp.	H								
Pseudoflavonifractor spp.	H	↑	↑	↑	↑	↑	↑	↑	↑
Roseburia spp.			↓						
Ruminococcus spp.	L	↓↑	↓	↓	↓	↓↑	↓↑	↓↑	↓↑
Veillonella spp.	H	↑	↑	↑	↑	↑	↑		↑
Actinobacteria Phylum									
Bifidobacterium spp.									
Bifidobacterium longum									
Collinsella aerofaciens		↓↑	↓↑	↓	↓↑	↓↑	↓↑	↓↑	↓↑
Proteobacteria Phylum									
Desulfovibrio piger									↑
Escherichia coli	H	↑	↑	↑	↑	↑	↑	↑	↑
Oxalobacter formigenes	H	↑		↑	↑				↑
Euryarchaeota Phylum									
Methanobrevibacter smithii	H	↑				↑			↑
Fusobacteria Phylum									
Fusobacterium spp.		↑	↑	↑	↑	↑	↑	↑	↑
Verrucomicrobia Phylum									
Akkermansia muciniphila		↓	↓	↓	↓	↓	↓	↓	↓

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- Features of this chart include:
  - A complete list of the 24 Commensal Bacteria (PCR) targets
  - A column indicating any patient result that is out of reference range – either high (H) or low (L)
  - Clinical Associations for eight specific disease states

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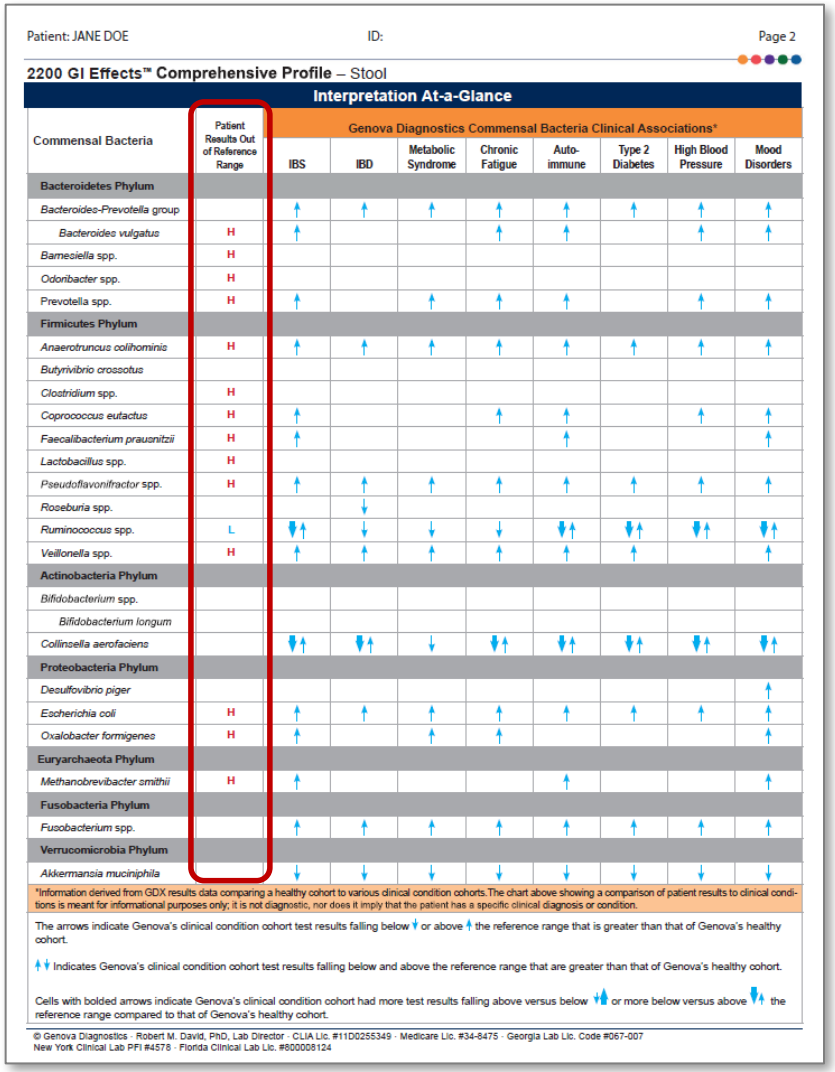
Commensal Bacteria Clinical Association Chart



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		IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
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Bacteroides-Prevotella group		↑	↑	↑	↑	↑	↑	↑	↑
Bacteroides vulgatus	H	↑			↑	↑		↑	↑
Barnesiella spp.	H								
Odoribacter spp.	H								
Prevotella spp.	H	↑		↑	↑	↑		↑	↑
Firmicutes Phylum									
Anaerotruncus colihominis	H	↑	↑	↑	↑	↑	↑	↑	↑
Butyrivibrio crossotus									
Clostridium spp.	H								
Coprococcus eutactus	H	↑			↑	↑		↑	↑
Faecalibacterium prausnitzii	H	↑				↑			↑
Lactobacillus spp.	H								
Pseudoflavonifractor spp.	H	↑	↑	↑	↑	↑	↑	↑	↑
Roseburia spp.			↓						
Ruminococcus spp.	L	↓↑	↓	↓	↓	↓↑	↓↑	↓↑	↓↑
Veillonella spp.	H	↑	↑	↑	↑	↑	↑		↑
Actinobacteria Phylum									
Bifidobacterium spp.									
Bifidobacterium longum									
Collinsella aerofaciens		↓↑	↓↑	↓	↓↑	↓↑	↓↑	↓↑	↓↑
Proteobacteria Phylum									
Desulfovibrio piger									↑
Escherichia coli	H	↑	↑	↑	↑	↑	↑	↑	↑
Oxalobacter formigenes	H	↑		↑	↑				↑
Euryarchaeota Phylum									
Methanobrevibacter smithii	H	↑				↑			↑
Fusobacteria Phylum									
Fusobacterium spp.		↑	↑	↑	↑	↑	↑	↑	↑
Verrucomicrobia Phylum									
Akkermansia muciniphila		↓	↓	↓	↓	↓	↓	↓	↓

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- Detailed Commensal Bacteria (PCR) patient results on a grid that also contains the details for GDX microbial clinical associations for various health conditions
  - Allows direct comparison of the patient's current commensal patterns to aggregate GDX commensal patterns seen in both healthy and unhealthy
  - "apples-to-apples" comparison provides more relevant information for results interpretation of commensal-bacteria status

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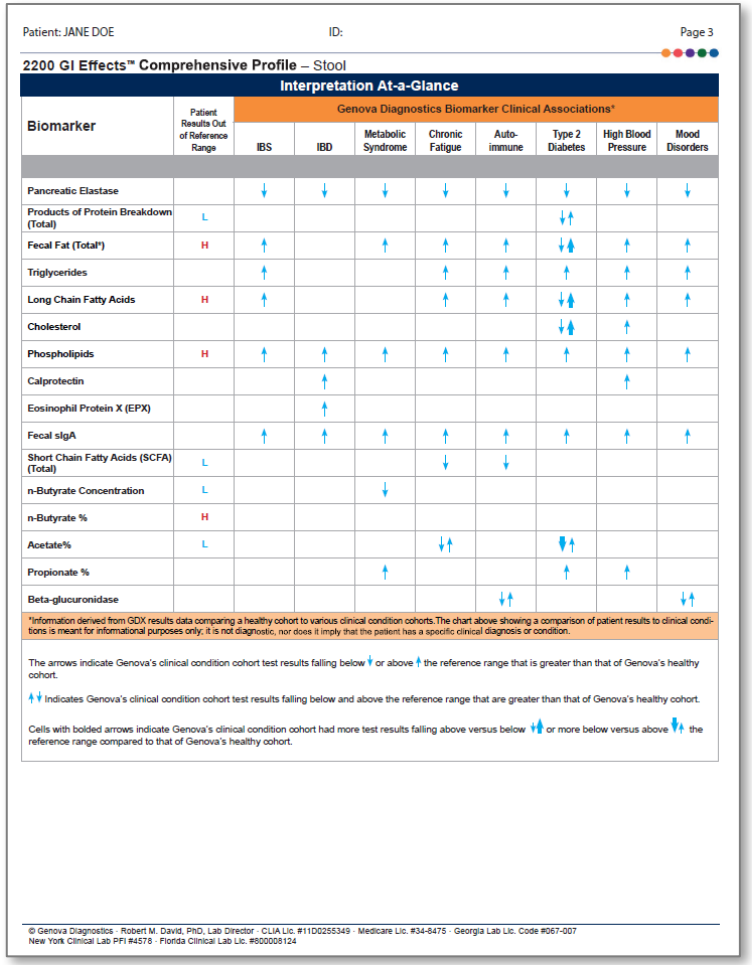
Biomarker Clinical Association Chart



Patient: JANE DOE ID: Page 3

2200 GI Effects™ Comprehensive Profile – Stool

Interpretation At-a-Glance

Biomarker	Patient Results Out of Reference Range	Genova Diagnostics Biomarker Clinical Associations*							
		IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
Pancreatic Elastase		↓	↓	↓	↓	↓	↓	↓	↓
Products of Protein Breakdown (Total)	L						↓↑		
Fecal Fat (Total*)	H	↑		↑	↑	↑	↓↑	↑	↑
Triglycerides		↑			↑	↑	↑	↑	↑
Long Chain Fatty Acids	H	↑			↑	↑	↓↑	↑	↑
Cholesterol							↓↑	↑	
Phospholipids	H	↑	↑	↑	↑	↑	↑	↑	↑
Calprotectin			↑					↑	
Eosinophil Protein X (EPX)			↑						
Fecal sIgA		↑	↑	↑	↑	↑	↑	↑	↑
Short Chain Fatty Acids (SCFA) (Total)	L				↓	↓			
n-Butyrate Concentration	L			↓					
n-Butyrate %	H								
Acetate%	L				↓↑		↓↑		
Propionate %				↑			↑	↑	
Beta-glucuronidase						↓↑			↓↑

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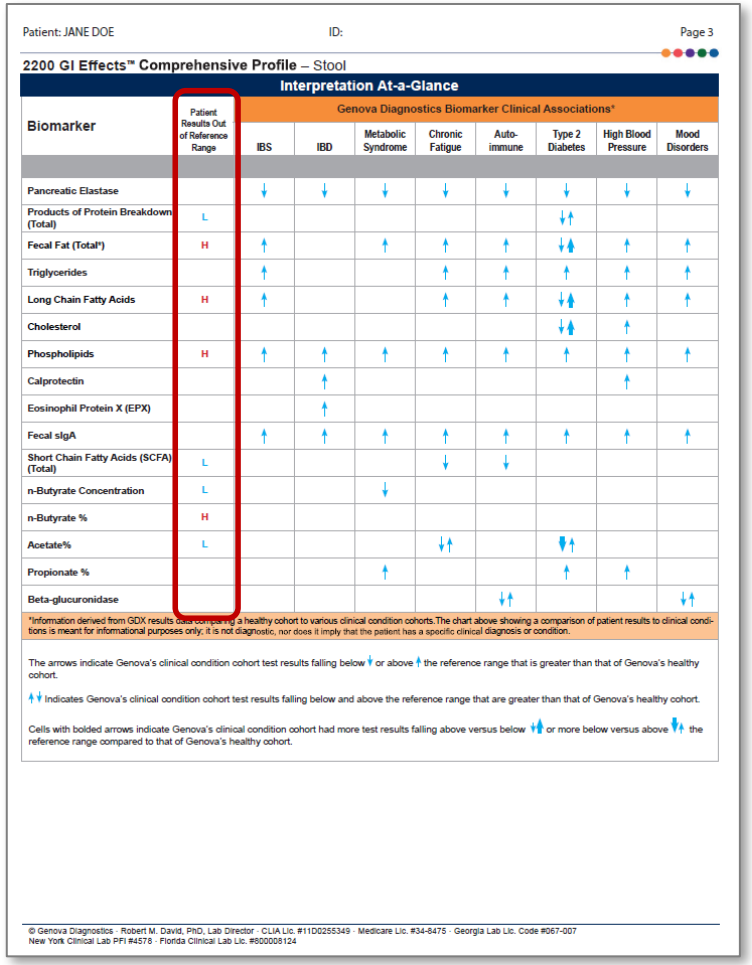
- Features of this chart include:
  - A list of 16 biomarkers providing insight into digestive insufficiency, inflammation and gut microbiome metabolic activity
  - A column indicating any patient result that is out of reference range – either high (H) or low (L)
  - Clinical Associations for eight specific disease states

GENOVA DIAGNOSTICS

Biomarker Clinical Association Chart



Patient: JANE DOE ID: Page 3

2200 GI Effects™ Comprehensive Profile – Stool

Interpretation At-a-Glance

Biomarker	Patient Results Out of Reference Range	Genova Diagnostics Biomarker Clinical Associations*							
		IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
Pancreatic Elastase		↓	↓	↓	↓	↓	↓	↓	↓
Products of Protein Breakdown (Total)	L						↓↑		
Fecal Fat (Total*)	H	↑		↑	↑	↑	↓↑	↑	↑
Triglycerides		↑			↑	↑	↑	↑	↑
Long Chain Fatty Acids	H	↑			↑	↑	↓↑	↑	↑
Cholesterol							↓↑	↑	
Phospholipids	H	↑	↑	↑	↑	↑	↑	↑	↑
Calprotectin			↑					↑	
Eosinophil Protein X (EPX)			↑						
Fecal sIgA		↑	↑	↑	↑	↑	↑	↑	↑
Short Chain Fatty Acids (SCFA) (Total)	L				↓	↓			
n-Butyrate Concentration	L			↓					
n-Butyrate %	H								
Acetate%	L				↓↑		↓↑		
Propionate %				↑			↑	↑	
Beta-glucuronidase						↓↑			↓↑

*Information derived from GDX results comparing a healthy cohort to various clinical condition cohorts. The chart above showing a comparison of patient results to clinical conditions is meant for informational purposes only, it is not diagnostic, nor does it imply that the patient has a specific clinical diagnosis or condition.

The arrows indicate Genova's clinical condition cohort test results falling below ↓ or above ↑ the reference range that is greater than that of Genova's healthy cohort.

↓↑ Indicates Genova's clinical condition cohort test results falling below and above the reference range that are greater than that of Genova's healthy cohort.

Cells with bolded arrows indicate Genova's clinical condition cohort had more test results falling above versus below ↓↑ or more below versus above ↓↑ the reference range compared to that of Genova's healthy cohort.

