GI Effects Comprehensive Stool Profile

Inflammation & Immunology: The Barrier to the Outside World

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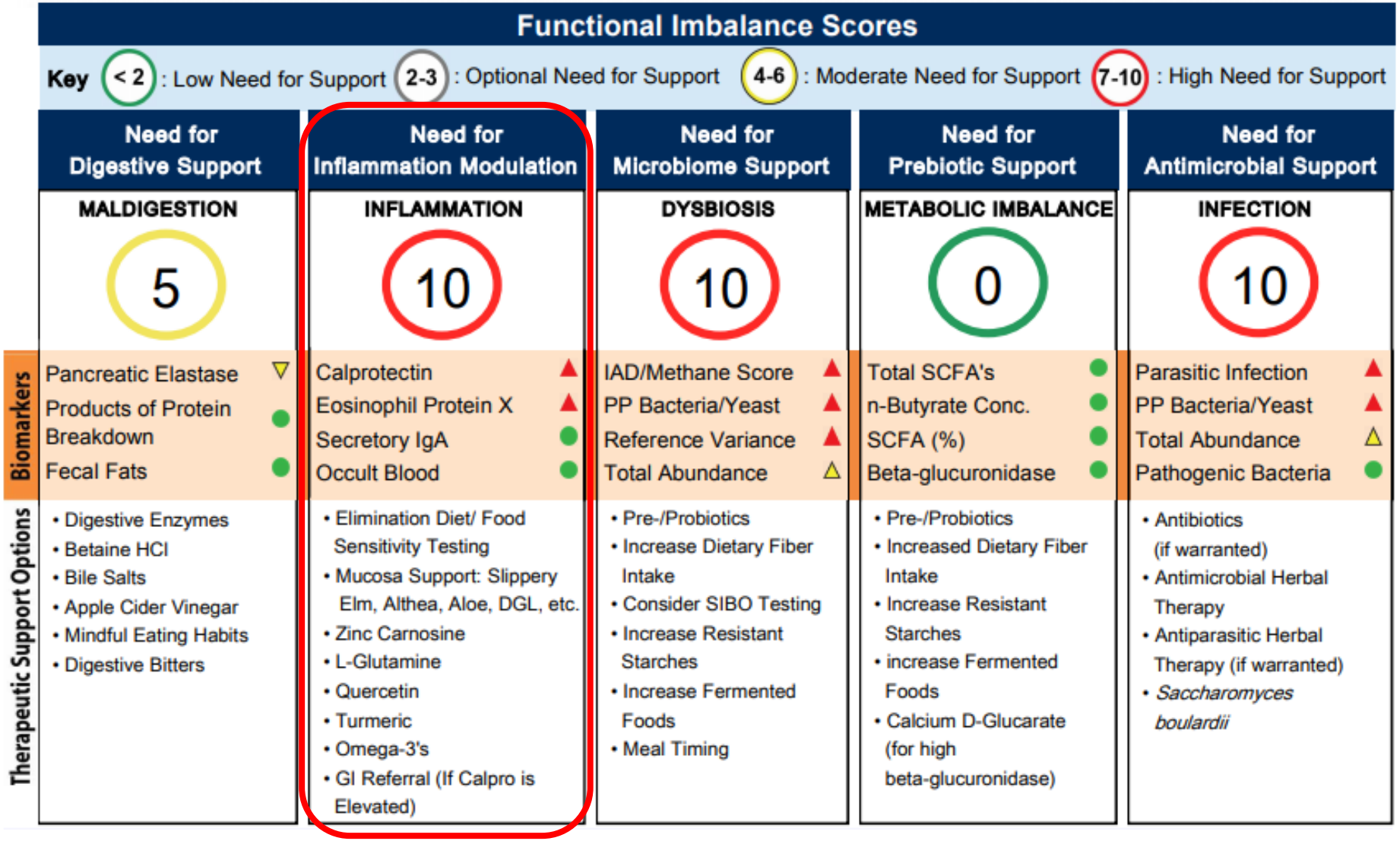
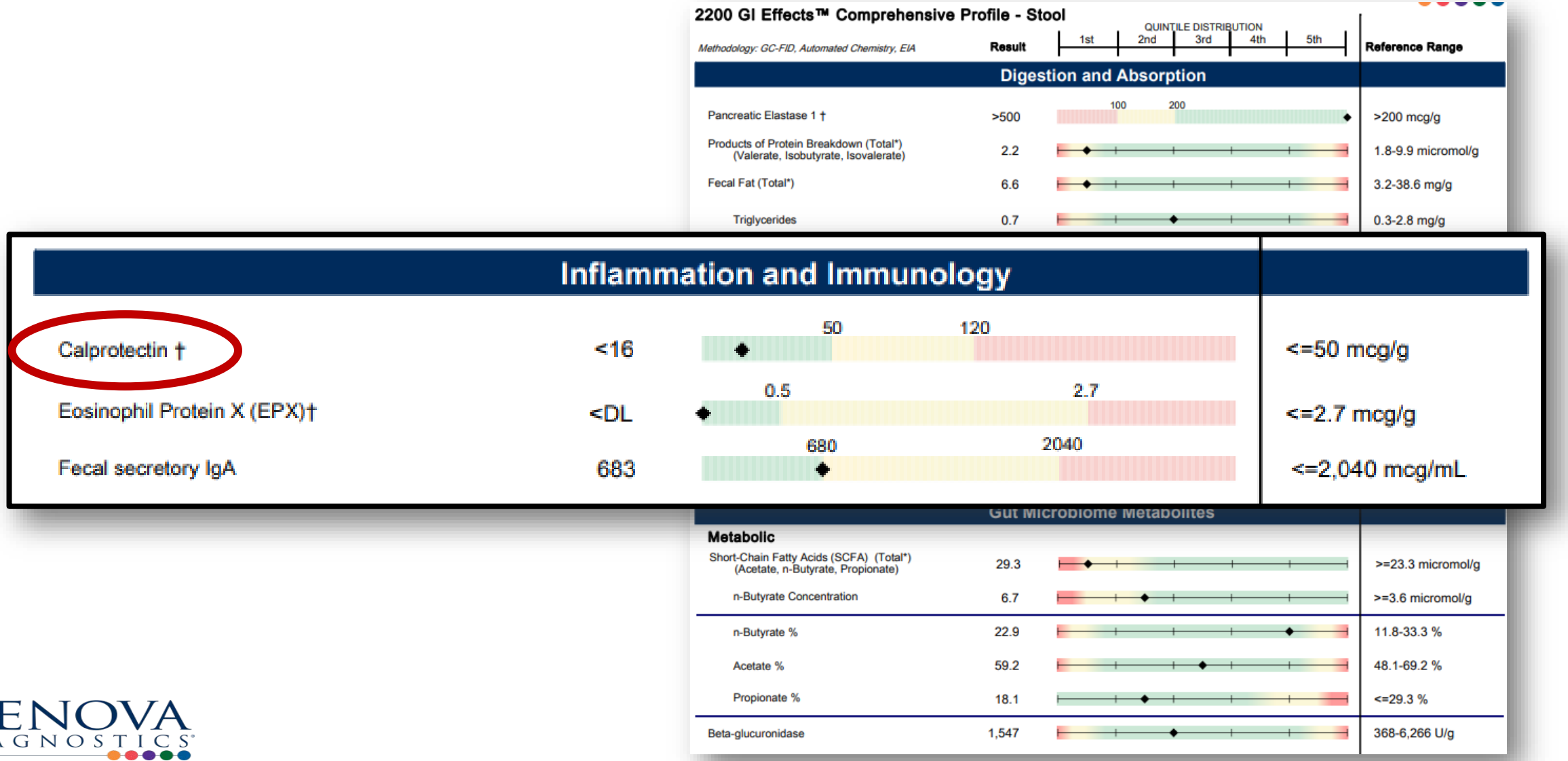
Functional Imbalance Scores



GENOVA DIAGNOSTICS

GI Effects Biomarkers For Inflammation & Immunology



GENOVA DIAGNOSTICS

Calprotectin

- Released from the intestinal mucosa, indicates neutrophil mediated inflammation
- Useful in differentiating IBS from IBD, ex. Ulcerative Colitis and Crohn's, and monitoring IBD treatment
- Considerations when elevated:
  - IBD, not in remission
  - Colorectal cancer and polyps
  - NSAID and PPI use
  - Infection
  - Diverticular disease
  - Bariatric surgery
  - Age, younger than 5 or greater than 60

GENOVA DIAGNOSTICS

Calprotectin

Calprotectin (mcg/g stool)	Interpretation	Follow up
≤50	Normal range	None
50 to 120	Suggestive of low grade inflammation	Address cause of inflammation (Suspected or history of IBD, Infection, Chronic NSAID or PPI use, Diverticular disease) Consider anti-inflammatories such as fish oil Re-evaluate at 4-6 weeks
>120	Abnormal	Refer to GI specialist to rule out IBD, malignancy, or other cause of significant GI inflammation

