

Metamatrix 2007 Teleconference Series

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With Assistance in Case Study Preparation by

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The Metamatrix GI Effects Stool Profile Microbial Detection by Phylum-, Genus-, Species- and Strain-Specific Genome Assay

Focus on Digestion, Absorption and Inflammation Markers

GI Effects Report Sections

1. Page 1
 - a. Predominant Bacteria
 - b. Opportunistic Bacteria
2. Page 2
 - a. Pathogens, Mycology & Parasitology
 - b. Adiposity Index
 - c. Drug Resistance Genes
3. Page 3
 - a. **Beneficial SCFA**
 - i. Severe suppression of intestinal bacteria by potent, wide-spectrum antibiotics causes **Total SCFA** and **n-Butyrate** to be low.
 - ii. The percentages (**Acetate %**, **Butyrate %**, **Propionate %** and **Valerate %**) shows a fingerprint of composite bacterial metabolism that can be monitored to see how changes in predominant bacteria and patient dietary intake of prebiotic sources affect their overall metabolic output.
 - b. **Inflammation**
 - i. **Lactoferrin** is an iron-binding glycoprotein released in IBD (Chron's, UC, infection) but not in non-inflammatory IBS.
 - ii. In certain stages of severe inflammation **WBC's** and heavy **Mucus** will appear, especially in diarrhea-dominant IBS.
 - c. **Immunology**
 - i. Chronic food intolerance or bacterial overgrowth can cause buildup of the intestinal immune barrier, producing elevated **Fecal sIgA** levels. On the other hand, sIgA is suppressed by cortisol, making low levels appear due to chronic stress.
 - ii. Specific antigen stimulation by gliadin is reflected in elevated fecal **Anti-gliadin sIgA**.
4. Page 4
 - a. **Additional Tests**
 - i. As SCFA levels fall or intestinal ammonia production increases, fecal **pH** tends to rise. Alternatively, high production of poorly-absorbed organic acids causes drop in pH. Profound D-lactic aciduria may be accompanied by low fecal pH.

- ii. Bleeding into the colon may be detected by the appearance of **Occult blood, RBCs** and characteristic **Color**. These tests are performed by visual examination.

b. Digestion

- i. Of the multiple enzymes secreted into the duodenum from the pancreas, **Elastase 1** is one of the most resistant to digestion. This property allows the digestion of dietary elastin in meats to continue far into the colon, while most amino acid and fat release is completed in the small intestine. The test is specific for human elastase, so therapy with digestive enzyme supplements does not interfere.
- ii. Maldigestion of fat causes appearance of undigested **Triglycerides** in stool, while protein maldigestion causes dietary peptides to persist into the colon where the amino acids are converted into **Putrefactive SCFA**. Observation of Vegetable Fibers simply provides a measure of heavy non-digestible fiber intake.

c. Absorption

- i. When pancreatic enzymes work properly to release fatty acids, but fat malabsorption is present, then fecal **LCFAs** are elevated along with **Total Fat** and **Cholesterol**.