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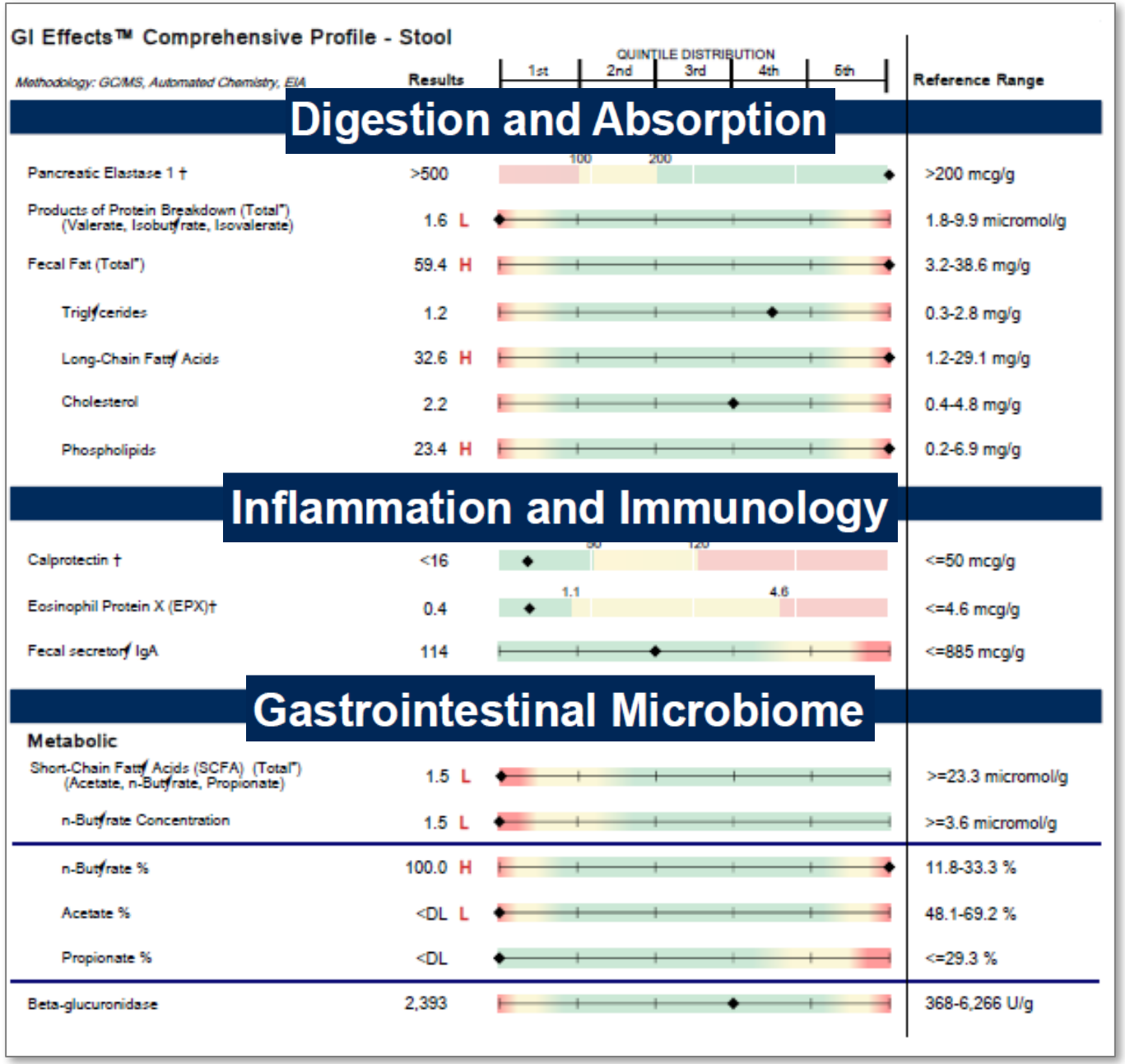
- Biomarkers found to have relevant associations with clinical conditions are displayed, along with the patient's results for these biomarkers
- These associations are:
  - Part of an ongoing research program
  - Will most likely evolve over time as data accrues and is refined
  - Reported for informational and clinical interest purposes only

GENOVA DIAGNOSTICS

D

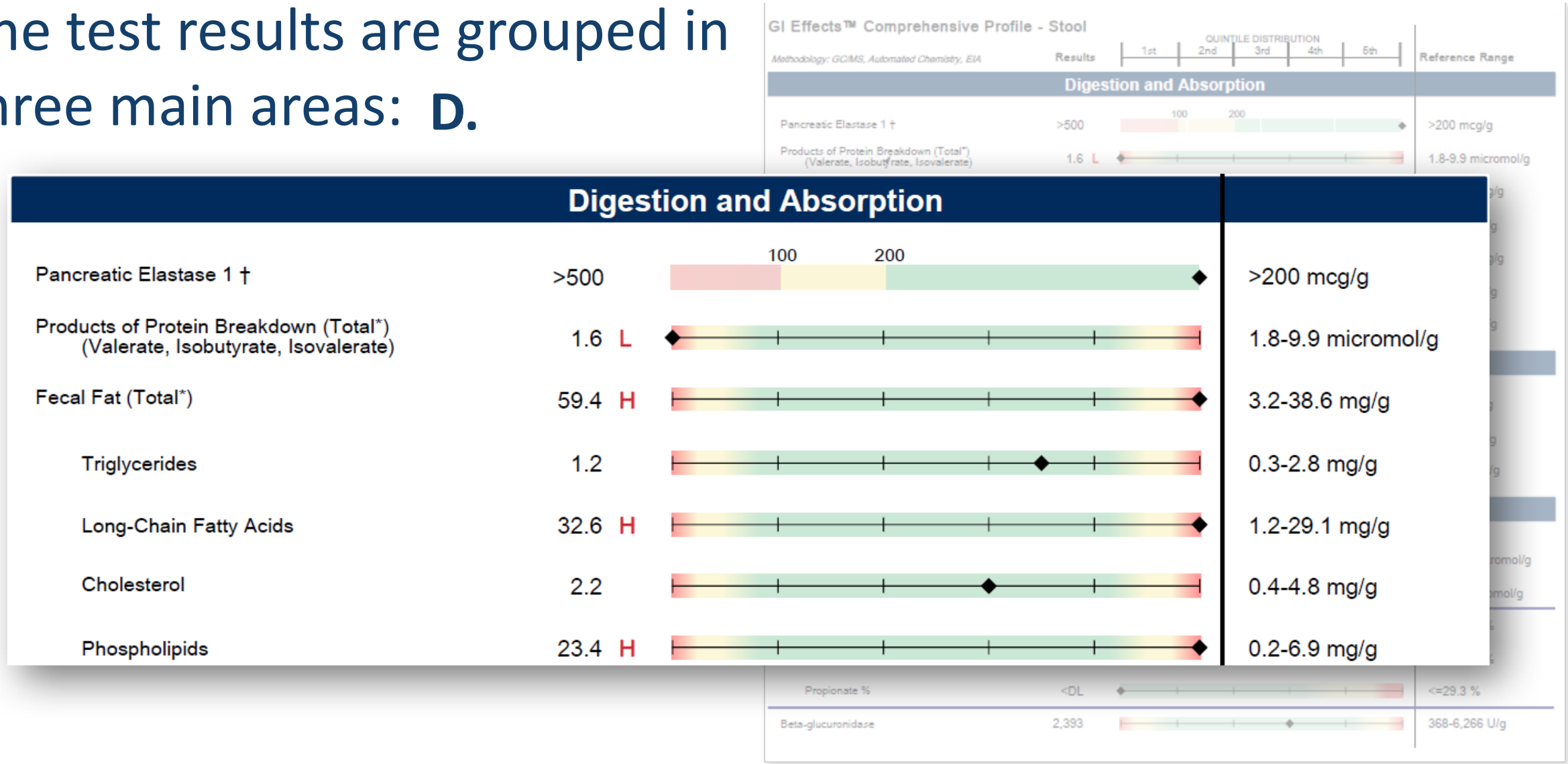
I

G



GENOVA DIAGNOSTICS

The test results are grouped in three main areas: **D.**



GENOVA DIAGNOSTICS

Pancreatic Elastase 1 (PE-1)

PE-1 is a proteolytic enzyme exclusively secreted by the pancreas

- Noninvasive biomarker of exocrine pancreatic function
- Reported according to Medical Decision Points

PE-1 (µ/g)	Interpretation
200 to >500	Normal pancreatic exocrine function
100 to 199	Mild-to-Moderate insufficiency
<100	Severe insufficiency

GENOVA DIAGNOSTICS

Pancreatic Exocrine Insufficiency (PEI)

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.3748/wjg.v19.i42.7258

World J Gastroenterol 2013 November 14; 19(42): 7258-7266
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TOPIC HIGHLIGHT

Asbjørn Mohr Drewes, MD, PhD, DMSc, Professor, *Series Editor*

Diagnosis and treatment of pancreatic exocrine insufficiency

Björn Lindkvist

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Accepted: September 16, 2013
Published online: November 14, 2013

spheres during meals. The dose should be in proportion to the fat content of the meal, usually 40-50000 lipase units per main meal, and half the dose is required for a snack. In cases that do not respond to initial treatment, the doses can be doubled, and proton inhibitors can be added to the treatment. This review focuses on current concepts of the diagnosis and treatment of pancreatic exocrine insufficiency.

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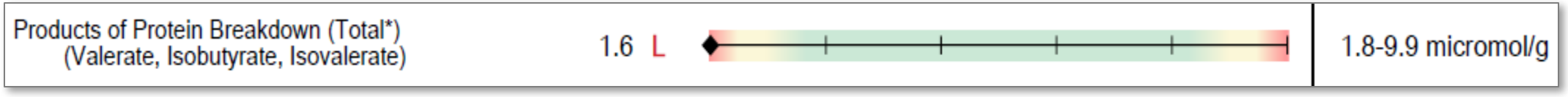
Key words: Chronic pancreatitis; Pancreatic exocrine insufficiency; Pancreatic enzyme replacement therapy

- Pancreatic exocrine insufficiency (PEI) is an important cause of maldigestion
- Symptoms in patients with PEI vary, depending on the degree and etiology of PEI
- Normal digestion requires adequate stimulation of pancreatic secretion, sufficient production of digestive enzymes by pancreatic acinar cells, and adequate mixing of pancreatic juice with ingested food
- The pancreatic juice plays a pivotal role in the digestion and absorption of nutrients

GENOVA DIAGNOSTICS

Drewes A. *World Journal of Gastroenterology*. 2013;19(42):7258-66.

Products of Protein Breakdown



Products of Protein Breakdown (Isovalerate, Valerate and Isobutyrate)

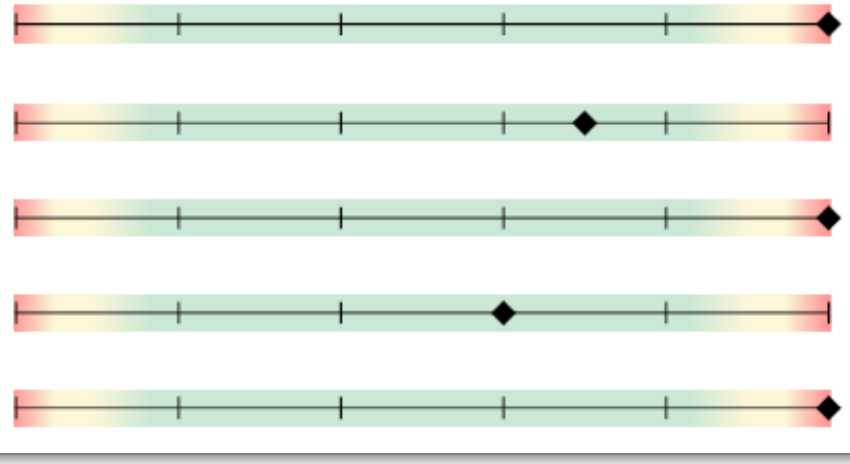
- Derived from bacterial fermentation of protein in the distal colon
- Normal protein digestion and absorption is relatively complete in the stomach and small intestine

Increased fecal Products of Protein Breakdown may be due to:

- Excessive delivery of protein to the colon, (ex: hypochlorhydria)
- Insufficient pancreatic proteases
- Excessive protein intake
- Increased colonic protein due to a GI bleed, excess mucus, bacterial overgrowth

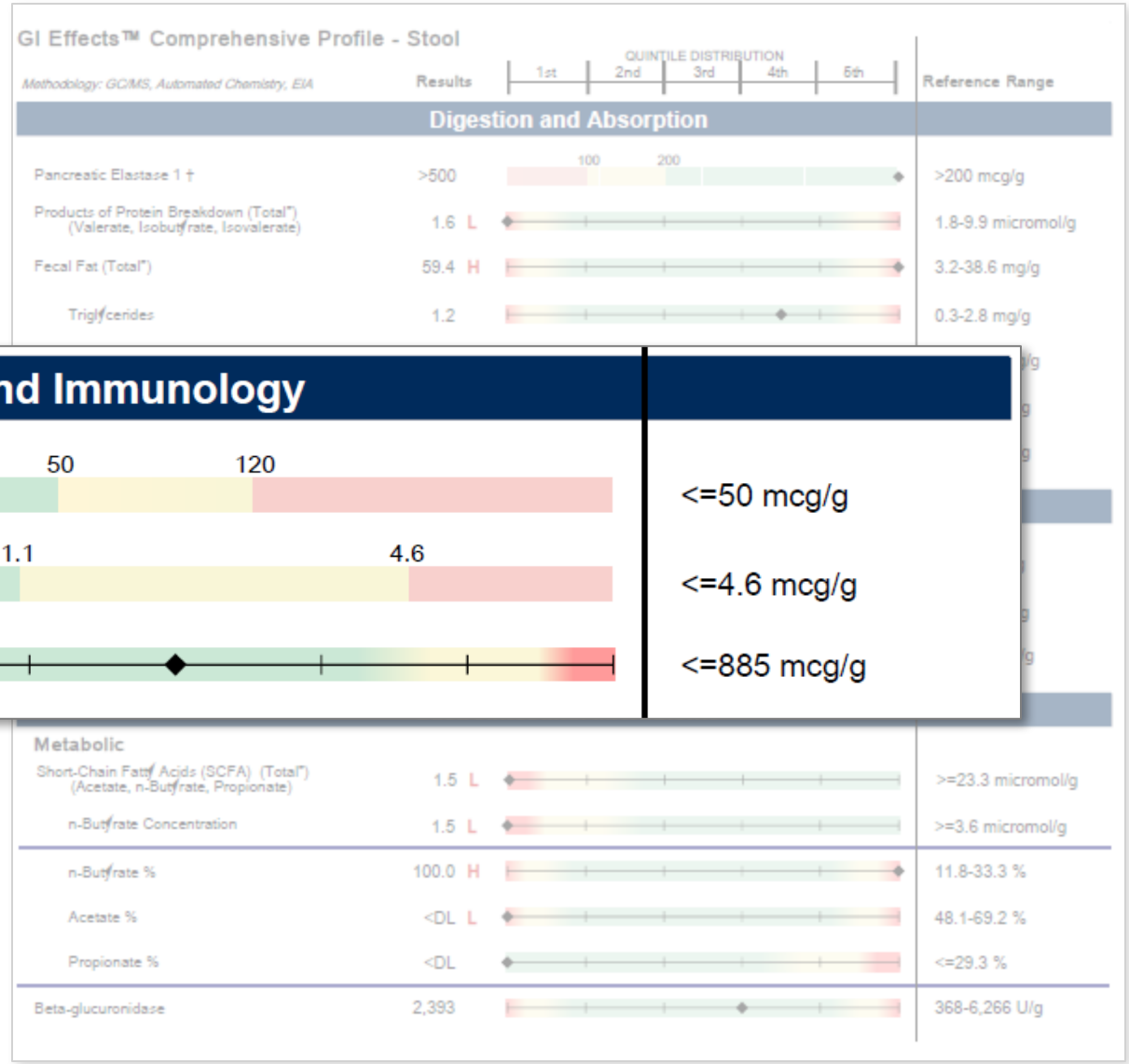
Fecal Fat (Total)