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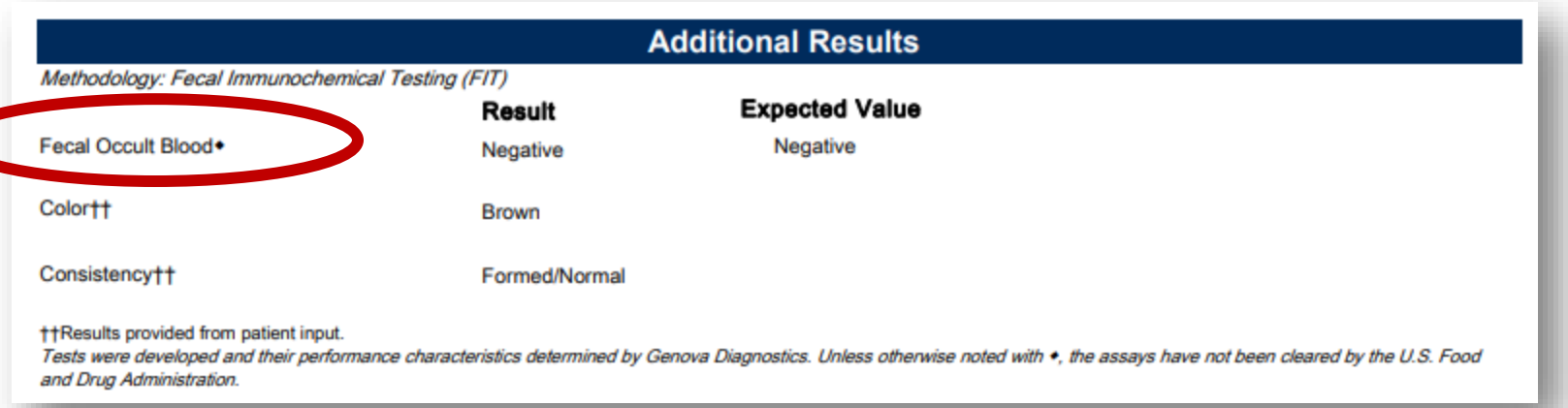
Next…

- Other markers to consider if Calprotectin is elevated.

Fecal Occult Blood

- Blood not evident to the naked eye and present in microscopic quantities only
- Inflammation
- Infection

- **Note:** Bleeding hemorrhoids or menstruation, may interfere with testing.



Additional Results

Methodology: Fecal Immunochemical Testing (FIT)

	Result	Expected Value
Fecal Occult Blood•	Negative	Negative
Color††	Brown	
Consistency††	Formed/Normal	

††Results provided from patient input.

Tests were developed and their performance characteristics determined by Genova Diagnostics. Unless otherwise noted with •, the assays have not been cleared by the U.S. Food and Drug Administration.

GENOVA DIAGNOSTICS

White Blood Cells

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
Protozoa	
Balantidium coli	Not Detected
Blastocystis spp.	**Many 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	

Additional Findings	
White Blood Cells	Not Detected
Charcot-Leyden Crystals	Not Detected

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White Blood Cells (WBC)

Indicates an immune response that can be seen in

infectious conditions or **inflammatory bowel disease (IBD).**

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Brief Review of Immunoglobulins

There are 5 types of immunoglobulins, also known as **antibodies**

IgA, IgD, IgE, IgG, and IgM

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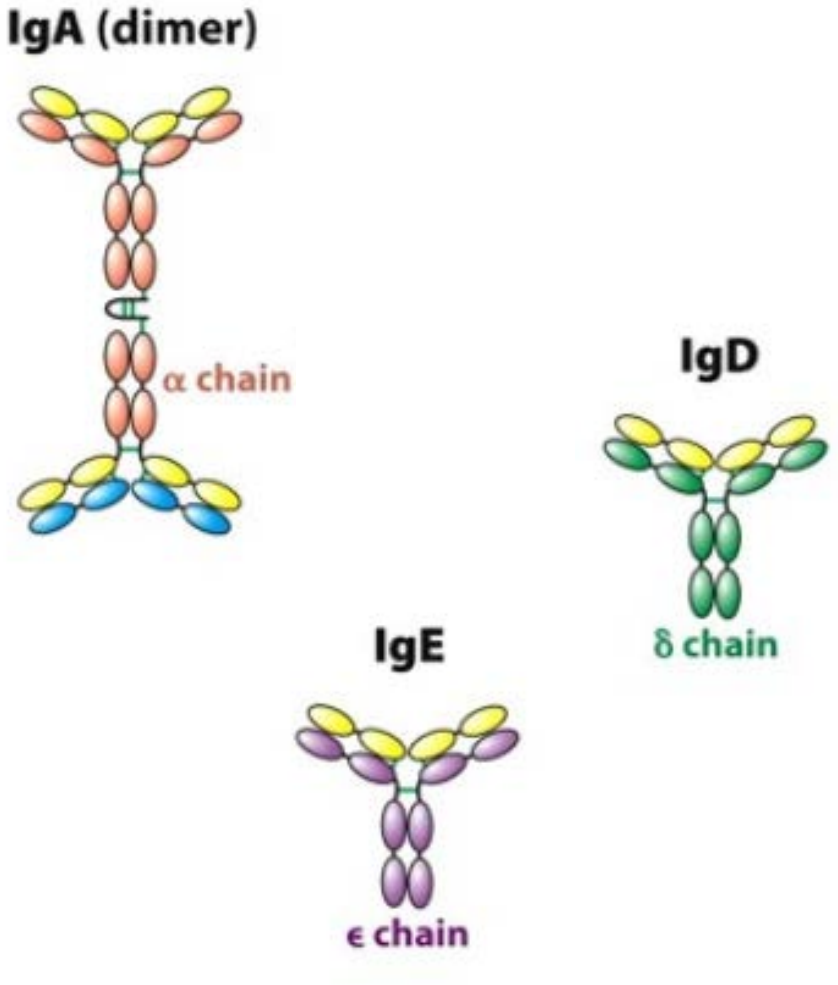
Immunoglobulins

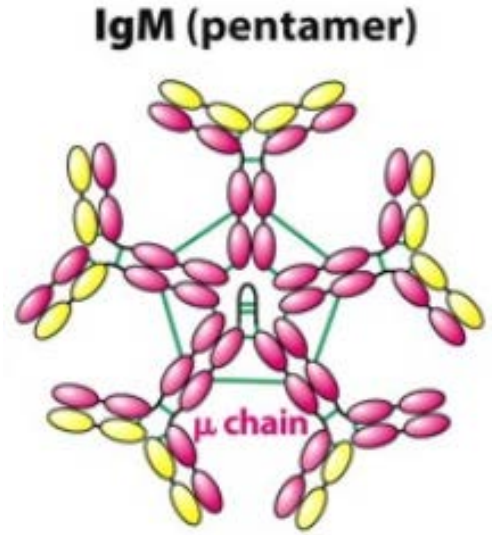


- Immunoglobulin A
  - First line defense against foreign pathogens
  - Found in fluids made by mucus membranes
    - tears, saliva, respiratory and intestinal secretions, and colostrum.
- Immunoglobulin D
  - Blood in small amounts
  - Function not clearly understood
- Immunoglobulin E
  - Associated with allergic reactions
  - Stimulates histamine release

GENOVA DIAGNOSTICS

Photo: https://www.creative-diagnostics.com/blog/index.php/clinical-significance-of-five-immunoglobulin-tests/

Immunoglobulins



- Immunoglobulin G
  - Protects against bacteria and viral infections
    - Secondary immune response
  - Most abundant circulating immunoglobulin
  - Found in blood and tissue fluids
- Immunoglobulin M
  - 1st antibody made to fight a new infection
  - Blood and lymph

GENOVA DIAGNOSTICS

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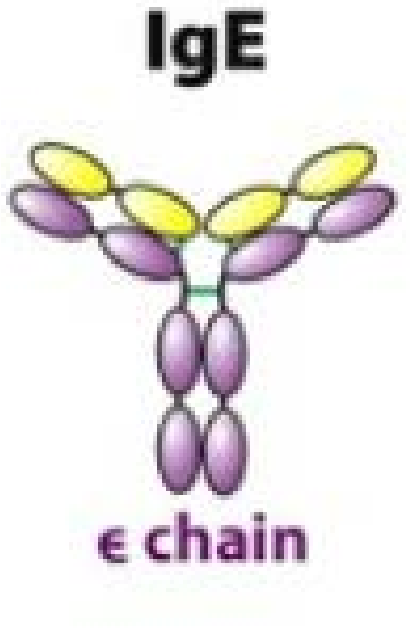
Food Allergy **vs Food Sensitivity**



