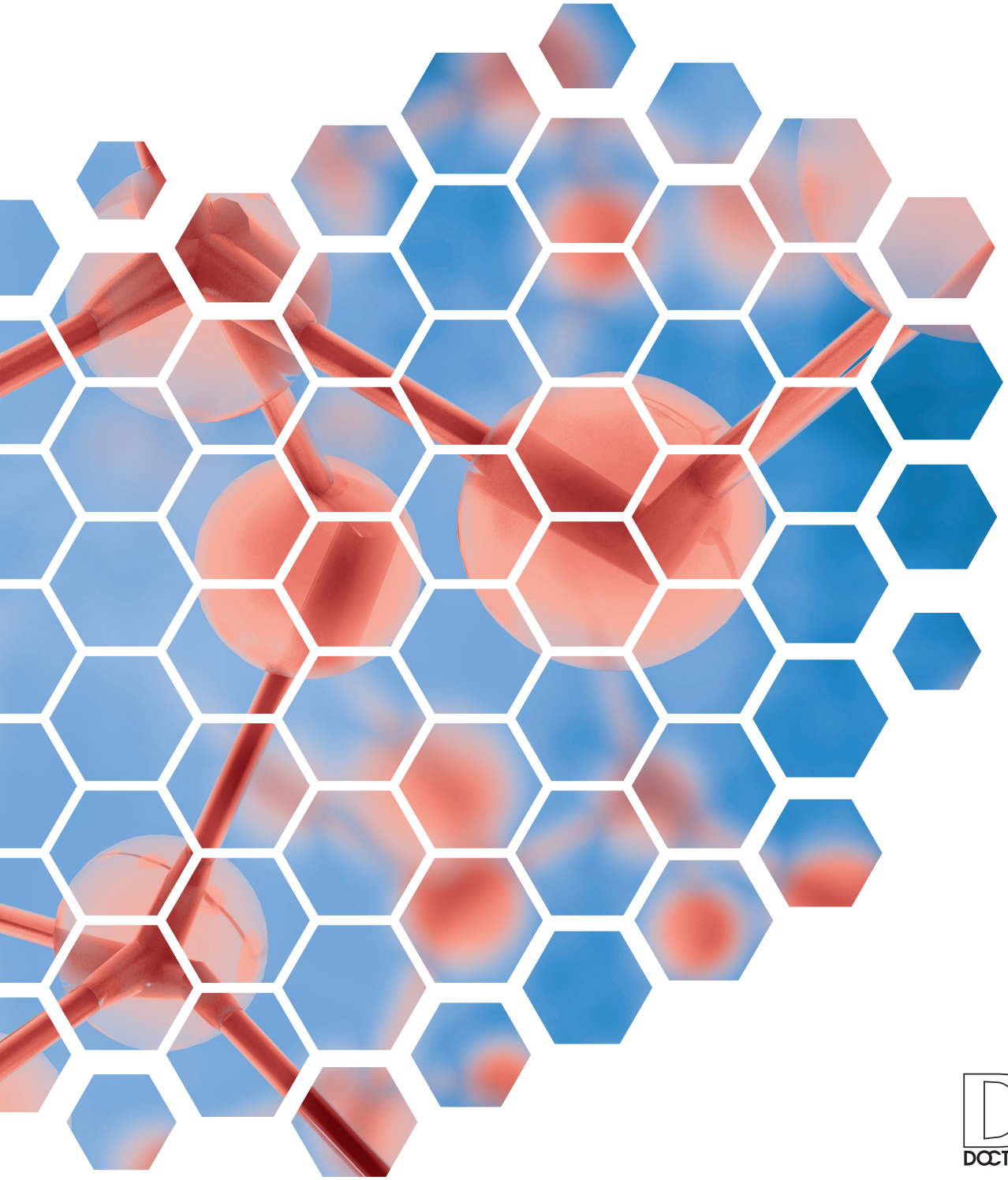
GI360™ Resource Guide



DOCTOR'S DATA INC.