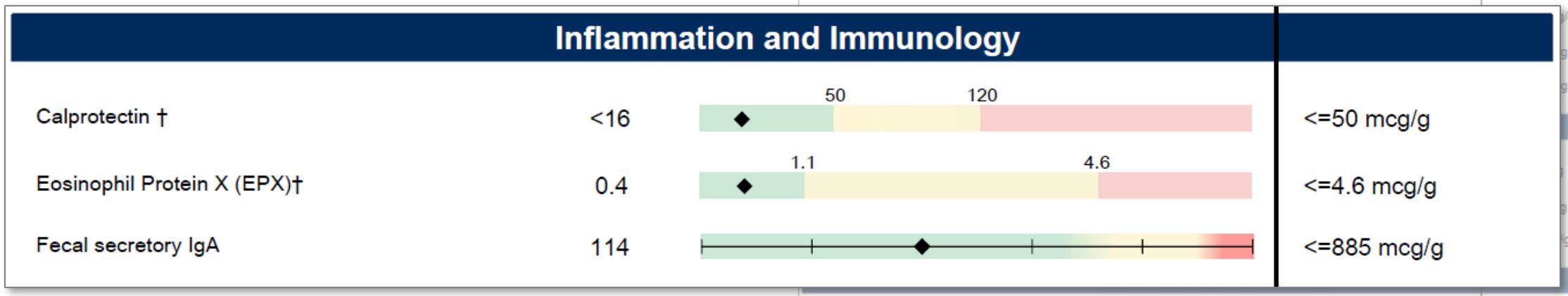
Fecal Fat (Total*)	59.4	H	3.2-38.6 mg/g
Triglycerides	1.2		0.3-2.8 mg/g
Long-Chain Fatty Acids	32.6	H	1.2-29.1 mg/g
Cholesterol	2.2		0.4-4.8 mg/g
Phospholipids	23.4	H	0.2-6.9 mg/g



- The Fecal Fat test is a fecal fat extraction method that results in a quantitative value
- Elevated fecal fat may indicate maldigestion, malabsorption, or steatorrhea
  - Also query: transit time and dietary intake

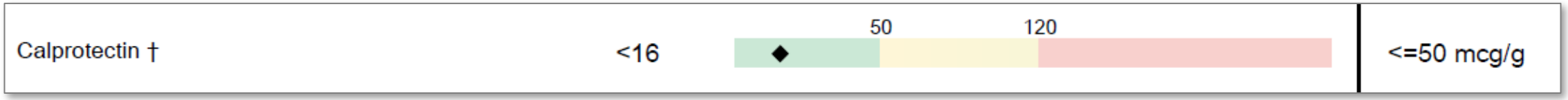
The test results are grouped in three main areas: **D. I.**





GENOVA DIAGNOSTICS

Calprotectin



- Neutrophil mediated inflammation in the gastrointestinal tract
  - Utilized for differentiating IBS from IBD
  - Quantify the degree of inflammation

Calprotectin (µg/g stool)	Interpretation	Follow up
≤50	Normal (no GI inflammation)	None
50 to 120	Borderline, suggestive of low grade inflammation	Re-evaluate at 4-6 weeks
>120	Abnormal	Determine source of inflammation and repeat test as clinically indicated
>250	Associated with high risk of clinical relapse	Adjust therapy accordingly

GENOVA DIAGNOSTICS

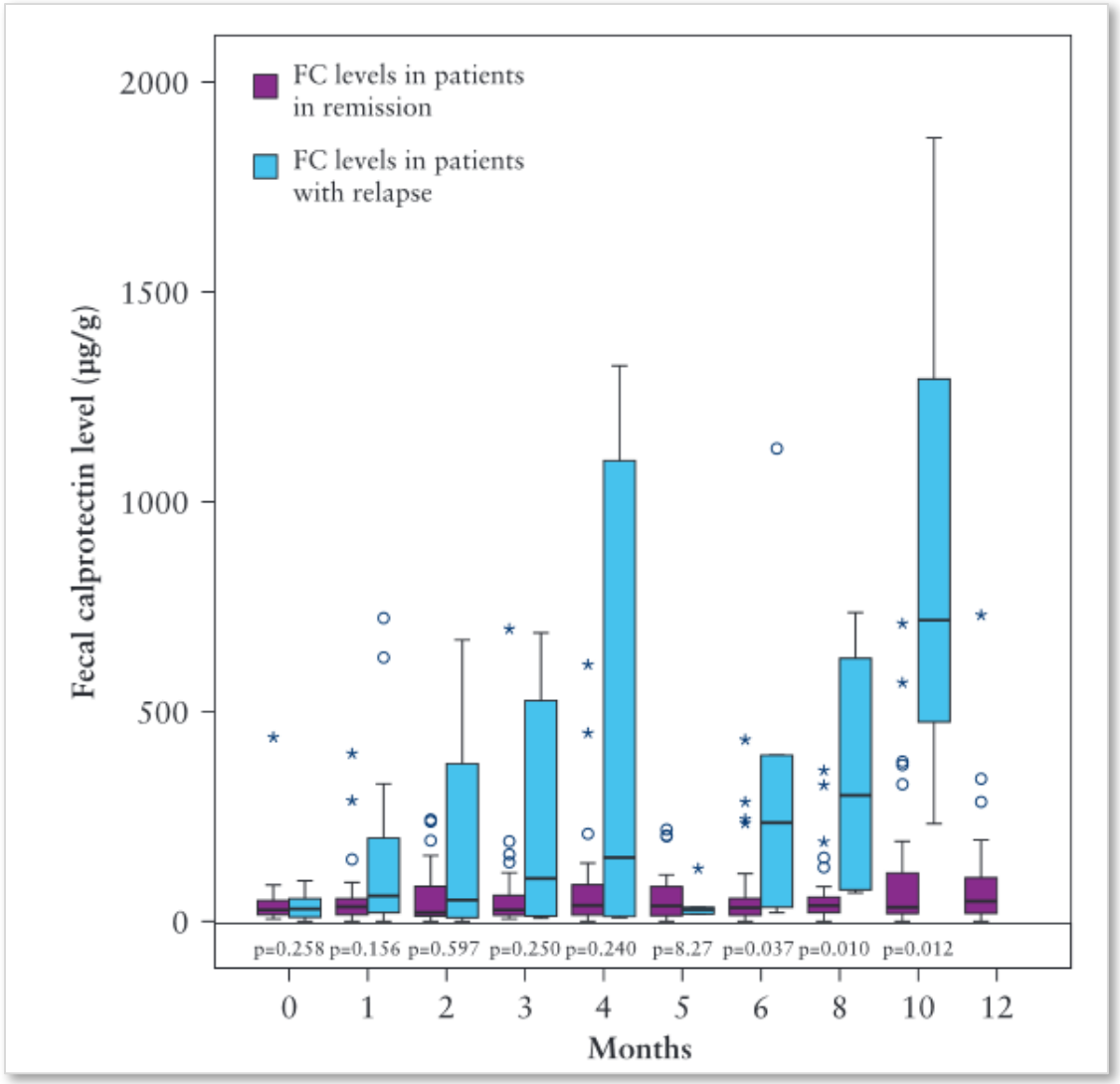
Calprotectin

Original Article

Does Fecal Calprotectin Predict Short-Term Relapse After Stopping Tnfα-Blocking Agents In Inflammatory Bowel Disease Patients In Deep Remission?

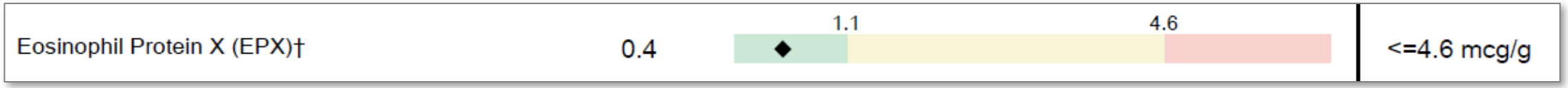
Pauliina Molander[a,*], Martti Färkkilä[b,m], Ari Ristimäki[c], Kimmo Salminen[d], Helena Kemppainen[d], Timo Blomster[e], Ritva Koskela[e], Airi Jussila[f], Henna Rautiainen[g], Markku Nissinen[h], Johanna Haapamäki[b], Perttu Arkkila[b], Urpo Nieminen[b], Juha Kuisma[i], Jari Punkkinen[j], Kaija-Leena Kolho[k,m], Harri Mustonen[l], Taina Sipponen[b]

FC seems to increase and remain elevated before clinical or endoscopic relapse, suggesting that it can be used as a surrogate marker for predicting and identifying patients requiring close follow-up in clinical practice.



Molander, P. et al. *Journal of Crohns Colitis*. 2015;9(1):33-40.

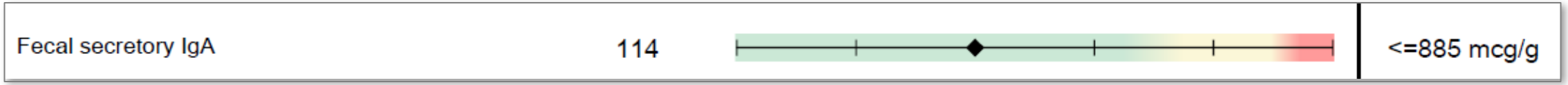
Eosinophil Protein X



- Eosinophil medicated inflammation in the gastrointestinal tract
  - Associated with inflammation and tissue damage

- Clinically, elevations in EPX indicate the presence of an IgE-mediated inflammatory process
  - Common associations are food allergies, parasitic infection, and IBD

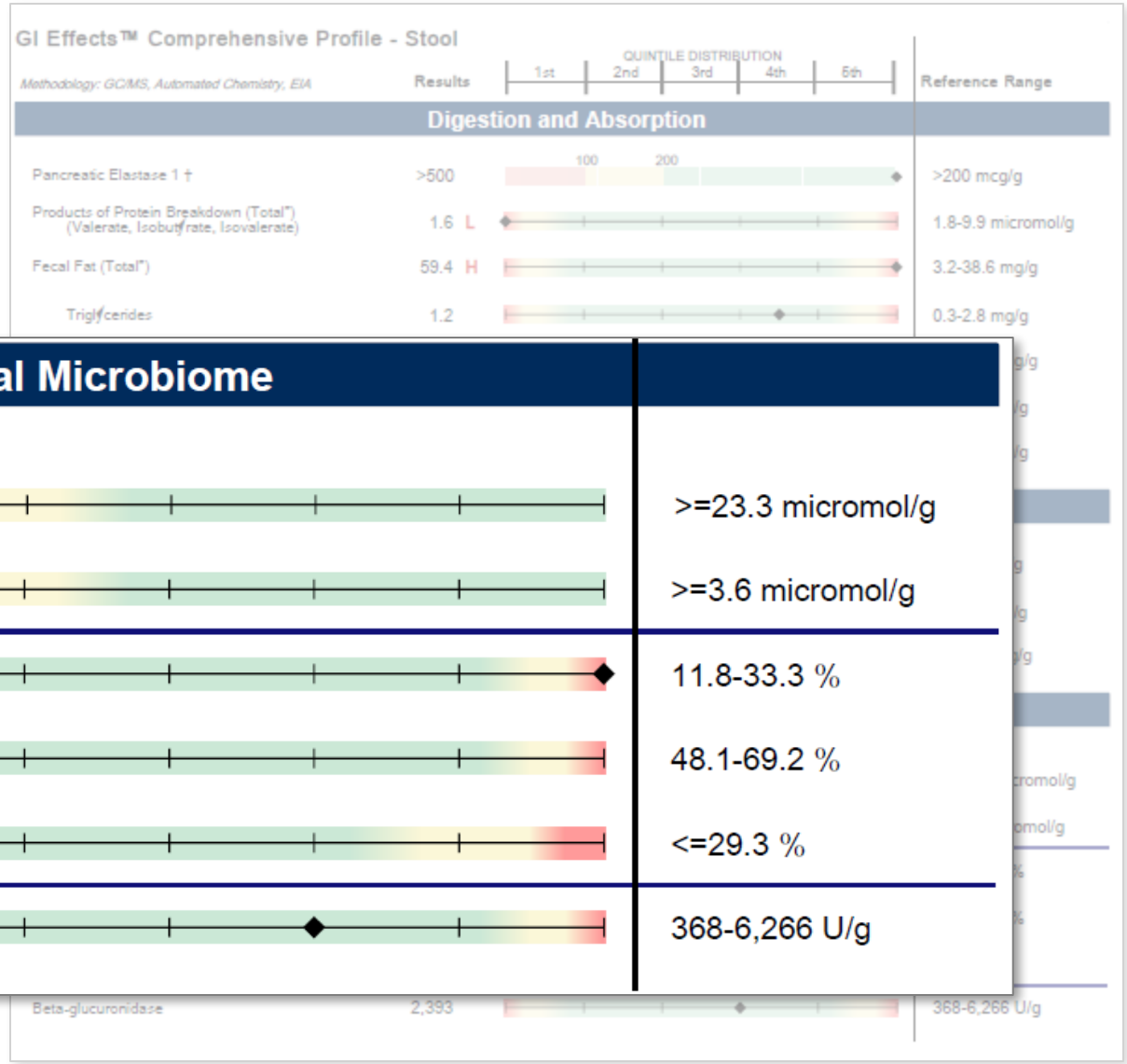
Fecal Secretory IgA



- Produced across mucosal surfaces, sIgA is the first line of defense in protecting the gastrointestinal epithelium from enteric toxins and pathogenic organisms