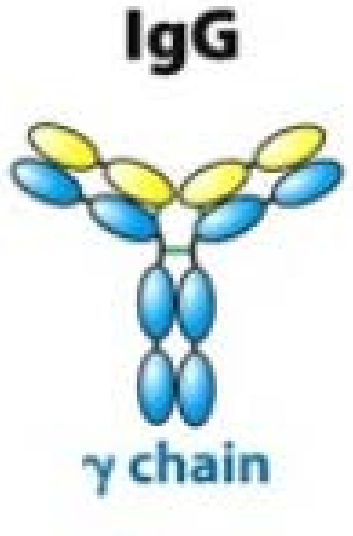
- Immediate onset (minutes to hours)
- Symptoms of Allergic Reaction
  - Hives, stuffy or itchy nose, sneezing, itchy, teary eyes, vomiting, stomach cramps or diarrhea, angioedema or swelling, shortness of breath or wheezing, anaphylaxis
- 90% of all food allergy reactions are cause by 8 foods
  - Milk, soy, eggs, wheat, peanuts, tree nuts, fish, and shellfish
- Treatment suggestion
  - Lifetime elimination
  - Allergen-specific immunotherapy

GENOVA DIAGNOSTICS

Photo: https://www.creative-diagnostics.com/blog/index.php/clinical-significance-of-five-immunoglobulin-tests/

Food Allergy vs *Food Sensitivity*

- Delayed onset, hours to days
- Symptoms of food sensitivities
  - Headache, itching, bloating, fatigue, diarrhea, constipation, joint pain, indigestion, or worsening of your chronic health complaints
- Often associated with “Leaky Gut”
- Treatment suggestion
  - Elimination Diet
  - Intestinal Permeability
  - Digestion/Absorption
  - Dysbiosis
  - Nutrition



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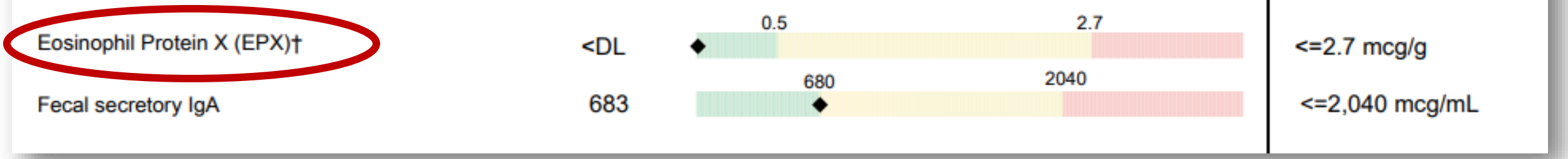
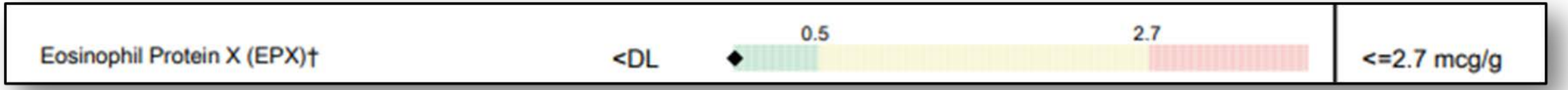
Mullin GE, et al. *Nutr Clin Pract*. 2010;25(2):192-98.
Schanahan F, et al. *Am J Gastroenterol*. 2005;100(7):1558-59.
Photo: https://www.creative-diagnostics.com/blog/index.php/clinical-significance-of-five-immunoglobulin-tests/

Eosinophil Protein X (EPX)

Inflammation and Immunology			
Calprotectin †	<16	50 / 120	<=50 mcg/g
Eosinophil Protein X (EPX)†	<DL	0.5 / 2.7	<=2.7 mcg/g
Fecal secretory IgA	683	680 / 2040	<=2,040 mcg/mL



Eosinophil Protein X (EPX)



- Eosinophil mediated inflammation
- Elevated with IgE mediated food allergies, atopic dermatitis, and immune-mediated food hypersensitivity
- Inflammatory Bowel Disease (IBD)
- Certain parasitic infections
- Microscopic colitis/gastritis (dx requires histological analysis)
- Eosinophilic gastrointestinal disorders
- Can be elevated in children younger than 4 years old

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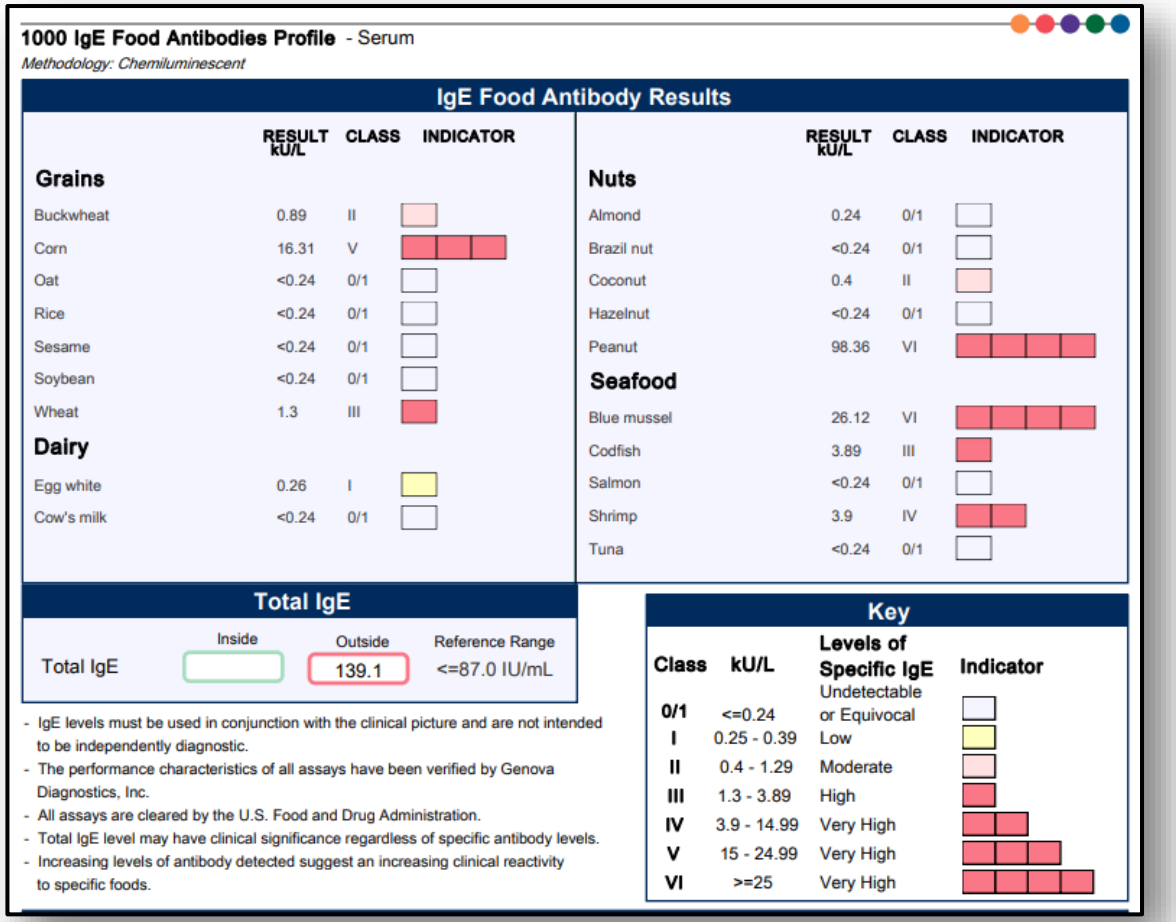
Therapeutic Considerations for Elevated EPX

- IgE-mediated allergy

  IgE Food Antibody panel
- IBD (review Calprotectin level)
- Evaluate for parasitic infection
- Anti-Inflammatories
- Mast Cell Stabilizers



1000 IgE Food Antibodies Profile - Serum

Methodology: Chemiluminescent

IgE Food Antibody Results

	RESULT kU/L	CLASS	INDICATOR
Grains			
Buckwheat	0.89	II	
Corn	16.31	V	
Oat	<0.24	0/1	
Rice	<0.24	0/1	
Sesame	<0.24	0/1	
Soybean	<0.24	0/1	
Wheat	1.3	III	
Dairy			
Egg white	0.26	I	
Cow's milk	<0.24	0/1	
Nuts			
Almond	0.24	0/1	
Brazil nut	<0.24	0/1	
Coconut	0.4	II	
Hazelnut	<0.24	0/1	
Peanut	98.36	VI	
Seafood			
Blue mussel	26.12	VI	
Codfish	3.89	III	
Salmon	<0.24	0/1	
Shrimp	3.9	IV	
Tuna	<0.24	0/1	

Total IgE

	Inside	Outside	Reference Range
Total IgE		139.1	<=87.0 IU/mL

- IgE levels must be used in conjunction with the clinical picture and are not intended to be independently diagnostic.
- The performance characteristics of all assays have been verified by Genova Diagnostics, Inc.
- All assays are cleared by the U.S. Food and Drug Administration.
- Total IgE level may have clinical significance regardless of specific antibody levels.
- Increasing levels of antibody detected suggest an increasing clinical reactivity to specific foods.