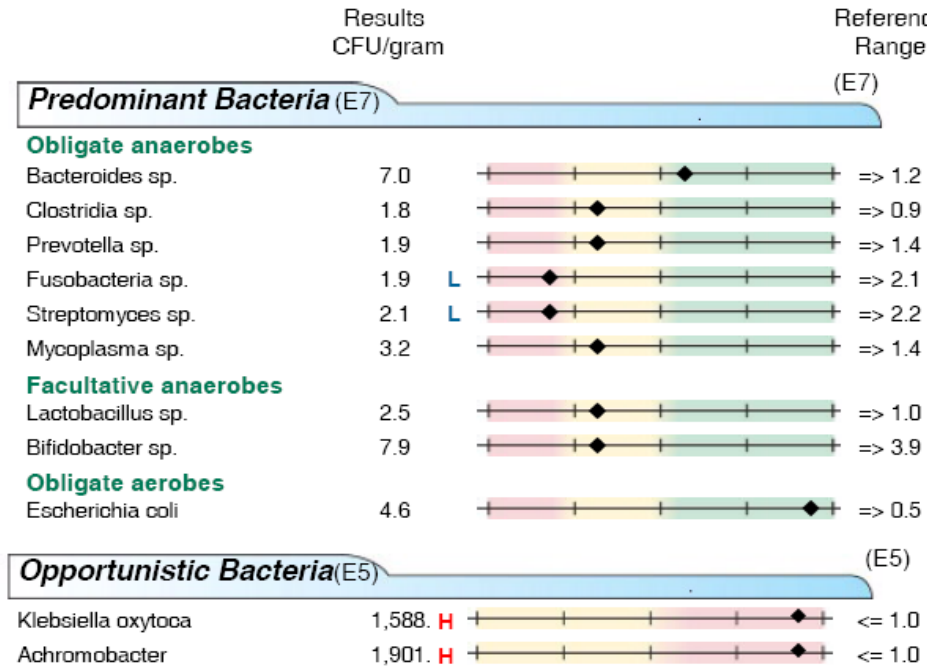
Case Studies

CASE 1 – 84/F with Chronic Diarrhea

An 84 y/o female presented with chronic, explosive diarrhea, onset 09/06, then again 01/07 until present, occurring primarily between midnight and 2AM.

Findings:

2100 Gastrointestinal Function Profile Methc



NOTE:
 Bacteroides = 7.0×10^7
 Bifidobacter = 7.9×10^7
 E. coli = 4.6×10^7 (Rel. High)
 K. oxytoca = 1.59×10^8
 Achromobacter = 1.90×10^8

Predominant Bacteria

Low abundance of Fusobacteria and Streptomyces (plus below normal Bifidobacter and Lactobacillus) with relative overgrowth of E. coli.

Opportunistic Bacteria (see NOTE showing genome abundances)

K. oxytoca

- known association with acute colitis caused by use of quinolone antibiotics.

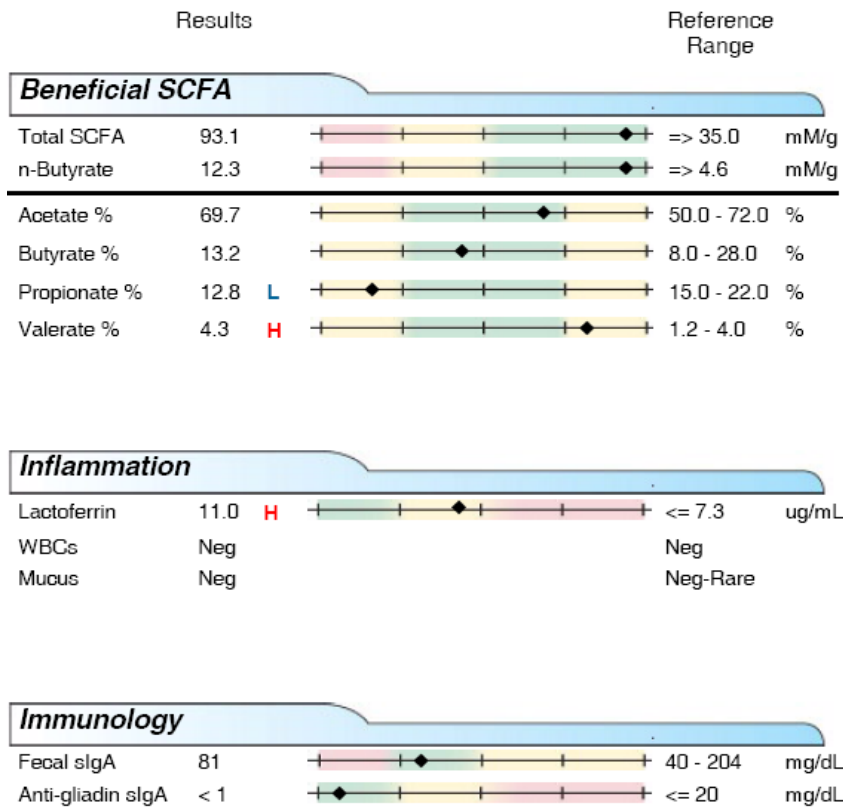
Achromobacter

- A. xylosoxidans is commonly found as a nosocomial infection in immunocompromised patients, so hospital exposure might be investigated, although numerous other specie may account for the elevation.

No pathogens, yeast, parasites or drug resistance genes are found.