- Provides information on the competence of mucosal immunity and the potential risk for epithelial barrier dysfunction

The test results are grouped in three main areas: **D. I. G.**



Gastrointestinal Microbiome

Metabolic

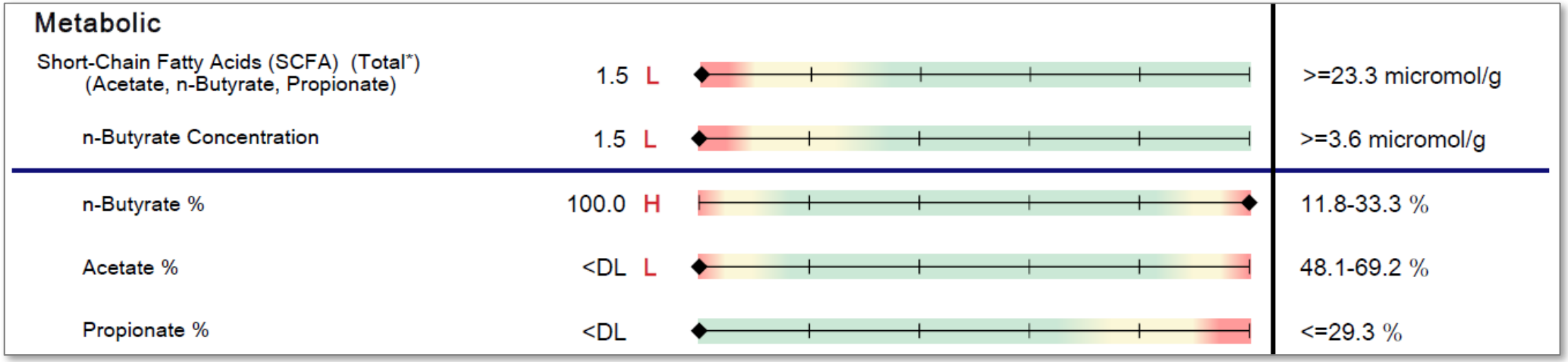
Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	1.5	L	>=23.3 micromol/g
n-Butyrate Concentration	1.5	L	>=3.6 micromol/g
n-Butyrate %	100.0	H	11.8-33.3 %
Acetate %	<DL	L	48.1-69.2 %
Propionate %	<DL		<=29.3 %
Beta-glucuronidase	2,393		368-6,266 U/g

GENOVA DIAGNOSTICS

G/Gastrointestinal Microbiome

- Metabolic
  - Short Chain Fatty Acids
  - Beta glucuronidase
- Commensal Bacteria (PCR)
- Bacteriology & Mycology (culture and MALDI-TOF)
  - Sensitivities provided if applicable
- Parasitology (microscopy and EIA)
- Pathogens (culture and MALDI-TOF, add-on via EIA)

Short Chain Fatty Acids

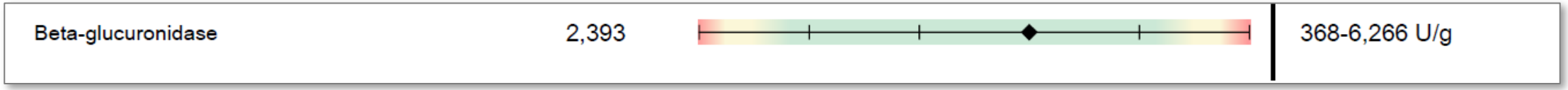


Metabolic			
Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	1.5 L		>=23.3 micromol/g
n-Butyrate Concentration	1.5 L		>=3.6 micromol/g
n-Butyrate %	100.0 H		11.8-33.3 %
Acetate %	<DL L		48.1-69.2 %
Propionate %	<DL		<=29.3 %

- Short Chain Fatty Acids (SCFA): Acetate, n-Butyrate and Propionate are produced by anaerobic bacterial fermentation of indigestible carbohydrate (fiber)
  - Important roles in maintaining colonic health and integrity
- Altered levels of fecal SCFA is an indicator of altered intestinal microbial composition

GENOVA DIAGNOSTICS

Beta-glucuronidase

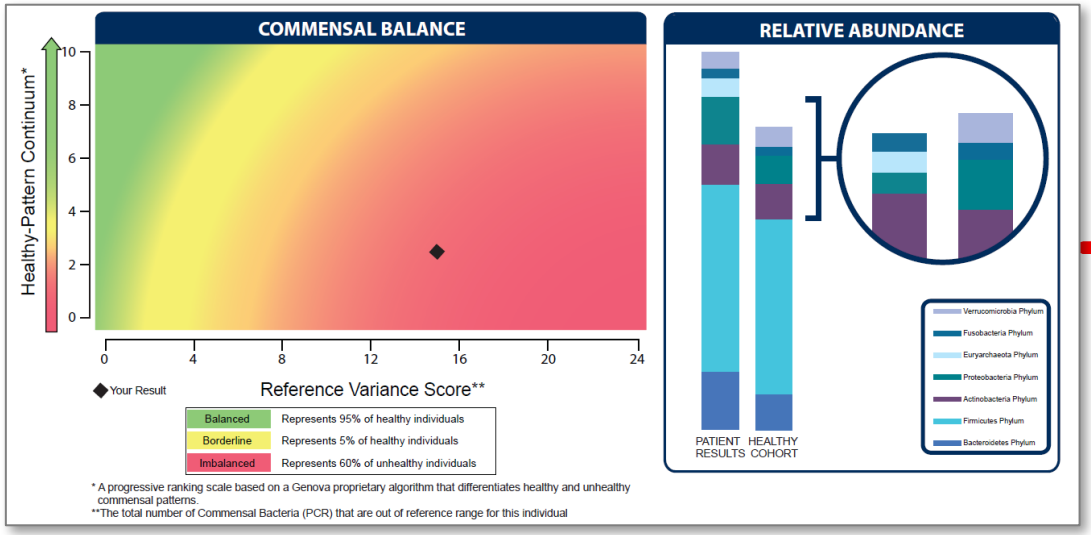


- Beta-glucuronidase is an enzyme induced by anaerobic bacteria
  - Many toxins, hormones, and drugs are excreted from the body after conjugation to a glucuronide molecule. Beta-glucuronidase can uncouple these conjugates, freeing these potential carcinogens in the bowel and promoting recirculation of these compounds through enterohepatic pathways
- A moderate level of Beta-glucuronidase activity is preferred as activity appears to be important for normal enterohepatic recirculation of endogenous compounds and vitamins
- Limited human studies show a relationship to colon cancer and hormone-related cancers
  - Calcium D-glucarate inhibits action of enzyme

G/Gastrointestinal Microbiome

24 Commensal targets (PCR technology)

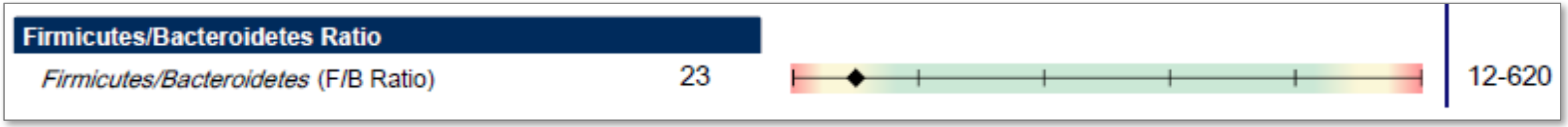
Commensal Balance and Relative Abundance graphics serve as broad markers of gut health



Commensal Bacteria (PCR)	Result CFU/g stool		QUINTILE DISTRIBUTION 1st 2nd 3rd 4th 5th	Reference Range CFU/g stool
Bacteroidetes Phylum				
Bacteroides-Prevotella group	1.4E9			3.4E6-1.5E9
Bacteroides vulgatus	4.8E9	H		<=2.2E9
Barnesiella spp.	4.3E8	H		<=1.6E8
Odoribacter spp.	2.3E8	H		<=8.0E7
Prevotella spp.	5.9E7	H		1.4E5-1.6E7
Firmicutes Phylum				
Anaerotruncus colihominis	4.3E7	H		<=3.2E7
Butyrivibrio crossotus	2.0E5			5.5E3-5.9E5
Clostridium spp.	2.6E10	H		1.7E8-1.5E10
Coprococcus eutactus	1.5E8	H		<=1.2E8
Faecalibacterium prausnitzii	1.2E10	H		5.8E7-4.7E9
Lactobacillus spp.	8.1E9	H		8.3E6-5.2E9
Pseudoflavonifractor spp.	4.3E8	H		4.2E5-1.3E8
Roseburia spp.	1.6E9			1.3E8-1.2E10
Ruminococcus spp.	5.2E7	L		9.5E7-1.6E9
Veillonella spp.	1.2E8	H		1.2E5-5.5E7
Actinobacteria Phylum				
Bifidobacterium spp.	3.2E9			<=6.4E9
Bifidobacterium longum	2.1E8			<=7.2E8
Collinsella aerofaciens	8.0E8			1.4E7-1.9E9
Proteobacteria Phylum				
Desulfovibrio piger	5.6E5			<=1.8E7
Escherichia coli	4.2E8	H		9.0E4-4.6E7
Oxalobacter formigenes	3.7E7	H		<=1.5E7
Euryarchaeota Phylum				
Methanobrevibacter smithii	2.0E8	H		<=8.6E7
Fusobacteria Phylum				
Fusobacterium spp.	1.8E4			<=2.4E5
Verrucomicrobia Phylum				
Akkermansia muciniphila	5.9E7			>=1.2E6

GENOVA DIAGNOSTICS

Firmicutes/Bacteroidetes Ratio



- The F/B Ratio is a comparison of the 2 largest phyla
- F/B Ratio has been associated with disruption of metabolic homeostasis
  - Type 2 diabetes
  - Non-alcoholic fatty liver disease
  - Body Mass Index (BMI)

Tehrani A, et al. *Neurogastroenterology & Motility.* 2012;24(4):305-311.
Koliada A, et al. *BMC Microbiology.* 2017;17(1):120-26.