Key

Class	kU/L	Levels of Specific IgE	Indicator
0/1	<=0.24	Undetectable or Equivocal	
I	0.25 - 0.39	Low	
II	0.4 - 1.29	Moderate	
III	1.3 - 3.89	High	
IV	3.9 - 14.99	Very High	
V	15 - 24.99	Very High	
VI	>=25	Very High	

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Fecal Secretory IgA

Inflammation and Immunology			
Calprotectin †	<16	50 120	<=50 mcg/g
Eosinophil Protein X (EPX)†	<DL	0.5 2.7	<=2.7 mcg/g
Fecal secretory IgA	683	680 2040	<=2,040 mcg/mL

GENOVA DIAGNOSTICS

Fecal Secretory IgA (fsIgA)

Fecal secretory IgA	683	680 2040	<=2,040 mcg/mL

- Non-specific indicator of an immune response at the gut barrier
- Recognized as a first line of defense in protecting the intestinal epithelium from enteric pathogens
- Considerations when elevated:
  - Infection/Potential Pathogen
  - IgG Food Sensitivity, Celiac disease
  - Defective Epithelial Barrier, Gut Permeability
  - Colon cancer, IBS and dysbiosis
- Low sIgA may reflect a loss of GI immune response resiliency

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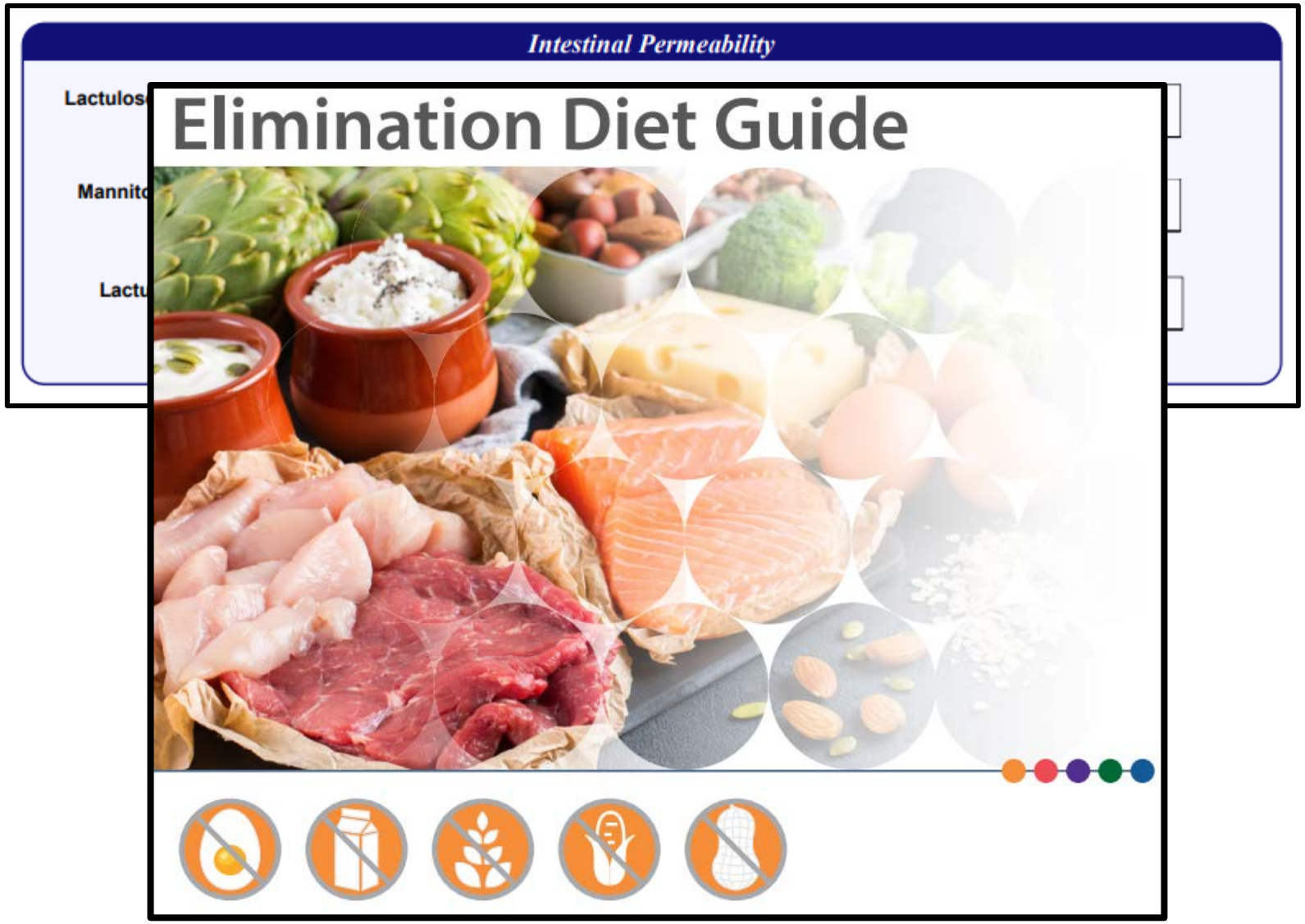
Therapeutic Considerations for Elevated fsIgA

- Address infection
- Assess for compromised intestinal barrier function
- Elimination Diet



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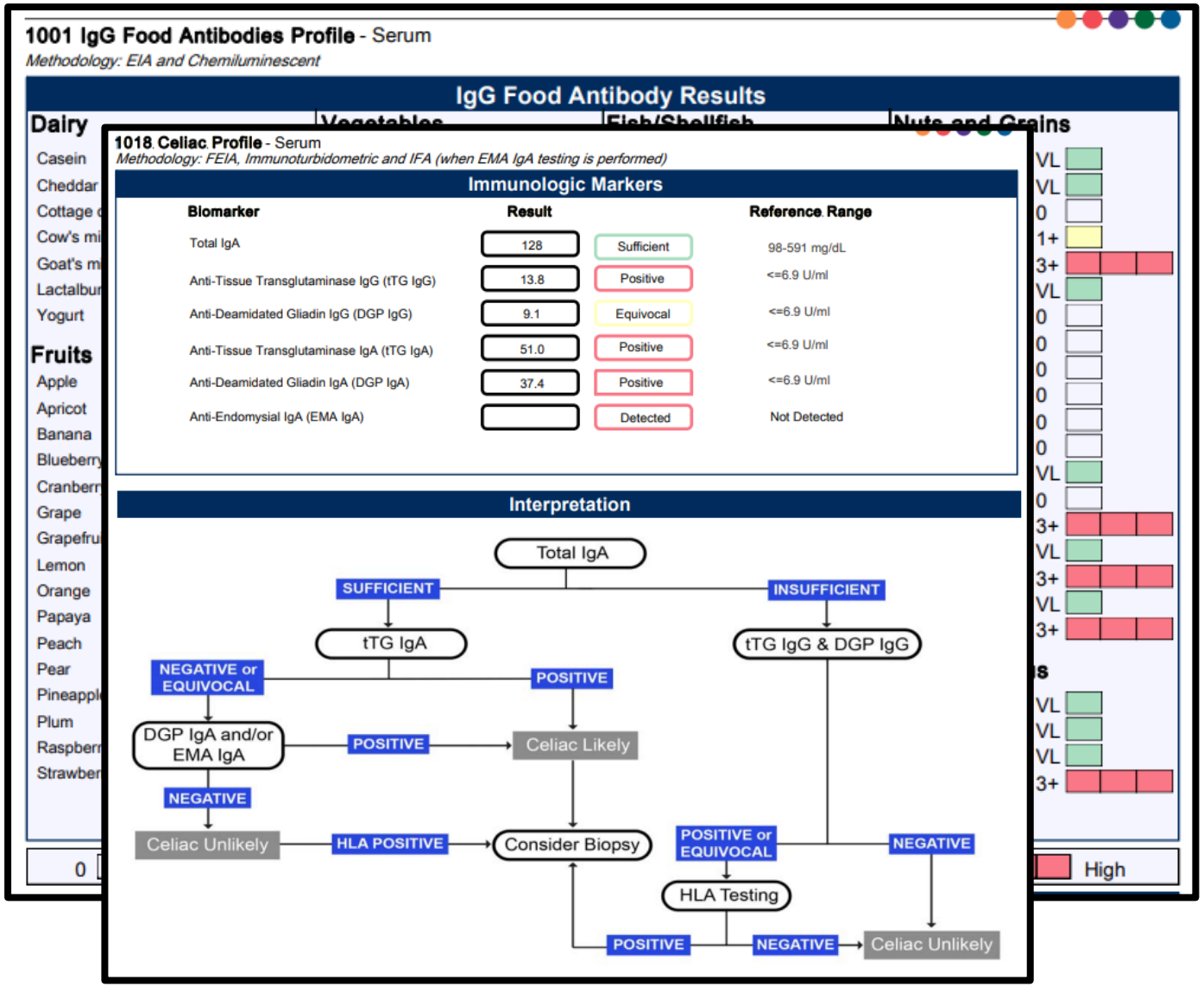
Therapeutic Considerations for Elevated fsIgA