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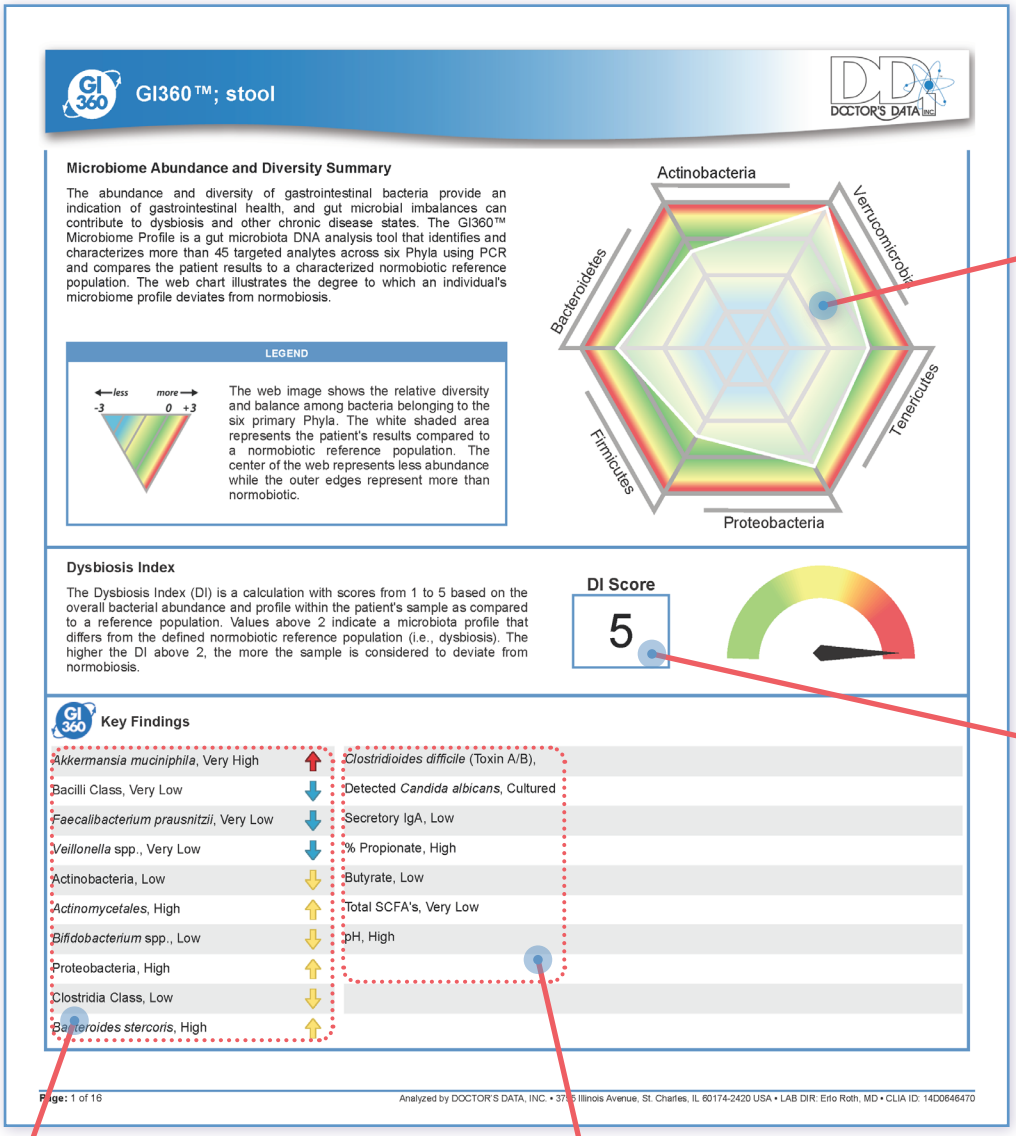

GI360™; stool

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Microbiome Abundance and Diversity Summary

The abundance and diversity of gastrointestinal bacteria provide an indication of gastrointestinal health, and gut microbial imbalances can contribute to dysbiosis and other chronic disease states. The GI360™ Microbiome Profile is a gut microbiota DNA analysis tool that identifies and characterizes more than 45 targeted analytes across six Phyla using PCR and compares the patient results to a characterized normobiotic reference population. The web chart illustrates the degree to which an individual's microbiome profile deviates from normobiosis.

LEGEND

less more
-3 0 +3

The web image shows the relative diversity and balance among bacteria belonging to the six primary Phyla. The white shaded area represents the patient's results compared to a normobiotic reference population. The center of the web represents less abundance while the outer edges represent more than normobiotic.

Actinobacteria
Verrucomicrobia
Bacteroidetes
Tenericutes
Firmicutes
Proteobacteria

Dysbiosis Index

The Dysbiosis Index (DI) is a calculation with scores from 1 to 5 based on the overall bacterial abundance and profile within the patient's sample as compared to a reference population. Values above 2 indicate a microbiota profile that differs from the defined normobiotic reference population (i.e., dysbiosis). The higher the DI above 2, the more the sample is considered to deviate from normobiosis.

DI Score
5

Key Findings

Akkermansia muciniphila, Very High	*Clostridioides difficile* (Toxin A/B),
Bacilli Class, Very Low	Detected *Candida albicans*, Cultured
Faecalibacterium prausnitzii, Very Low	Secretory IgA, Low
Veillonella spp., Very Low	% Propionate, High
Actinobacteria, Low	Butyrate, Low
Actinomycetales, High	Total SCFA's, Very Low
Bifidobacterium spp., Low	pH, High
Proteobacteria, High	
Clostridia Class, Low	
Bacteroides stercoris, High	

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Patients results at a glance compared to the normobiotic reference population. Deviation from a hexagonal shape indicates variant abundance and diversity within the microbial community.