Firmicutes/Bacteroidetes Ratio

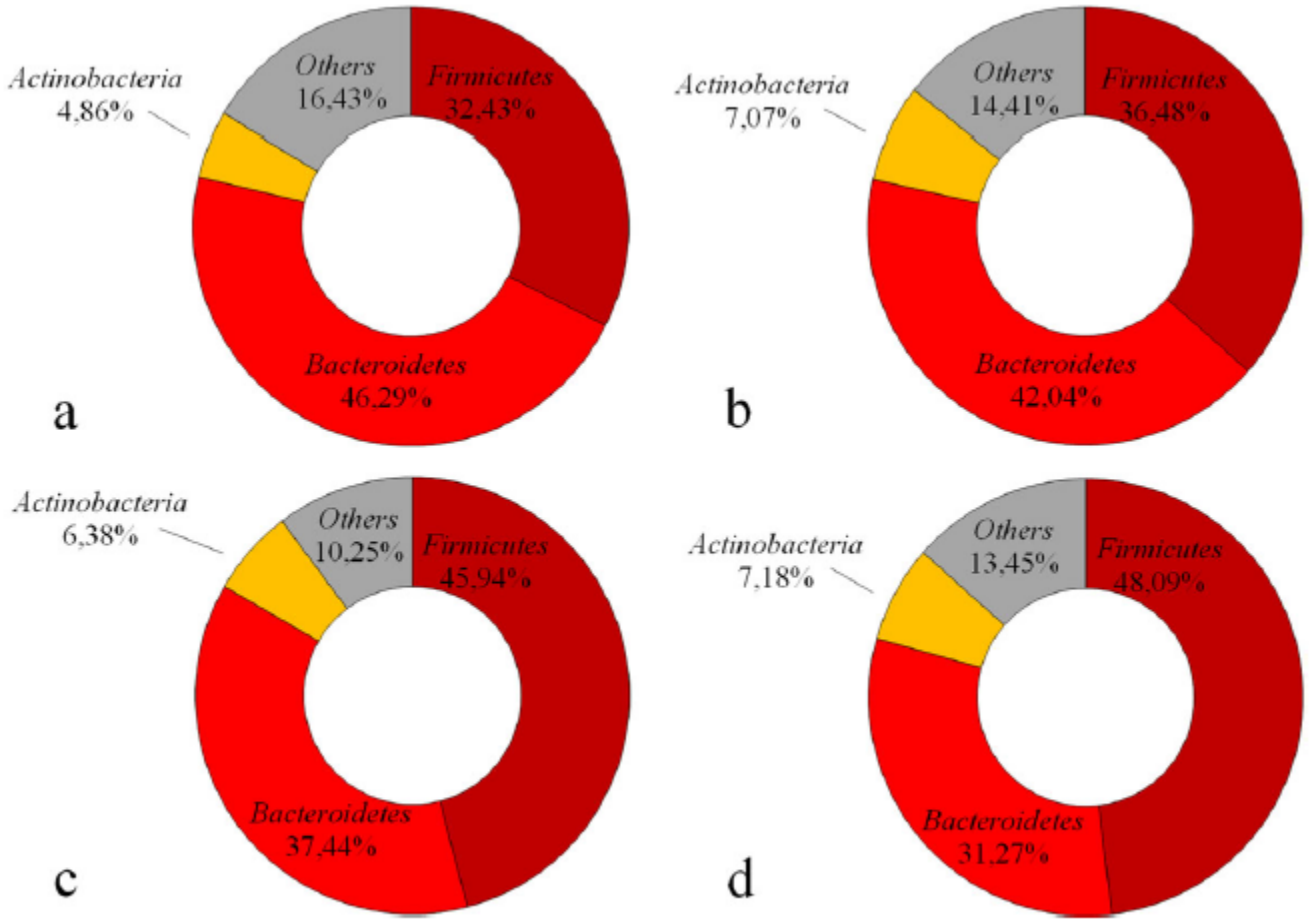
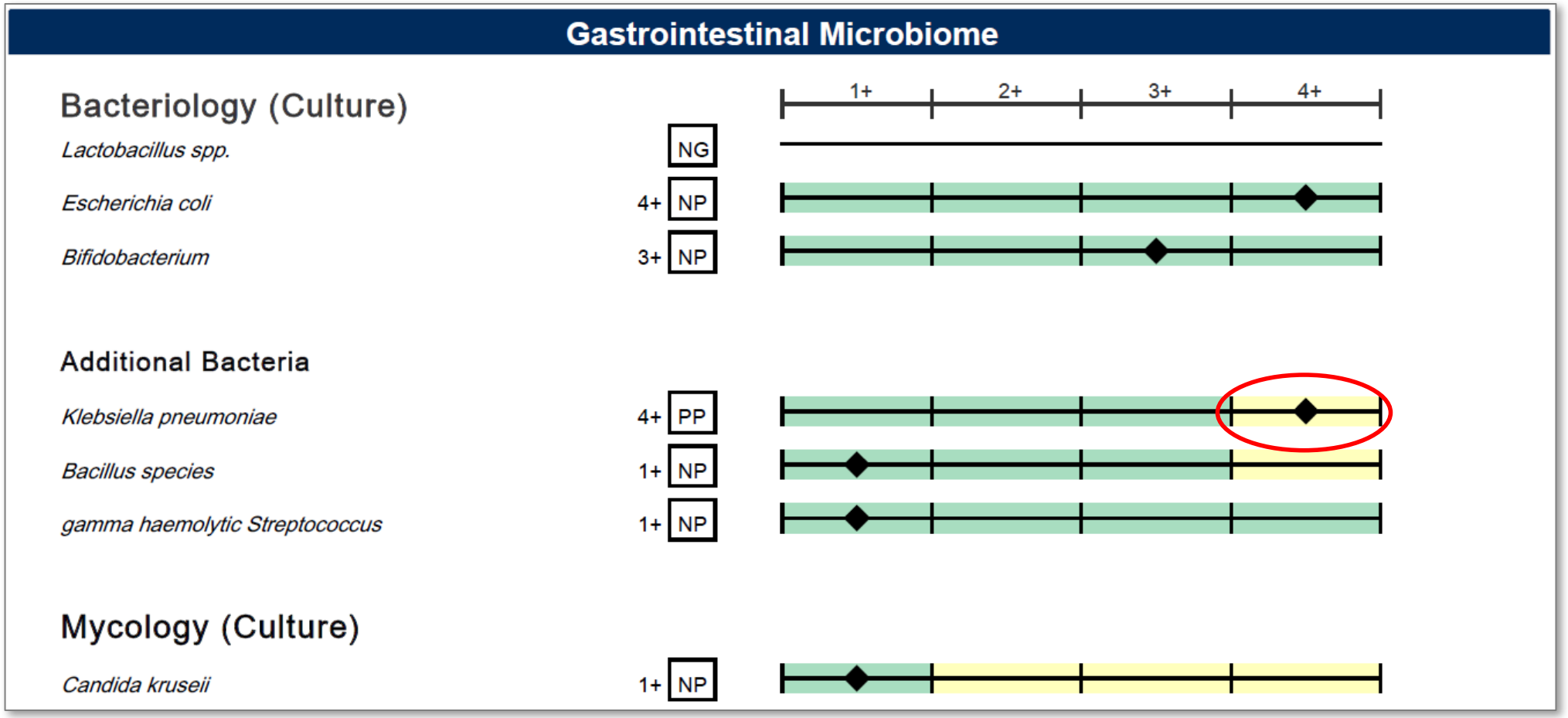


Fig. 1 The relative abundance of the major microbial phyla in different BMI categories (**a** BMI < 18.5, **b** BMI 18.5–24.9, **c** BMI 25–29.9 and **d** BMI ≥30)

GENOVA DIAGNOSTICS

Koliada, A, et al. *BMC Microbiology.* 2017;17:120-26.

Gastrointestinal Microbiome



Microbiology Legend

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

GENOVA DIAGNOSTICS

Bacteriology and Mycology

- Methodology is culture with MALDI-TOF MS identification
  - Vitex – MS (mass spectrometry) is an FDA-cleared platform for the rapid identification of bacteria and yeast from isolated culture colonies
  - Matrix Assisted Laser Desorption Ionization Time-Of-Flight

- MALDI-TOF examines patterns of proteins detected from intact bacteria and yeast isolated cultures
  - Technology ionizes chemical compounds (a combination of the culture sample to be examined and an assay matrix) to generate charged molecules, which provide a ‘tell-tale’ signature for identification

GENOVA DIAGNOSTICS

Bacteria Sensitivity

Prescriptive Agents

Klebsiella pneumoniae	R	I	S-DD	S	NI
Ampicillin	R				
Amox./Clavulanic Acid				S	
Cephalothin				S	
Ciprofloxacin				S	
Tetracycline				S	
Trimethoprim/Sulfa				S	

Natural Agents

Klebsiella pneumoniae	LOW INHIBITION	HIGH INHIBITION
Berberine		
Oregano		
Plant Tannins		
Uva-Ursi		

GENOVA DIAGNOSTICS

Mycology Sensitivity

Azole Antifungals

Candida kruseii	R	I	S-DD	S	NI
Fluconazole					32
Voriconazole				0.25	

Non-absorbed Antifungals

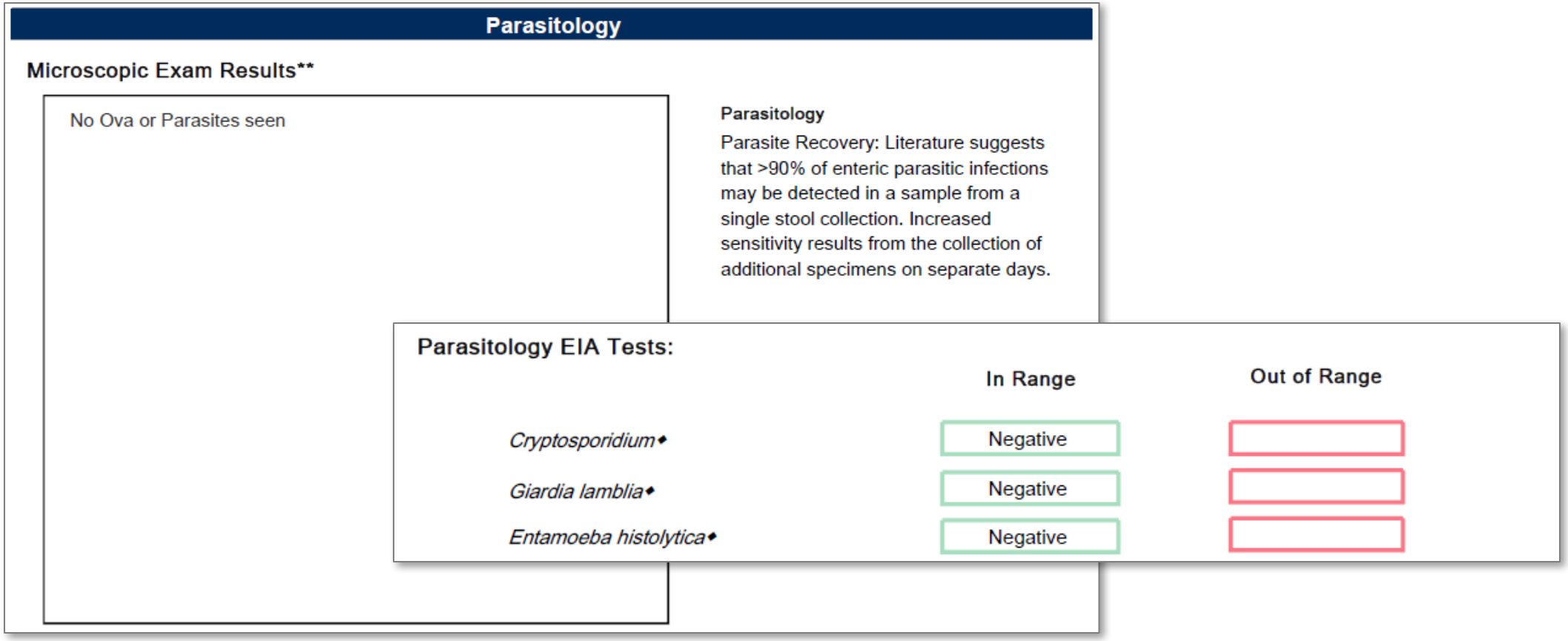
Candida kruseii	LOW INHIBITION	HIGH INHIBITION
Nystatin		

Natural Agents

Candida kruseii	LOW INHIBITION	HIGH INHIBITION
Berberine		
Caprylic Acid		
Garlic		
Undecylenic Acid		
Plant tannins		
Uva-Ursi		

GENOVA DIAGNOSTICS

G/Gastrointestinal Microbiome – Parasitology



Parasitology

Microscopic Exam Results**

No Ova or Parasites seen

Parasitology

Parasite Recovery: Literature suggests that >90% of enteric parasitic infections may be detected in a sample from a single stool collection. Increased sensitivity results from the collection of additional specimens on separate days.

Parasitology EIA Tests:

	In Range	Out of Range
Cryptosporidium◆	Negative	
Giardia lamblia◆	Negative	
Entamoeba histolytica◆	Negative	

GENOVA DIAGNOSTICS

Additional Results

Additional Results		
	Result	**Expected Value**
Fecal Occult Blood◆	Negative	Negative
Color††	Brown	
Consistency††	Formed/Normal	

Macroscopic Exam for Worms**
No larvae seen. Specimen contains mucus/mucosal material.

GENOVA DIAGNOSTICS

Zonulin (add-on)

Stool Zonulin		
	Result	Reference Range
Zonulin, Stool	62.5	22.3-161.1 ng/mL

- Zonulin is a protein modulator of intestinal tight junctions (cellular structures that are critical for maintaining the integrity of the intestinal barrier)
- Release of zonulin has been shown to alter intestinal barrier integrity
- When tight junctions open, the intestinal barrier becomes permeable (intestinal permeability)
  - This is important for normal physiologic function
  - Prolonged release of zonulin is correlated with a dysfunctional intestinal barrier

Zonulin (add-on)

- Prolonged compromise of intestinal barrier function (altered intestinal permeability) has been associated with a wide range of conditions encompassing gastrointestinal as well as systemic conditions
- Patients in whom zonulin testing may be most useful include:
  - Celiac Disease and other GI disorders (ex: non-celiac gluten sensitivity, IBS)
  - Cardiometabolic diseases including diabetes, obesity, NAFLD, insulin resistance, and associations with cardiometabolic risk and inflammatory markers

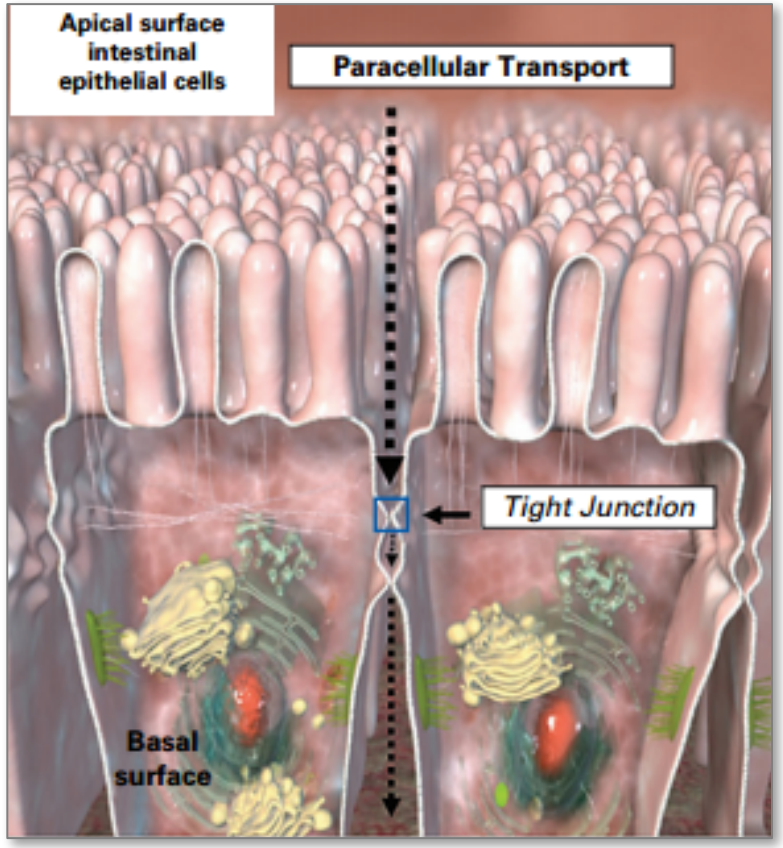


Image courtesy of: http://www.massgeneral.org/children/assets/pdf/cascades_winter2013.pdf