- IgG Food antibody testing
- Celiac testing



Next…

- **Optional Add On Markers**

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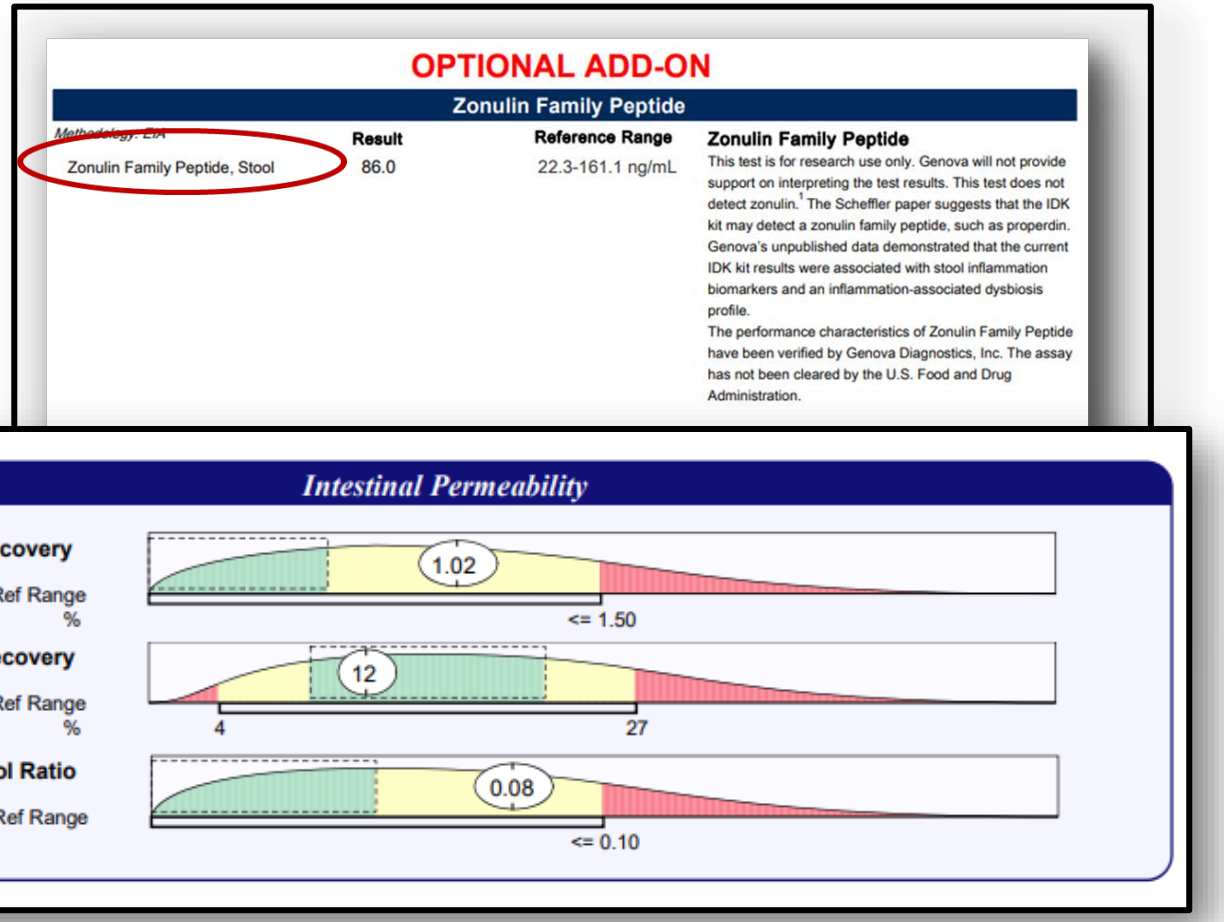
Zonulin Family Peptide

- Non-specific indicator associated with intestinal inflammation
- Clinical significance of an elevated Zonulin Family Peptide is unknown
- May relate to increased intestinal permeability. Consider follow up testing with the Intestinal Permeability Assessment.



OPTIONAL ADD-ON

Zonulin Family Peptide

	Result	Reference Range
Zonulin Family Peptide, Stool	86.0	22.3-161.1 ng/mL

Zonulin Family Peptide

This test is for research use only. Genova will not provide support on interpreting the test results. This test does not detect zonulin.[1] The Scheffler paper suggests that the IDK kit may detect a zonulin family peptide, such as properdin. Genova's unpublished data demonstrated that the current IDK kit results were associated with stool inflammation biomarkers and an inflammation-associated dysbiosis profile.

The performance characteristics of Zonulin Family Peptide have been verified by Genova Diagnostics, Inc. The assay has not been cleared by the U.S. Food and Drug Administration.

Intestinal Permeability

Lactulose Percent Recovery

Ref Range %

1.02

<= 1.50

Mannitol Percent Recovery

Ref Range %

12

4

27

Lactulose/Mannitol Ratio

Ref Range

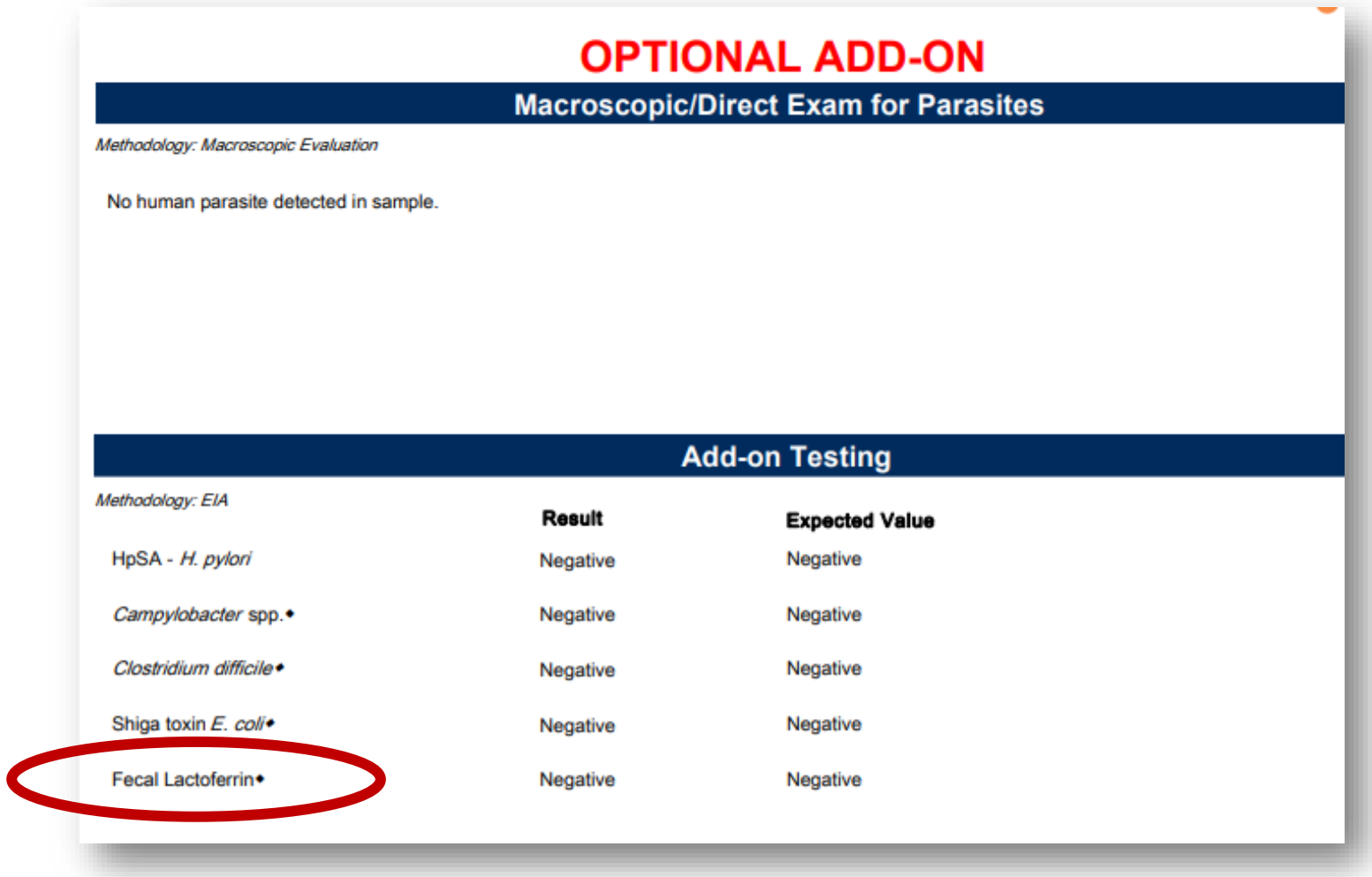
0.08

<= 0.10

GENOVA DIAGNOSTICS

Fecal Lactoferrin

- Biomarker of neutrophil mediated inflammation.
- Expressed a Positive or Negative finding.
- Calprotectin is a more sensitive biomarker.