CASE 1 – 84/F with Chronic Diarrhea (continued)

2100 Gastrointestinal Function Profile Methc



Here we see the metabolic fingerprint of the bacterial dysbiosis in the imbalanced propionate (L) and valerate (H) reflecting shifted total bacterial metabolic output. We also note the high Lactoferrin produced by intra-epithelial leukocytes to sequester iron as means of lowering the inflammation due to bacterial growth. The Metamatrix Celiac Profile might be ordered to further diagnose this patient.

2100 Gastrointestinal Function Profile *Methc*

Results		Reference Range
Additional Tests		
pH	6.9	6.1 - 7.2
Occult blood	Neg	Neg
RBCs	Neg	Neg
Color	Brown	
Digestion		
Elastase 1	183 L	=> 200 ug/mL
Triglycerides	67	<= 400 mg/dL
Putrefactive SCFA	2.9	0.7 - 4.8 mM/g
Vegetable Fibers	None	None-Few
Absorption		
LCFAs	2.3	0.3 - 12.0 mmol/L
Total Fat	3.4	0.7 - 24.0 mmol/L
Cholesterol	14	<= 70 mg/dL

Low Elastase 1 indicates that pancreatic insufficiency is suggested as one etiological origin of the disturbance. With this information we might construct the medical hypothesis that, as cortisol falls in the evening, the GI immune system activity increases with inflammatory responses. Concurrently, dietary proteins are passing undigested into the lower regions of the gut where they may be presenting as antigenic stimulants. Toxic bacterial metabolic products can increase sharply, along with direct bacterial enterotoxic effects, producing gasses and stimulating peristalsis that manifests as explosive diarrhea.

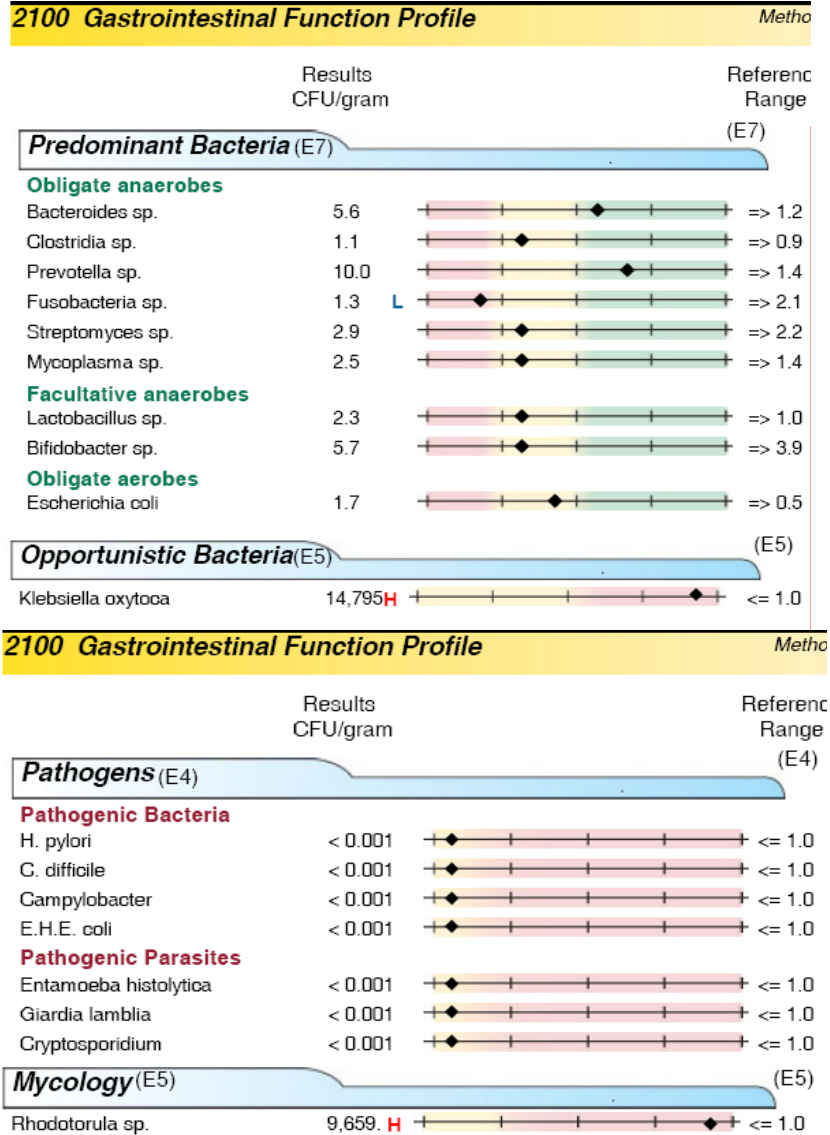
Rec:

- Submit Celiac Disease Profile specimen
- Address diet – investigate food sensitivities with IgG profile
- Add digestive enzymes and pre & pro biotics
- Alternate high dose allicyn, berberine and oil of oregano (thymol)

CASE 2 – 5/M Autistic

This 5 y/o boy developed IBS symptoms at 18 months old, with intermittent constipation. His gross motor development is normal, but he has been diagnosed as autistic with severe behavior and allergic manifestations.

Findings:



Here we find an even more severe overgrowth of *K. oxytoca*. Note that the assay is able to keep on counting target genomes over many orders of magnitude. Here the oxytoca is present at 1.48×10^9 /gm, and this child also shows concurrent extremely high levels of *Rhodotorula* sp. Incidence of *Rhodotorula* in our results is higher than from culture techniques, probably because it is identified microscopically, but grows poorly in usual culture media.

Drug Resistance Genes			
aacA, aphD	Pos	gyrB, ParE	Neg
mecA	Neg	PBP1a, 2B	Neg
vanA, B, and C	Neg		

The drug resistance gene (aacA, aphD) indicates that Erythromycin and Levofloxacin or other amino glycoside class antibiotics should be avoided, even though *K. oxytoca* in this patient is sensitive to these drugs. Drugs like Amoxicillin may be used to reduce the *K. oxytoca* and Rhodotorula is sensitive to Amphotericin B or Fluconazole.

2100 Gastrointestinal Function Profile Methc

Results		Reference Range
Beneficial SCFA		
Total SCFA	93.1	=> 35.0 mM/g
n-Butyrate	10.1	=> 4.6 mM/g
Acetate %	66.4	50.0 - 72.0 %
Butyrate %	10.9	8.0 - 28.0 %
Propionate %	21.3	15.0 - 22.0 %
Valerate %	1.4	1.2 - 4.0 %
Inflammation		
Lactoferrin	7.1	<= 7.3 ug/mL
WBCs	Neg	Neg
Mucus	Neg	Neg-Rare
Immunology		
Fecal sIgA	37 L	40 - 204 mg/dL
Anti-gliadin sIgA	5	<= 20 mg/dL

Plenty of metabolic activity is shown by the abundant SCFA and balanced percentages. Note the normal level of Lactoferrin, showing lack of inflammatory involvement currently in this patient with very active IBS due to profound microbial overgrowth. The immune barrier is weakened as shown by low sIgA.

Rec:

- Antibacterials and antifungals according to sensitivities
- Immune support with glutamine (consider colostrums) and glutathione
- Investigate food intolerances to lower the loading of GALT