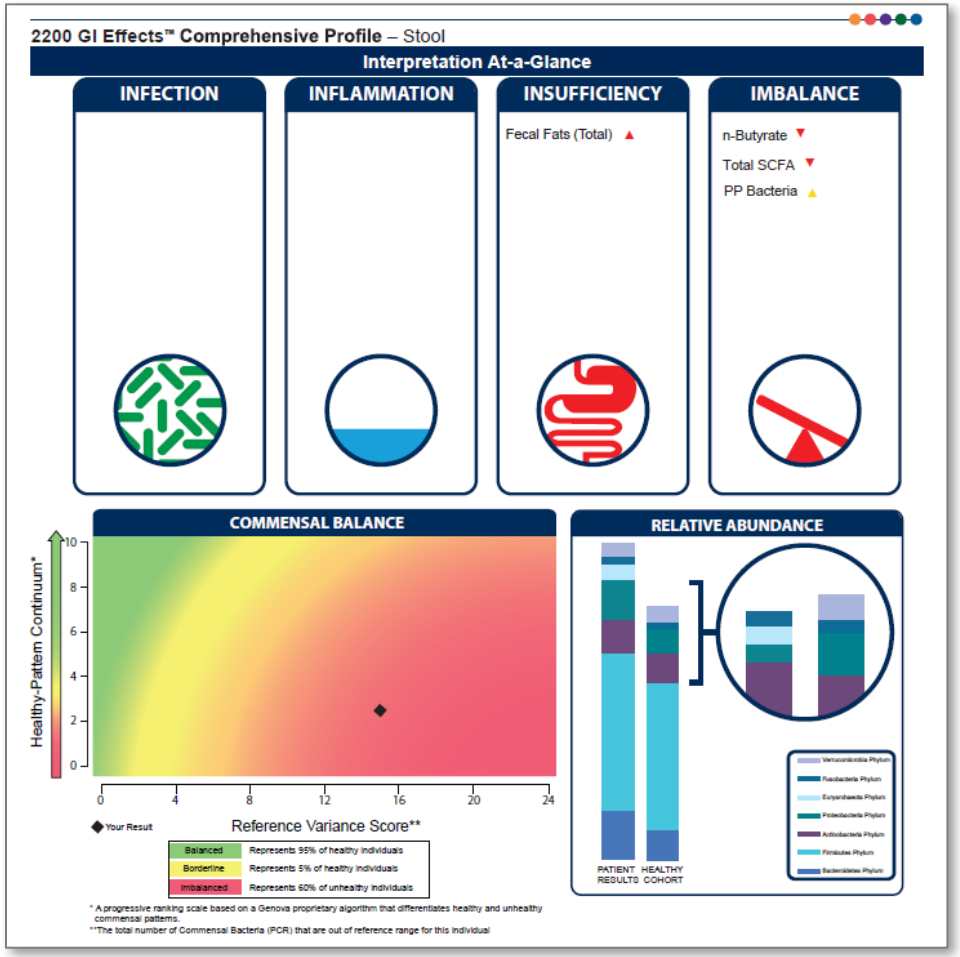
Fecal Occult Blood

	Result	Expected Value
Fecal Occult Blood◆	Negative	Negative

- Identifies microscopic amounts of blood in the stool
- Hemosure diagnostic kit utilizes immunochemical technology (FIT)
- Higher sensitivity and specificity
  - No medication, supplement, or dietary restrictions
  - Specific to human hemoglobin (monoclonal and polyclonal antibodies)
  - One positive test warrants further investigation

Case Example

- 52 y/o female with bloating and excessive belching and flatulence
- Constipation; uses a laxative three times per week to achieve a bowel movement
- Cerebellar ataxia
- Overweight
- Sedentary lifestyle
- WNL thyroid function
- Eats a standard American diet; sugar cravings & avoids fiber (aggravates symptoms)



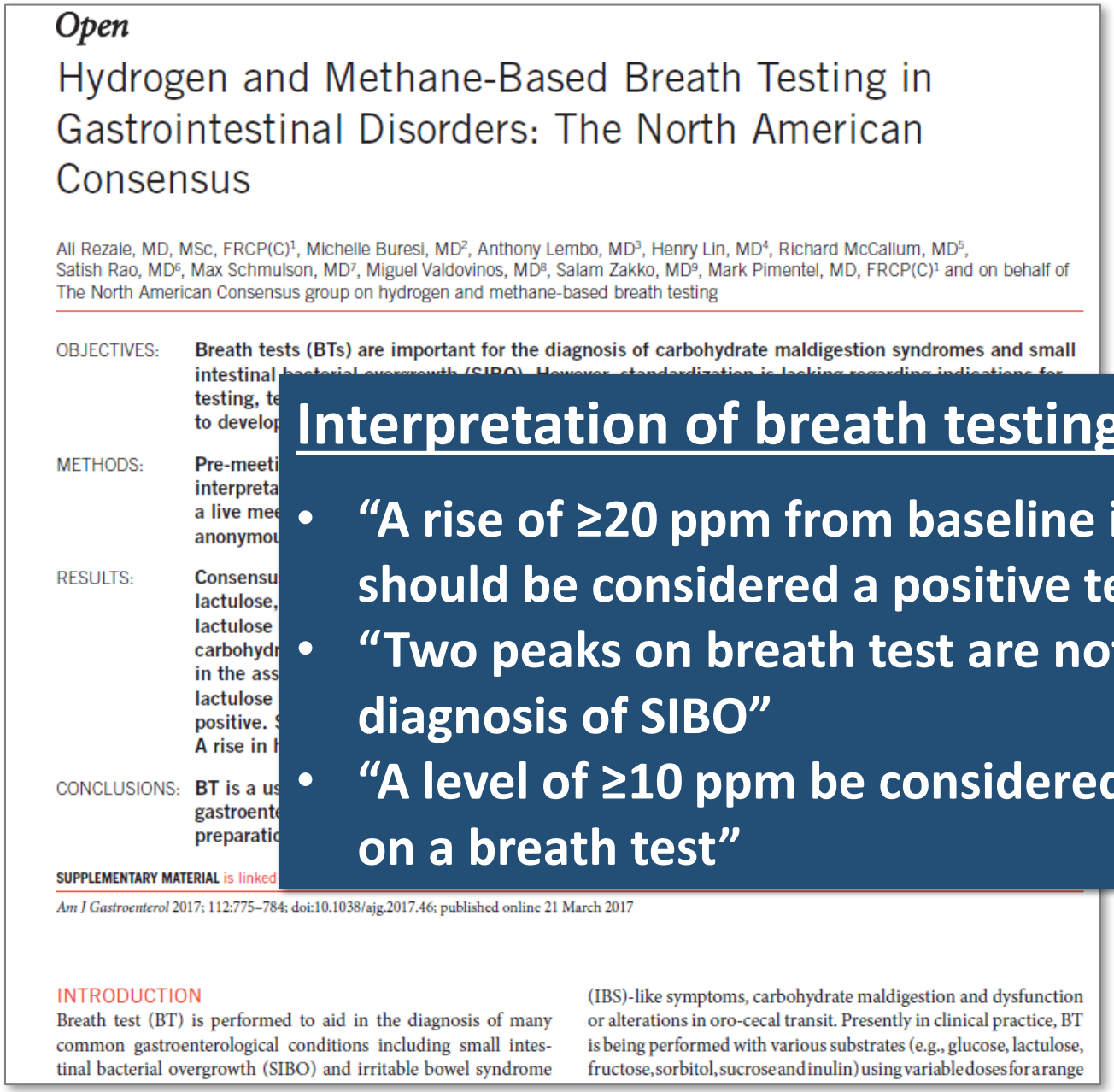
GENOVA DIAGNOSTICS

GI Effects® Stool Profile Overview & Considerations

- Maldigestion/absorption of fats
- Low dietary intake of meat; evaluate protein consumption
- Low SCFA; patient avoids fiber because it leads to an increase in gas and bloating
- PP Bacteria Klebsiella
- Elevated relative abundance compared to healthy cohort

GI Effects profile can not identify SIBO, however there are markers that can be suggestive in the right patient population

GENOVA DIAGNOSTICS



Open

Hydrogen and Methane-Based Breath Testing in Gastrointestinal Disorders: The North American Consensus

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OBJECTIVES: **Breath tests (BTs) are important for the diagnosis of carbohydrate maldigestion syndromes and small intestinal**

METHODS: **Pre-meeti**

RESULTS: **Consensu**

CONCLUSIONS: **BT is a us**

SUPPLEMENTARY MATERIAL is linked

Am J Gastroenterol 2017; 112:775–784; doi:10.1038/ajg.2017.46; published online 21 March 2017

INTRODUCTION

Breath test (BT) is performed to aid in the diagnosis of many common gastroenterological conditions including small intestinal bacterial overgrowth (SIBO) and irritable bowel syndrome (IBS)-like symptoms, carbohydrate maldigestion and dysfunction or alterations in oro-cecal transit. Presently in clinical practice, BT is being performed with various substrates (e.g., glucose, lactulose, fructose, sorbitol, sucrose and inulin) using variable doses for a range

Interpretation of breath testing results:

- "A rise of ≥20 ppm from baseline in hydrogen by 90 min should be considered a positive test for SIBO"
- "Two peaks on breath test are not required for the diagnosis of SIBO"
- "A level of ≥10 ppm be considered positive for methane on a breath test"

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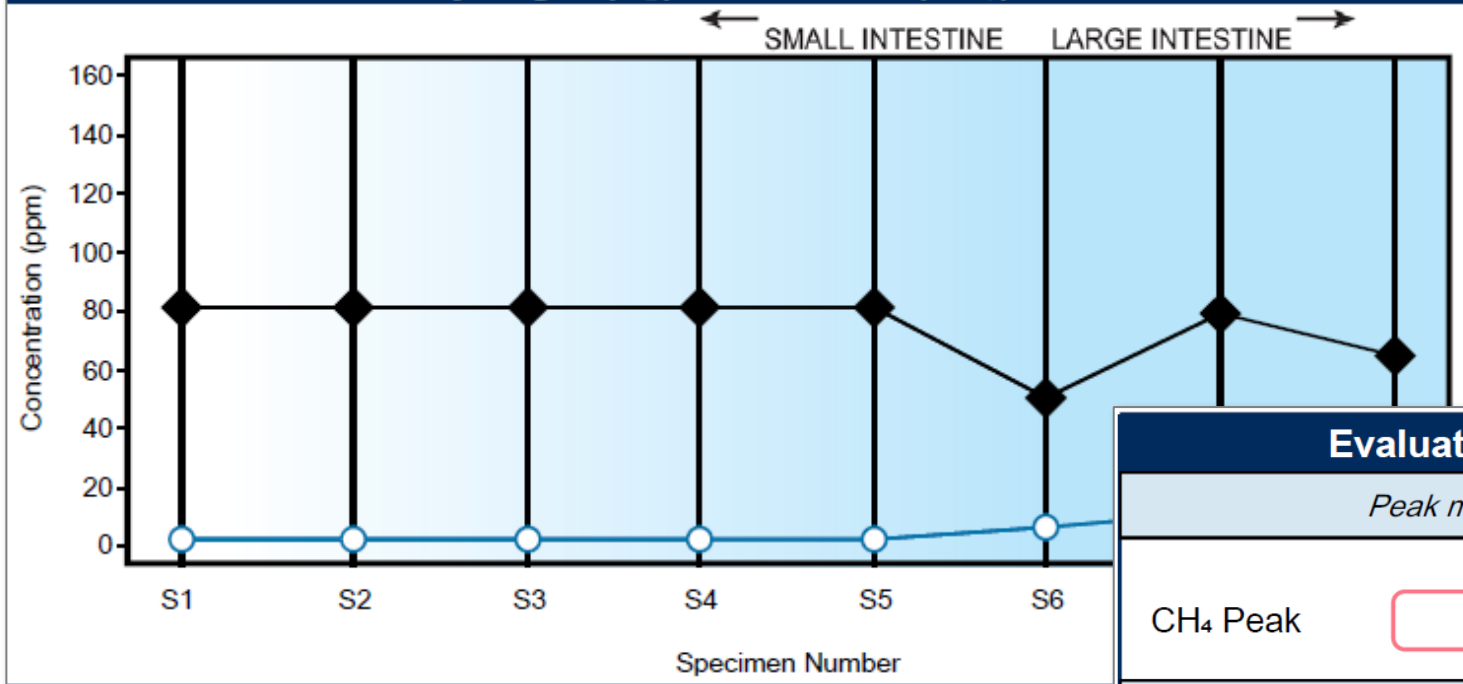
Rezaie A, et. al. *The American Journal of Gastroenterology*. 2017;112:775-84.

Hydrogen (H_2) and Methane (CH_4) Breath Gases



Evaluation for Methane (CH_4)

Peak methane level at any point

	Result		Expected Value
CH_4 Peak	>81	H	<10 ppm

A peak methane level ≥ 10 ppm at any point is indicative of a methane-positive result.

Hydrogen (H_2), Methane (CH_4) and Carbon Dioxide

	Baseline 0 min (S1)	20 min (S2)	40 min (S3)	60 min (S4)	90 min (S5)	120 min (S6)	150 min (S7)	180 min (S8)
H_2	2	<2	<2	<2	<2	6	11	7
CH_4	>81	>81	>81	>81	>81	50	78	64
$H_2 + CH_4$	NR	NR	NR	NR	NR	56	89	71
CO_2 **	✓	✓	✓	✓	✓	✓	✓	✓
Actual Collection Times								
Actual Time	6:10	6:30	6:50	7:10	7:40	8:10	8:40	9:10
Actual Interval	0 min	20 min	40 min	60 min	90 min	120 min	150 min	180 min

**CO_2 is measured for quality assurance. ✓ indicates the CO_2 level is acceptable. ✗ indicates room air contamination exceeding acceptable limits.