OPTIONAL ADD-ON

Macroscopic/Direct Exam for Parasites

Methodology: Macroscopic Evaluation

No human parasite detected in sample.

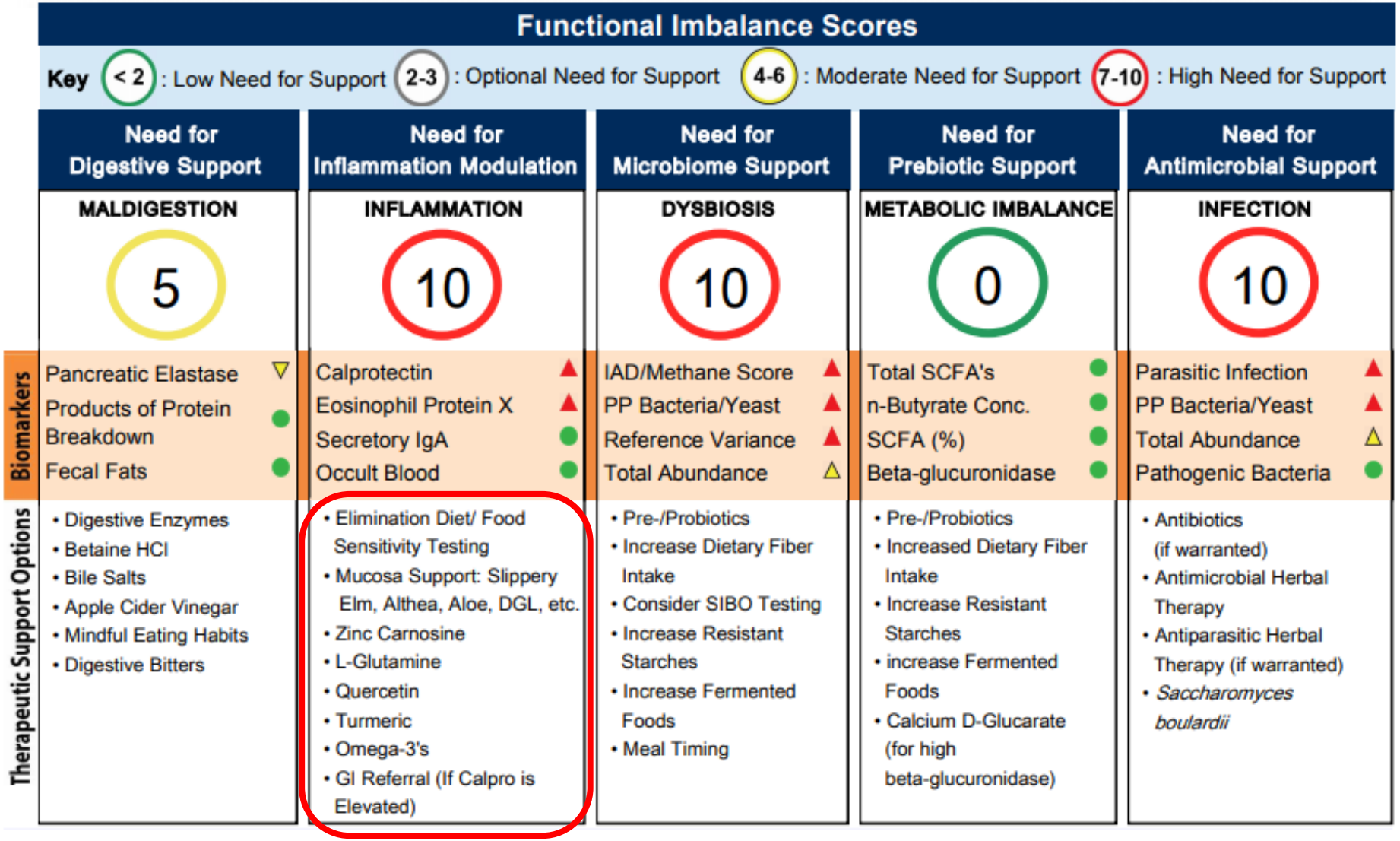
Add-on Testing

Methodology: EIA

	Result	Expected Value
HpSA - *H. pylori*	Negative	Negative
Campylobacter spp.•	Negative	Negative
Clostridium difficile•	Negative	Negative
Shiga toxin *E. coli*•	Negative	Negative
Fecal Lactoferrin•	Negative	Negative

GENOVA DIAGNOSTICS

Functional Imbalance Scores



GENOVA DIAGNOSTICS

Case Study 1

- 39 yo M
- Hx of IBD, Ulcerative Colitis
- Carnivore diet

Inflammation and Immunology			
Calprotectin †	203 H	50 — 120	<=50 mcg/g
Eosinophil Protein X (EPX)†	4.1 H	0.5 — 2.7	<=2.7 mcg/g
Fecal secretory IgA	316	680 — 2040	<=2,040 mcg/mL

Additional Findings	
White Blood Cells	Detected
Charcot-Leyden Crystals	Not Detected

Additional Results

Methodology: Fecal Immunochemical Testing (FIT)

	Result	Expected Value
Fecal Occult Blood•	Positive	Negative
Consistency††	Watery/Diarrhea	

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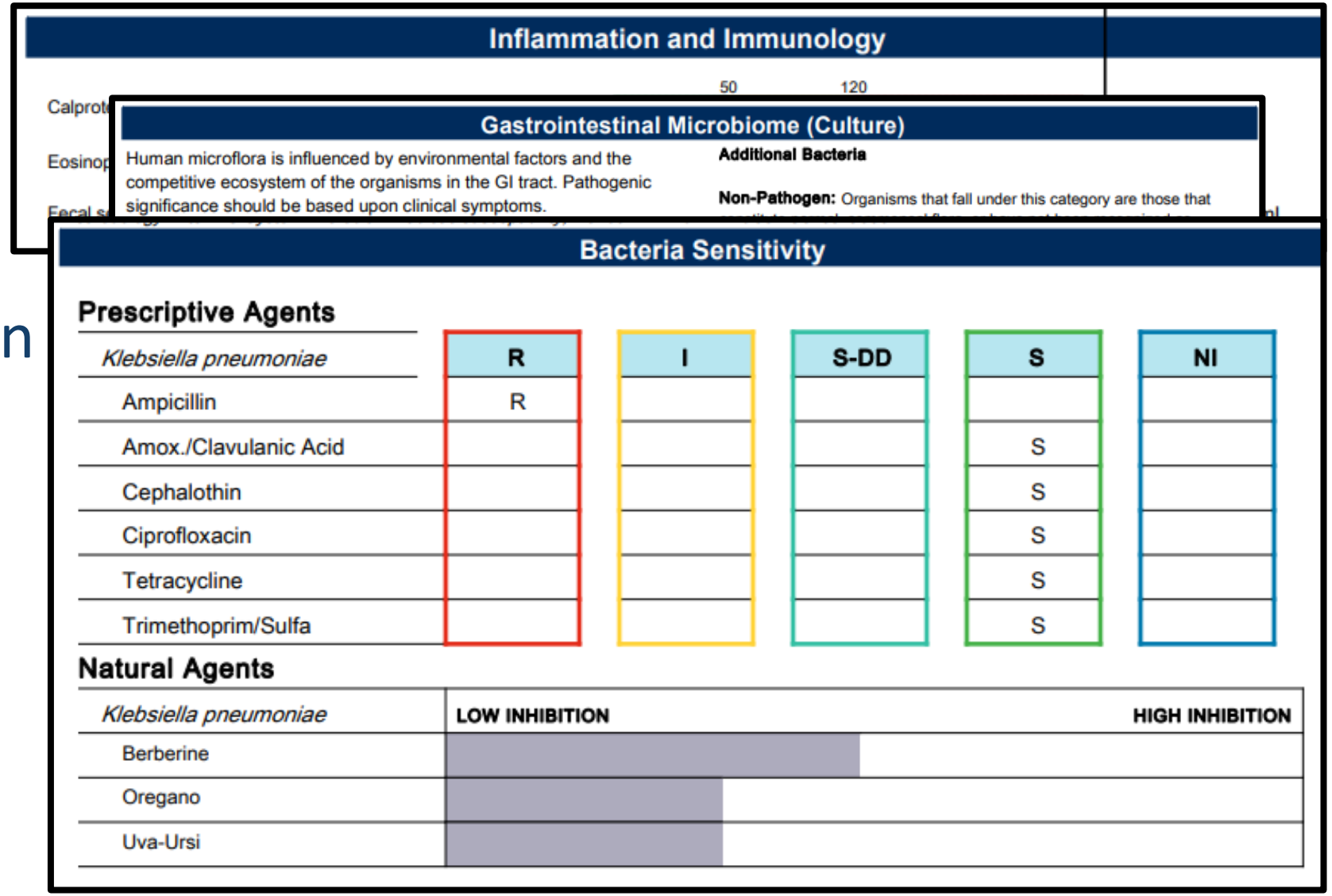
Case Study 2

- 84 yo F
- Diarrhea
- Abdominal pain
- Weight loss
- Hives



Inflammation and Immunology

50 120

Calprot

Eosinop

Fecal s

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.