CASE 3 – Carbohydrate Intolerant

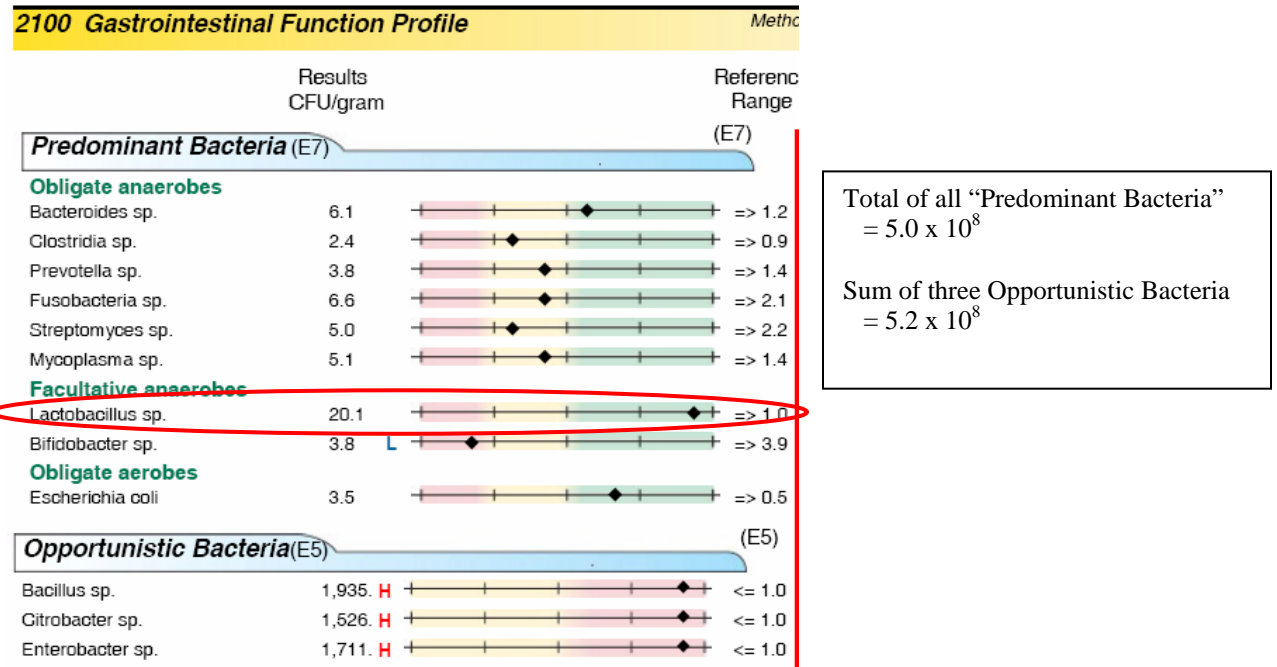
This 26 y/o female presented with severe carbohydrate intolerance—including gas, bloating, intense upper GI pain dysglycemia and mood swings. She reported severe vaginal candidiasis associated with sugar intake and amenorrhea for the past year. She has gained 60lb of body weight over past few years and she is unable to lose weight (currently 190 lbs, formerly 130 lbs).

Past medical history: adult: ACTH-secreting pituitary adenoma developed Cushing disease. Adenectomy x 1 year ago. History of anorexia and binge eating, use of prescription diet pills. Childhood: colitis. Regular use of antibiotics.

Current treatment (before GIFX): agave juice fast resulted in greatly reduced GI pain and she is starting to lose some weight. Other interventions have stimulated menstruation, although it is irregular, generally occurring about every other month. Numerous supplements and diets- including low carbohydrate- have been intolerant to this patient.

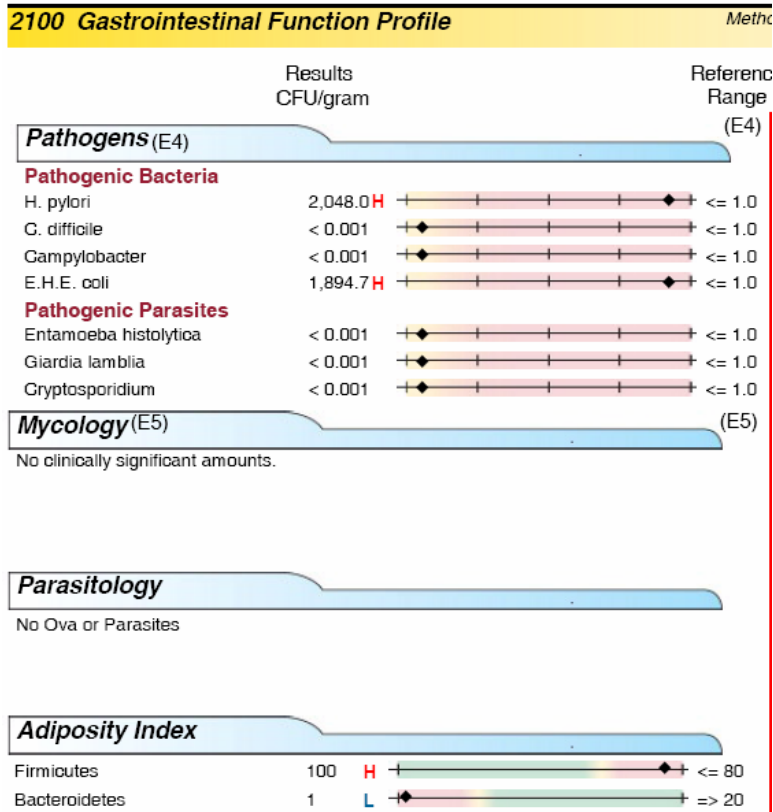
Patient has recently submitted for a full GI endoscopy- results pending.