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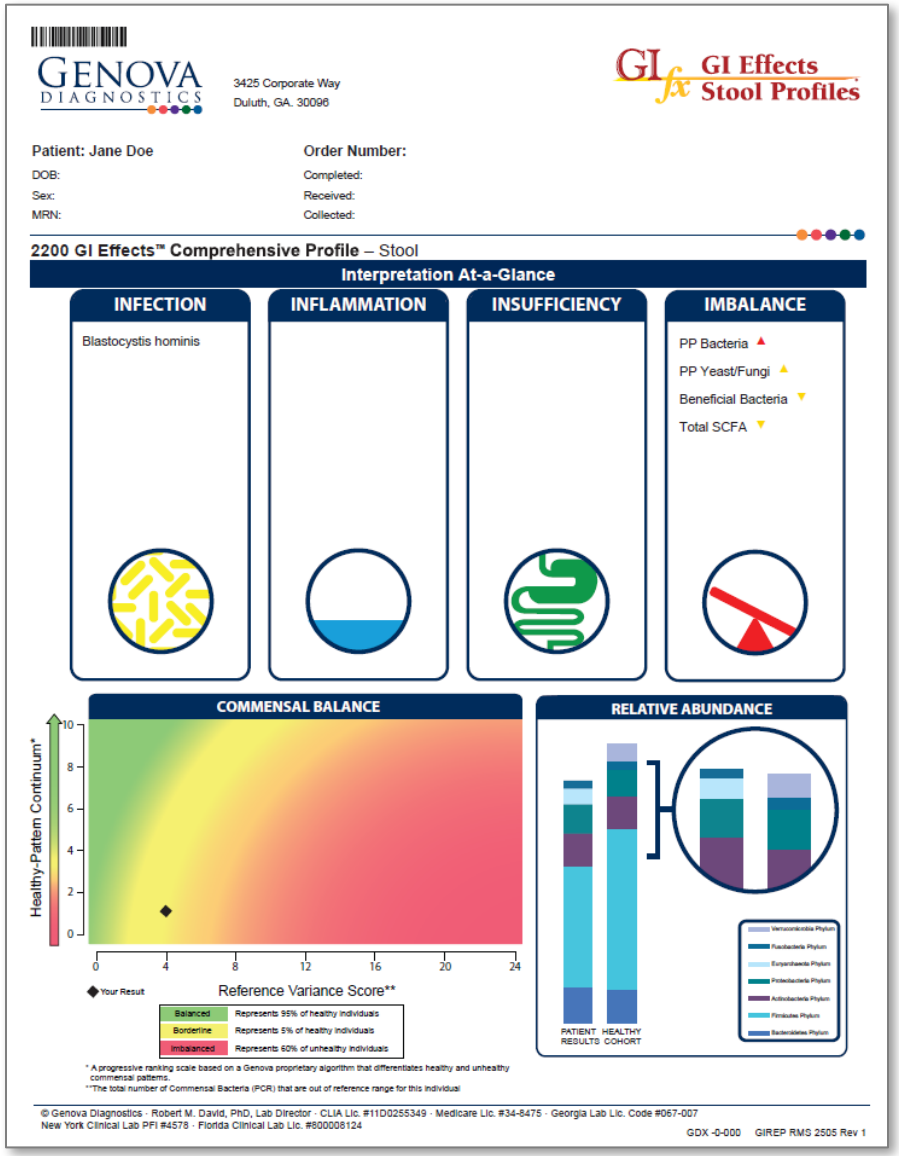
Treatment

- Botanical Therapy for SIBO and PPs: garlic, goldenseal (berberine), and oregano x 4 weeks
- Iberogast, a prokinetic, was recommended
- Magnesium and exercise to assist with regularity
- Recommended increasing water intake to at least 1-2 L daily
- While fiber is important for constipation, it was avoided initially to address SIBO
- Encouraged increasing plant-based foods, healthy fats, and moderate amount of clean and lean meats
- Probiotics 4 hours away from botanical therapy

Case Example #2

- 61 y/o female with IBS diagnosis
- History of bowel obstruction
- Bloating and alternating stools



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3425 Corporate Way
Duluth, GA. 30096

GI fx GI Effects Stool Profiles

Patient: Jane Doe
DOB:
Sex:
MRN:

Order Number:
Completed:
Received:
Collected:

2200 GI Effects™ Comprehensive Profile – Stool

Interpretation At-a-Glance

INFECTION
Blastocystis hominis

INFLAMMATION

INSUFFICIENCY

IMBALANCE
PP Bacteria ▲
PP Yeast/Fungi ▲
Beneficial Bacteria ▼
Total SCFA ▼

COMMENSAL BALANCE
Healthy-Pattern Continuum*
0 2 4 6 8 10
0 4 8 12 16 20 24
Your Result
Reference Variance Score**
Balanced Represents 95% of healthy individuals
Borderline Represents 5% of healthy individuals
Imbalanced Represents 60% of unhealthy individuals

RELATIVE ABUNDANCE
PATIENT RESULTS
HEALTHY COHORT

* A progressive ranking scale based on a Genova proprietary algorithm that differentiates healthy and unhealthy commensal patterns.
**The total number of Commensal Bacteria (PCR) that are out of reference range for this individual

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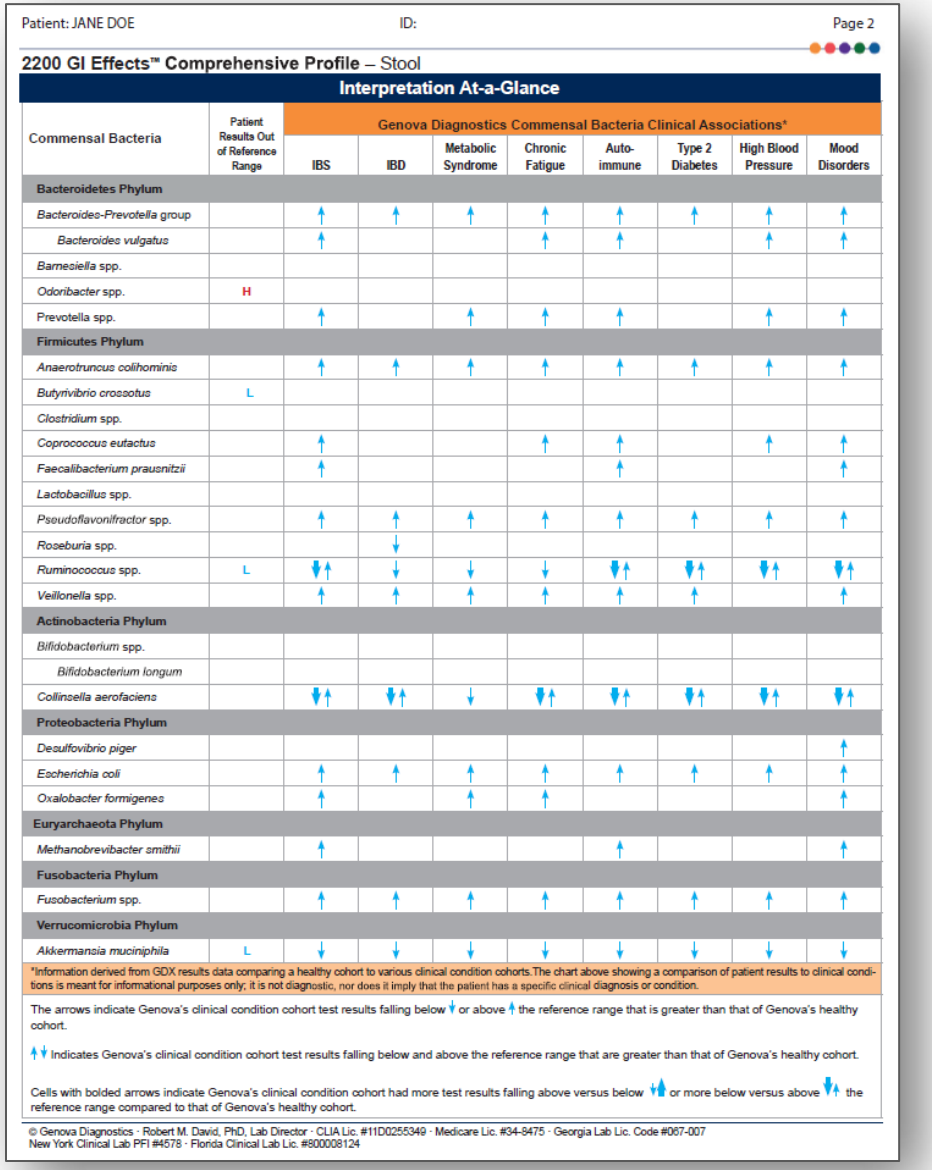
Commensal and Biomarker Clinical Association Findings Charts



Patient: JANE DOE ID: Page 2

2200 GI Effects™ Comprehensive Profile – Stool

Interpretation At-a-Glance

Commensal Bacteria	Patient Results Out of Reference Range	Genova Diagnostics Commensal Bacteria Clinical Associations*							
		IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
Bacteroidetes Phylum									
Bacteroides-Prevotella group		↑	↑	↑	↑	↑	↑	↑	↑
Bacteroides vulgatus		↑			↑	↑		↑	↑
Barnesiella spp.									
Odoribacter spp.	H								
Prevotella spp.		↑		↑	↑	↑		↑	↑
Firmicutes Phylum									
Anaerotruncus colihominis		↑	↑	↑	↑	↑	↑	↑	↑
Butyrivibrio crossotus	L								
Clostridium spp.									
Coprococcus eutactus		↑			↑	↑		↑	↑
Faecalibacterium prausnitzii		↑				↑			↑
Lactobacillus spp.									
Pseudoflavonifractor spp.		↑	↑	↑	↑	↑	↑	↑	↑
Roseburia spp.			↓						
Ruminococcus spp.	L	↓↑	↓	↓	↓	↓↑	↓↑	↓↑	↓↑
Veillonella spp.		↑	↑	↑	↑	↑	↑		↑
Actinobacteria Phylum									
Bifidobacterium spp.									
Bifidobacterium longum									
Collinsella aerofaciens		↓↑	↓↑	↓	↓↑	↓↑	↓↑	↓↑	↓↑
Proteobacteria Phylum									
Desulfovibrio piger									↑
Escherichia coli		↑	↑	↑	↑	↑	↑	↑	↑
Oxalobacter formigenes		↑		↑	↑				↑
Euryarchaeota Phylum									
Methanobrevibacter smithii		↑				↑			↑
Fusobacteria Phylum									
Fusobacterium spp.		↑	↑	↑	↑	↑	↑	↑	↑
Verrucomicrobia Phylum									
Akkermansia muciniphila	L	↓	↓	↓	↓	↓	↓	↓	↓

*Information derived from GDX results data comparing a healthy cohort to various clinical condition cohorts.The chart above showing a comparison of patient results to clinical conditions is meant for informational purposes only; it is not diagnostic, nor does it imply that the patient has a specific clinical diagnosis or condition.

The arrows indicate Genova's clinical condition cohort test results falling below ↓ or above ↑ the reference range that is greater than that of Genova's healthy cohort.

↓↑ Indicates Genova's clinical condition cohort test results falling below and above the reference range that are greater than that of Genova's healthy cohort.

Cells with bolded arrows indicate Genova's clinical condition cohort had more test results falling above versus below ↓↑ or more below versus above ↓↑ the reference range compared to that of Genova's healthy cohort.

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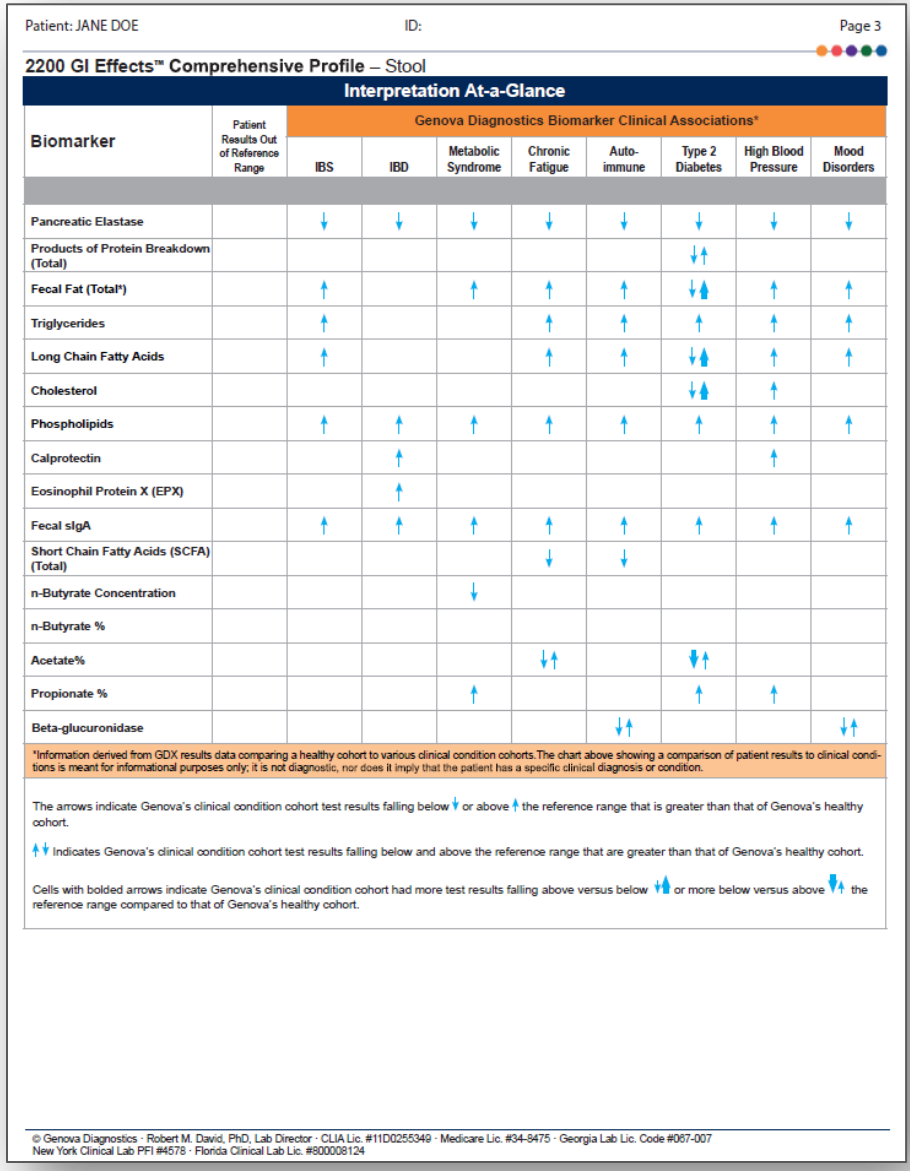

Patient: JANE DOE ID: Page 3

2200 GI Effects™ Comprehensive Profile – Stool

Interpretation At-a-Glance

Biomarker	Patient Results Out of Reference Range	Genova Diagnostics Biomarker Clinical Associations*							
		IBS	IBD	Metabolic Syndrome	Chronic Fatigue	Auto-immune	Type 2 Diabetes	High Blood Pressure	Mood Disorders
Pancreatic Elastase		↓	↓	↓	↓	↓	↓	↓	↓
Products of Protein Breakdown (Total)							↓↑		
Fecal Fat (Total¹)		↑		↑	↑	↑	↓↑	↑	↑
Triglycerides		↑			↑	↑	↑	↑	↑
Long Chain Fatty Acids		↑			↑	↑	↓↑	↑	↑
Cholesterol							↓↑	↑	
Phospholipids		↑	↑	↑	↑	↑	↑	↑	↑
Calprotectin			↑					↑	
Eosinophil Protein X (EPX)			↑						
Fecal sIgA		↑	↑	↑	↑	↑	↑	↑	↑
Short Chain Fatty Acids (SCFA) (Total)					↓	↓			
n-Butyrate Concentration				↓					
n-Butyrate %									
Acetate%					↓↑		↓↑		
Propionate %				↑			↑	↑	
Beta-glucuronidase						↓↑			↓↑

*Information derived from GDX results data comparing a healthy cohort to various clinical condition cohorts.The chart above showing a comparison of patient results to clinical conditions is meant for informational purposes only; it is not diagnostic, nor does it imply that the patient has a specific clinical diagnosis or condition.