Additional Bacteria

Non-Pathogen: Organisms that fall under this category are those that

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	

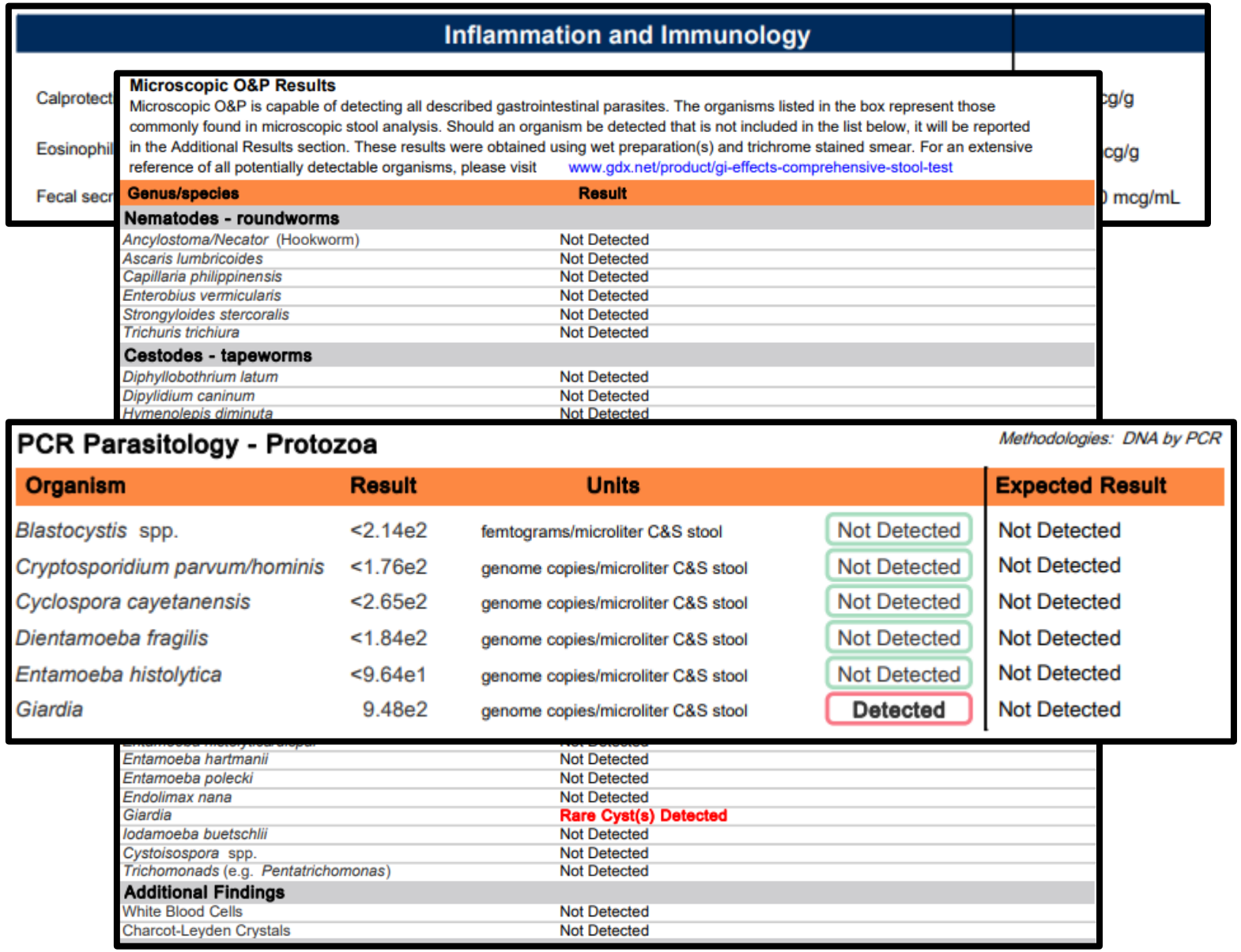
Natural Agents

Klebsiella pneumoniae	LOW INHIBITION	HIGH INHIBITION
Berberine		
Oregano		
Uva-Ursi		

GENOVA DIAGNOSTICS

Case Study 3

- 64 yo M
- Urgent diarrhea
- Gas, cramping
- Recently returned from a 2 week backpacking trip



Inflammation and Immunology

Calprotect
Eosinophil
Fecal secr
cg/g
cg/g
mcg/mL

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	
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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

PCR Parasitology - Protozoa

Methodologies: DNA by PCR

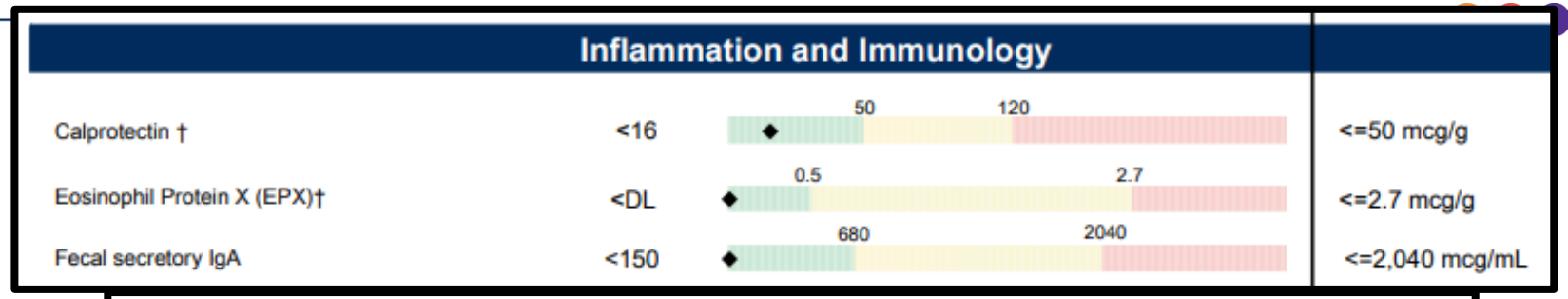
Organism	Result	Units		Expected Result
Blastocystis spp.	<2.14e2	femtograms/microliter C&S stool	Not Detected	Not Detected
Cryptosporidium parvum/hominis	<1.76e2	genome copies/microliter C&S stool	Not Detected	Not Detected
Cyclospora cayetanensis	<2.65e2	genome copies/microliter C&S stool	Not Detected	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	9.48e2	genome copies/microliter C&S stool	Detected	Not Detected

Entamoeba hartmanii	Not Detected
Entamoeba polecki	Not Detected
Endolimax nana	Not Detected
Giardia	Rare Cyst(s) 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

GENOVA DIAGNOSTICS

Case Study 4

- 27 yo F
- Alternating constipation, diarrhea
- Gas, bloating
- Preparing for pregnancy
- Takes PERT



Inflammation and Immunology

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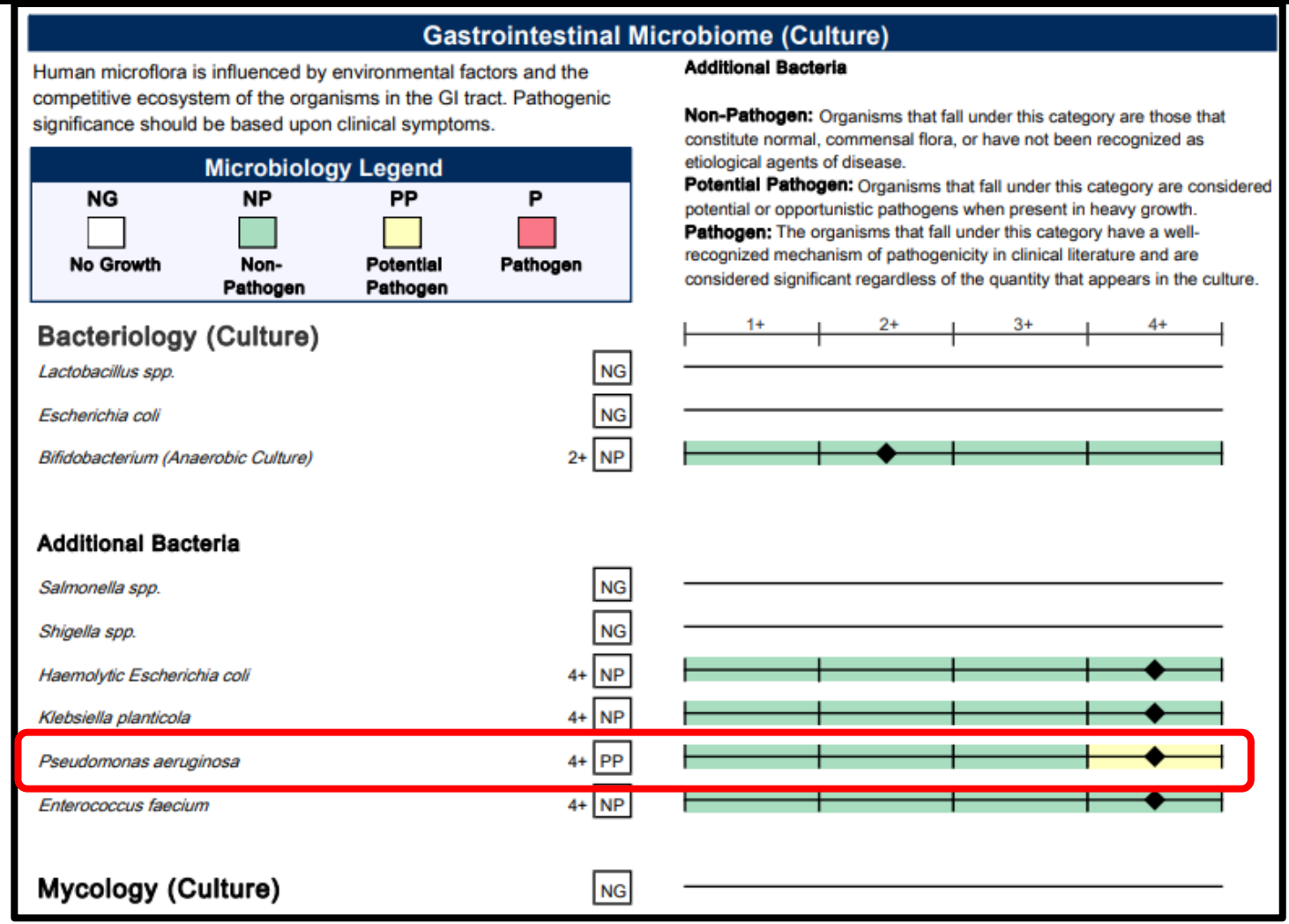