Findings:



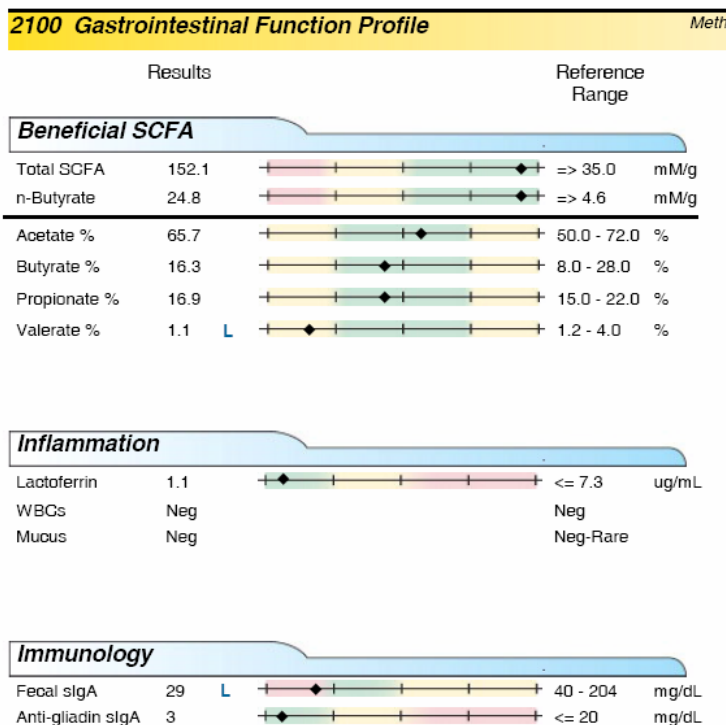
The results show a severe, multiple genus colonic bacterial overgrowth. The three genera of opportunistic organisms are present at numbers exceeding all of the normal predominant genera measured by the assay. This is in spite of grossly high levels of Lactobacillus accompanied by suppression of Bifidobacter sp. This patient has a severe colonic bacterial imbalance of the type that may now be seen from the perspective of the full magnitude of normal bacterial balance disturbance. As therapy proceeds, the quantitative results will allow progressive inspection of how successful each step is toward the goal of bring down the opportunistic numbers and restoring more normal numbers of both aerobic and anaerobic Bifidobacter sp.

With this picture in mind, we can go on to inspect for pathogens and other signs of imbalance.