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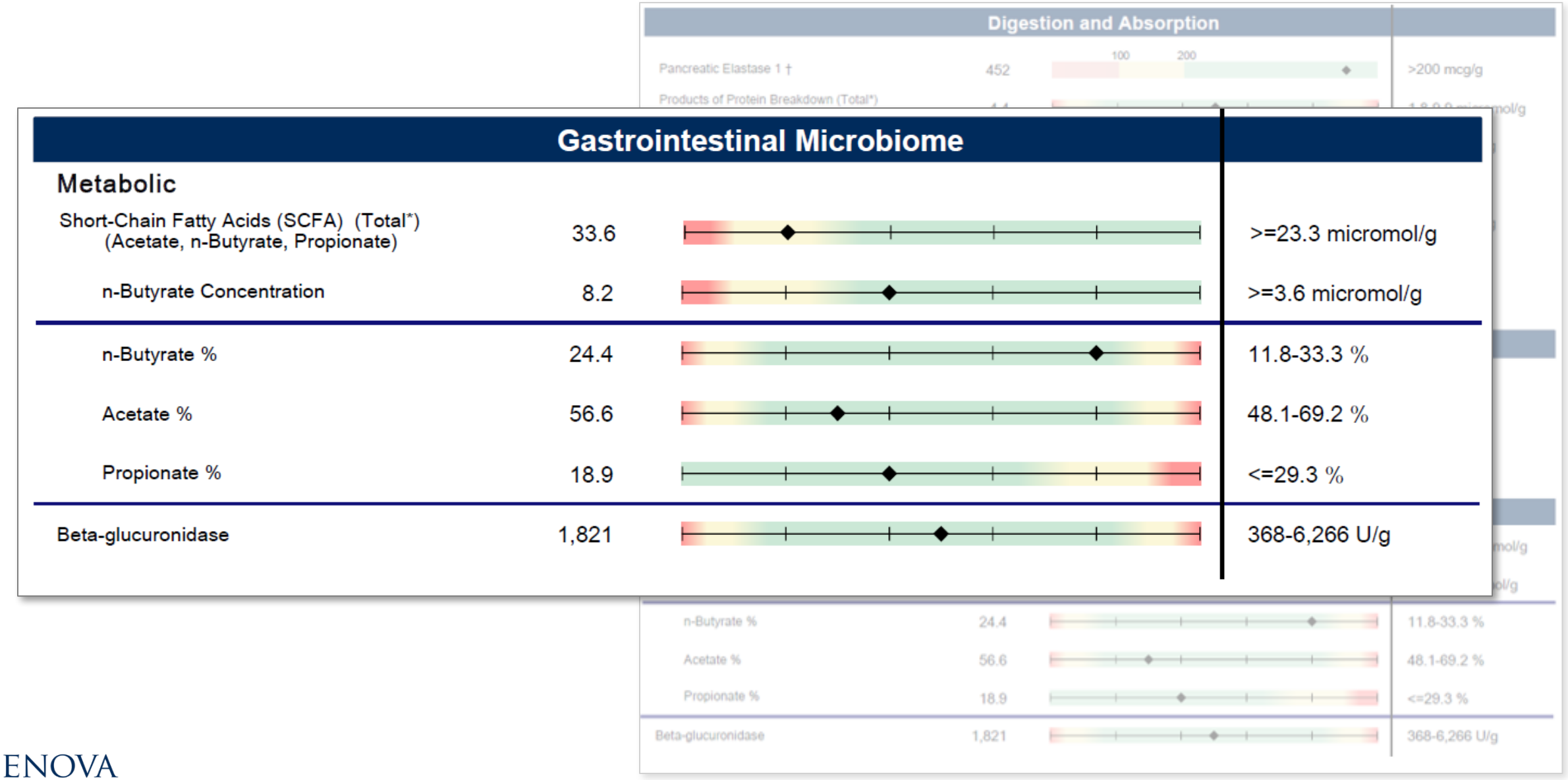

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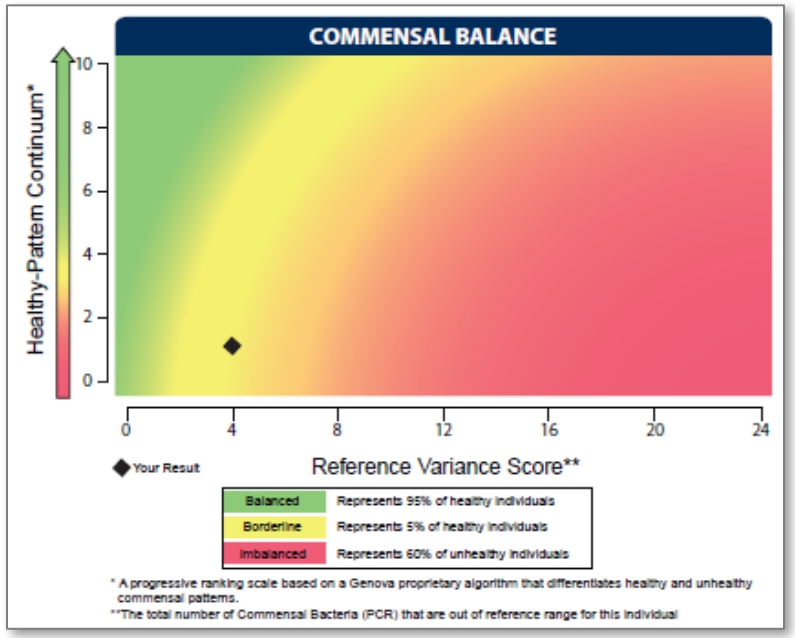

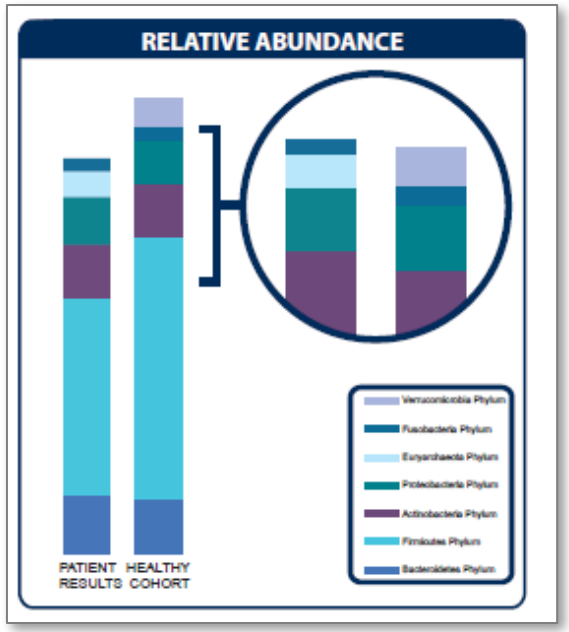

Gastrointestinal Microbiome

Commensal Bacteria (PCR)	Result CFU/g stool		QUINTILE DISTRIBUTION 1st 2nd 3rd 4th 5th	Reference Range CFU/g stool
Bacteroidetes Phylum				
Bacteroides-Prevotella group	2.2E8			3.4E6 - 1.5E9
Bacteroides vulgatus	1.3E9			<=2.2E9
Barnesiella spp.	<DL			<=1.6E8
Odoribacter spp.	1.6E8	H		<=8.0E7
Prevotella spp.	7.1E6			1.4E5 - 1.6E7
Firmicutes Phylum				
Anaerotruncus colihominis	6.9E6			<=3.2E7
Butyrivibrio crossotus	<DL	L		5.5E3 - 5.9E5
Clostridium spp.	1.7E9			1.7E8 - 1.5E10
Coprococcus eutactus	<DL			<=1.2E8
Faecalibacterium prausnitzii	1.3E9			5.8E7 - 4.7E9
Lactobacillus spp.	1.9E9			8.3E6 - 5.2E9
Pseudoflavonifractor spp.	7.1E7			4.2E5 - 1.3E8
Roseburia spp.	6.0E8			1.3E8 - 1.2E10
Ruminococcus spp.	<DL	L		9.5E7 - 1.6E9
Veillonella spp.	4.1E6			1.2E5 - 5.5E7
Actinobacteria Phylum				
Bifidobacterium spp.	7.0E7			<=6.4E9
Bifidobacterium longum	9.0E6			<=7.2E8
Collinsella aerofaciens	6.7E7			1.4E7 - 1.9E9
Proteobacteria Phylum				
Desulfovibrio piger	<DL			<=1.8E7
Escherichia coli	2.3E7			9.0E4 - 4.6E7
Oxalobacter formigenes	4.9E6			<=1.5E7
Euryarchaeota Phylum				
Methanobrevibacter smithii	2.9E7			<=8.6E7
Fusobacteria Phylum				
Fusobacterium spp.	6.4E3			<=2.4E5
Verrucomicrobia Phylum				
Akkermansia muciniphila	<DL			>=1.2E6

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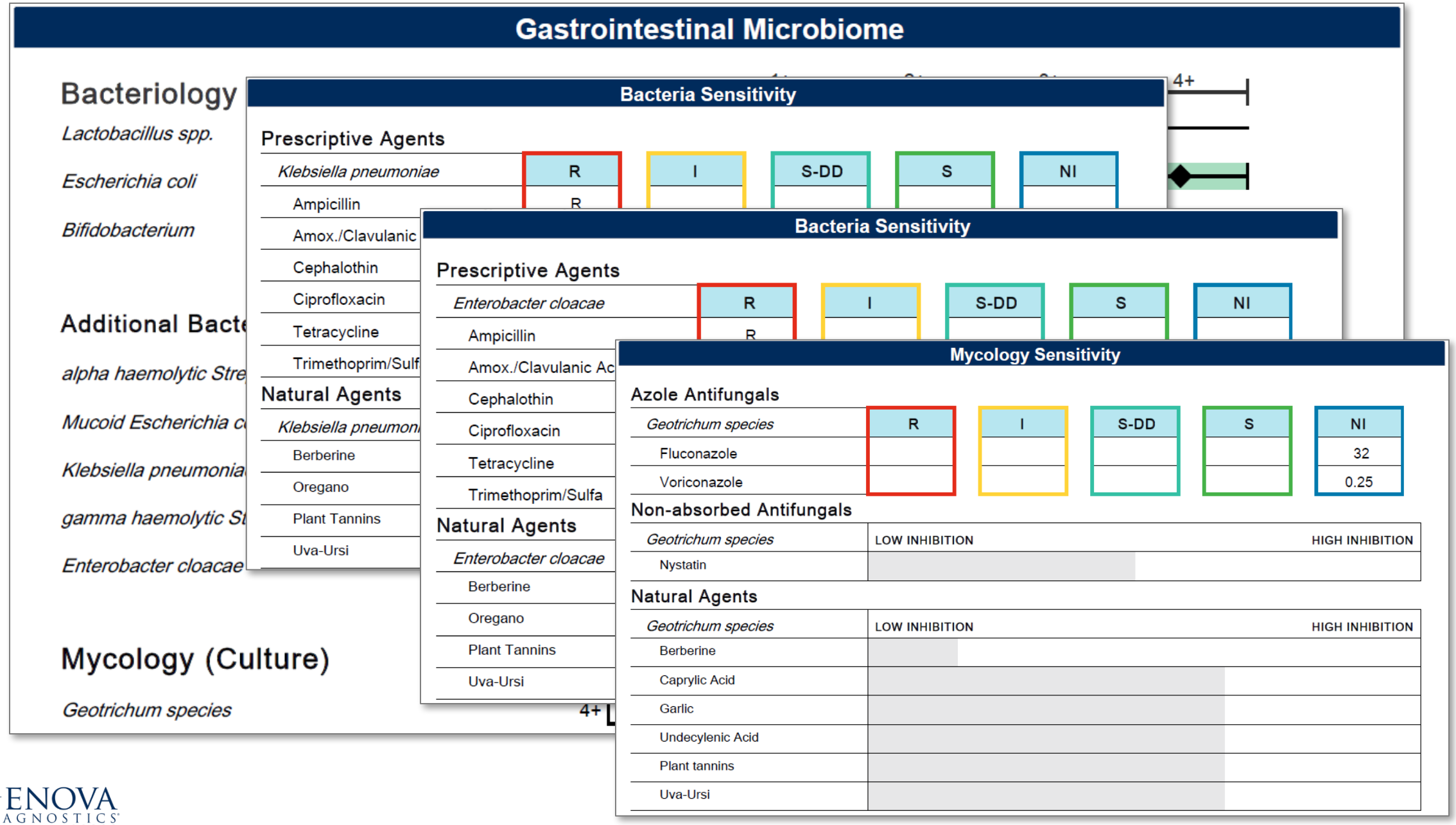

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Parasitology

Microscopic Exam Results**

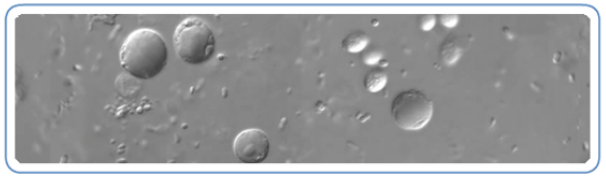
Blastocystis hominis: Many

Parasitology EIA Tests:

	In Range	Out of Range
Cryptosporidium◆	Negative	
Giardia lamblia◆	Negative	
Entamoeba histolytica◆	Negative	

Parasites - Blastocystis spp. infection

- *Blastocystis spp.*
- Frequently Asked Questions (FAQs)
- Biology
- Resources for Health Professionals
- Publications



Blastocystis is a common microscopic organism that inhabits the intestine and is found throughout the world. A full understanding of the biology of *Blastocystis* and its relationship to other organisms is not clear, but is an active area of research.

Image: B. spp. *cyst-like forms in wet mounts under differential interference contrast (DIC) microscopy. Credit:* DPDx

BLASTOCYSTIS SPP. FAQS
Most common questions answered...

PUBLICATIONS
Related articles and journal entries...

BIOLOGY
Stages of parasite development...

RESOURCES FOR HEALTH PROFESSIONALS
What you need to know...

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Website Image: www.cdc.gov

Treatment

- Parasite Treatment - Alinia BID x 7 days
  - Retest in 6 weeks
- Botanicals: Oil of oregano, horse chestnut, and berberine x 4 weeks
- Probiotics 4 hours away from therapy
- Decrease sugar, yeasted and molded foods
- Insoluble fiber and encourage food prebiotics (onion, garlic, artichoke, etc…)

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Lahnor Powell, ND, MPH
Moderator

Michelle Maddux, ND
Presenter

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LIVE GDX

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GI fx GI Effects® Stool Profiles

The GI Effects® Advanced Interpretation

Digging Deeper

Michelle Maddux, ND

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