Gastrointestinal Microbiome (Culture)

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		NG	
Bifidobacterium (Anaerobic Culture)	2+	NP	

Additional Bacteria

Salmonella spp.		NG	
Shigella spp.		NG	
Haemolytic Escherichia coli	4+	NP	
Klebsiella planticola	4+	NP	
Pseudomonas aeruginosa	4+	PP	
Enterococcus faecium	4+	NP	

Mycology (Culture)

		NG

GENOVA DIAGNOSTICS

Case Study 4 cont.

- 27 yo F
- Alternating constipation, diarrhea
- Gas, bloating
- Preparing for pregnancy
- Takes PERT

Parasitology

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Genus/species	Result
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Ancylostoma/Necator (Hookworm)	Not Detected
Ascaris lumbricoides	Not Detected

PCR Parasitology - Protozoa

Methodologies: DNA by PCR

Organism	Result	Units		Expected Result
Blastocystis spp.	1.99e4	femtograms/microliter C&S stool	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

Protozoa	
Balantidium coli	Not Detected
Blastocystis spp.	**Many 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

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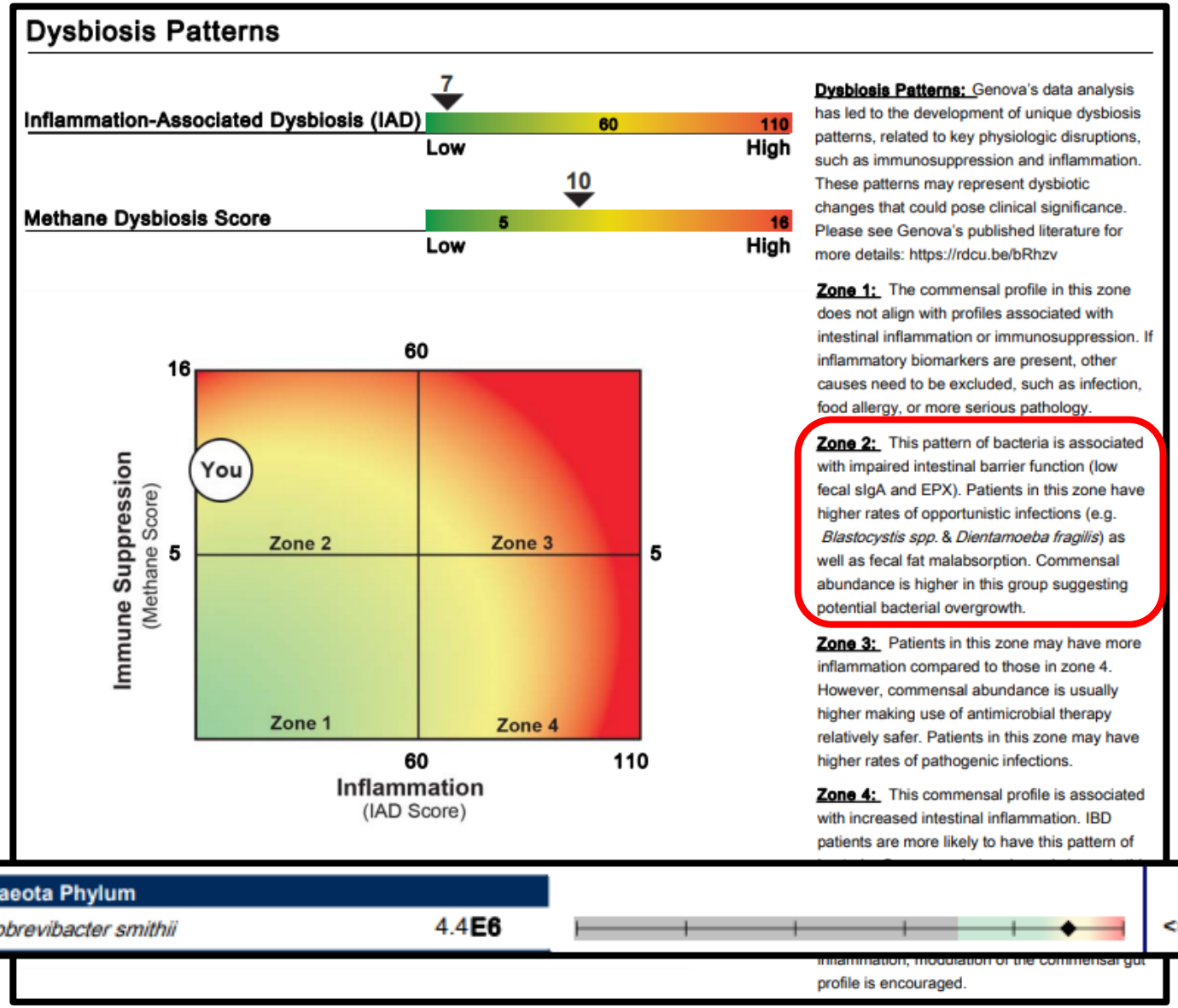
Case Study 4 cont.

- 27 yo F
- Alternating constipation, diarrhea
- Gas, bloating
- Preparing for pregnancy
- Takes PERT



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Case Study 4 cont.

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- Alternating constipation, diarrhea
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Inflammation and Immunology			
Calprotectin †	<16	50 120	<=50 mcg/g
Eosinophil Protein X (EPX)†	<DL	0.5 2.7	<=2.7 mcg/g
Fecal secretory IgA	<150	680 2040	<=2,040 mcg/mL

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Additional Educational Resources

www.GDX.net

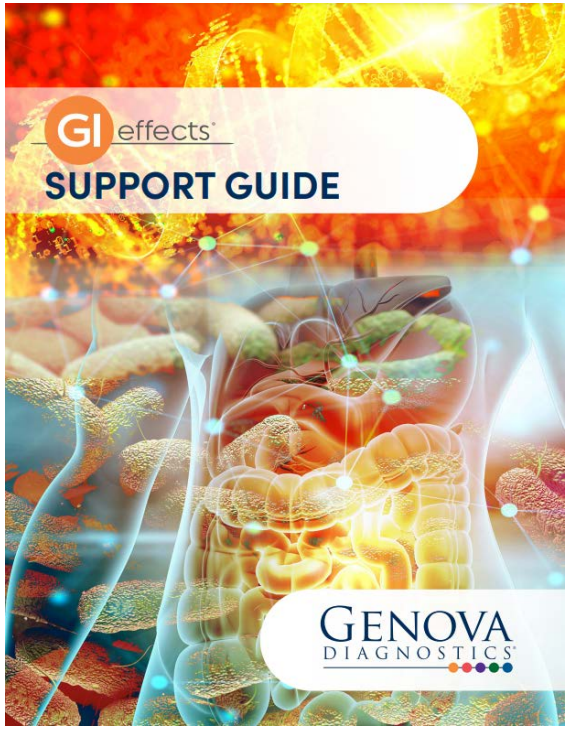
- GI Effects Support Guide and organism charts
- Learning Library video modules
- Test Prep information

The Lab Report Podcast

- Available on Apple Podcasts and GDX.net

Medical Education Consultations

- Schedule online through myGDX
- Call Client Services 800-522-4762
- 1:1 and group consultations



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Inflammation & Immunology: The Barrier to the Outside World

Donna J Smith, ND, L.Ac
Medical Education Specialist| Genova Diagnostics

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