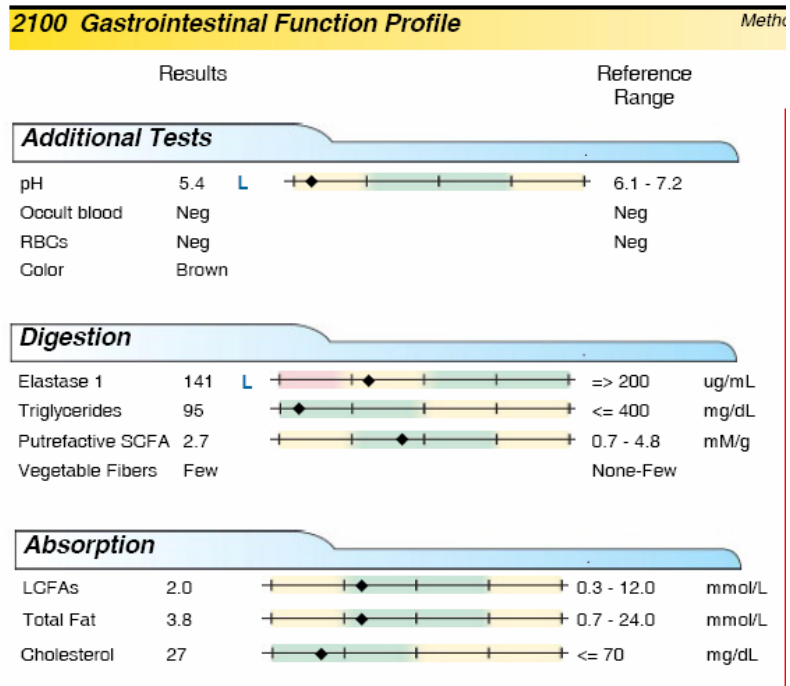


The bacterial situation is amplified by finding very high levels of H. pylori and Enterohemorrhagic E. Coli, two species that are known to elaborate toxic products. With the history in this case, the data provide ample evidence of intestinal bacterial overgrowth that needs to be brought under control. The reported symptoms of vaginal yeast are not accompanied by detection of fecal yeast. Possibly, urinary excretion of bacterial toxins is impairing vaginal immune suppression of yeast.

Note the extreme imbalance in the Adiposity Index that greatly restricts weight loss because of increased caloric yield from dietary caloric sources. Successful therapy should focus on raising Bifidobacter levels since Lactobacilli are among the members of the Firmicutes phylum, and Lactobacilli are currently strongly elevated.



The low valerate shows that the opportunists are not valerate producers, and that they have suppressed normal species that do make this SCFA. The low Fecal sIgA is no surprise, but it also provides a good record for comparison as follow up test is done to monitor progress with the immune barrier competence that should improve as bacterial populations become more favorable to healthy GALT function.



The bacterial overgrowth in this case appears to be so intense that the rate of production of organic acid metabolic products is causing a lowering of fecal pH. Pancreatic digestive function impairment is revealed by the low Elastase 1 level.

Microbial Sensitivity Profile	Microbial Sensitivity Profile	Microbial Sensitivity Profile
Bacillus sp.	Enterobacter sp.	Citrobacter sp.
Sensitive	Sensitive	Sensitive
Pharmaceuticals Amoxicillin S Ampicillin S Augmentin S Cefpodoxime S Cefuroxime S Ciprofloxacin S Clindamycin S Erythromycin S Levofloxacin S Penicillin S Potassium Clavula S Sulfamethoxazole S Tetracyclin S Trimeth-Sulfa S	Pharmaceuticals Amoxicillin S Ampicillin S Augmentin S Cefuroxime S Ciprofloxacin S Clindamycin S Erythromycin S Levofloxacin S Penicillin S Potassium Clavula S Sulfamethoxazole S Tetracyclin S Trimeth-Sulfa S	Pharmaceuticals Erythromycin S Levofloxacin S Penicillin S Potassium Clavula S Sulfamethoxazole S Tetracyclin S Trimeth-Sulfa S Amoxicillin S Ampicillin S Augmentin S Cefuroxime S Ciprofloxacin S Clindamycin S
Botanicals Aliin S Berberine S Black walnut S Caprylic acid (Octanoic) S Goldenseal root S Grapefruit seed extract S	Botanicals Aliin S Berberine S Black walnut S Caprylic acid (Octanoic) S Goldenseal root S Grapefruit seed extract S	Botanicals Aliin S Berberine S Black walnut S Caprylic acid (Octanoic) S Goldenseal root S Grapefruit seed extract S

Quick inspection of the sensitivity report allows us to see that botanicals and pharmaceuticals like Amoxicillin may answer for opportunistic as well as pathogenic *H. pylori*. Quinoline antibiotics may be needed to bring the *E.H.E. coli* under control. If so, we know to watch follow up GI Effects profiles for the appearance of *K. oxytoca* that might exacerbate recovery with appearance of increased diarrheal episodes.

Digestive intervention with pancreatic enzymes and a trial of betaine hydrochloride is suggested. This patient can be encouraged to look forward to better success with weight control as the digestive and bacterial situation is normalized. She can renew her efforts toward dietary balance and caloric restriction with expectation of success. Those efforts will hasten the improvement in the Adiposity Index. This effect may be due to caloric restriction causing growth of species that have more varied energy substrate requirements. Under reduced energy intake, they more successfully compete to establish permanent colonies, generating improved microbial diversity.

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