There are different types of dysbiosis. The Dysbiosis Index is calculated strictly from the Microbiota Abundance analytes, and does not include specific pathogenic and dysbiotic bacteria, yeast, parasites and viruses that may be identified in subsequent sections of the GI360™.

Significant findings at-a-glance from the PCR-based Microbiome Profile, with detailed results on the following 3 pages of the report.

Significant findings at-a-glance from all other sections of the report

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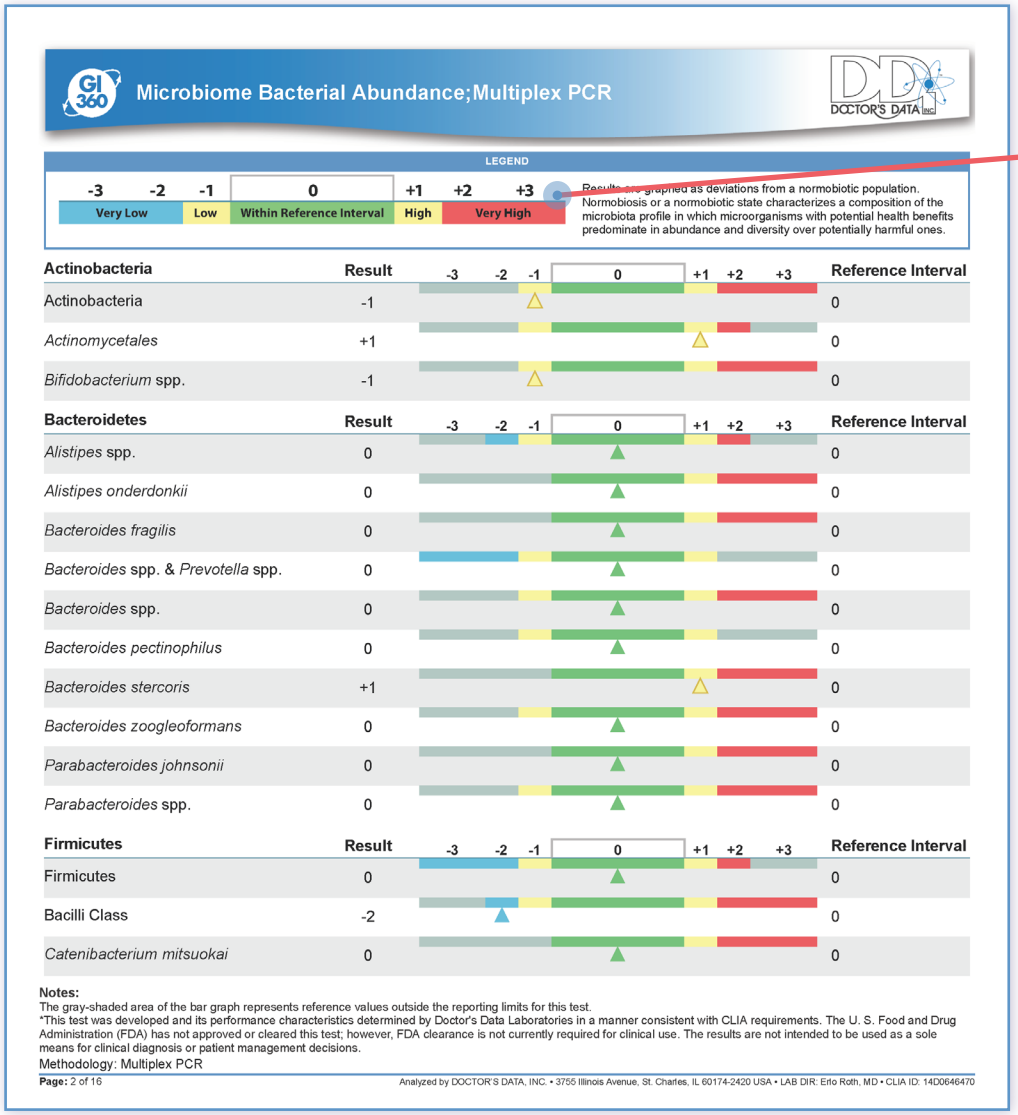

Microbiome Bacterial Abundance;Multiplex PCR

LEGEND

-3	-2	-1	0	+1	+2	+3
Very Low		Low	Within Reference Interval	High	Very High	

Results are graphed as deviations from a normobiotic population. Normobiosis or a normobiotic state characterizes a composition of the microbiota profile in which microorganisms with potential health benefits predominate in abundance and diversity over potentially harmful ones.

Actinobacteria	Result	Reference Interval
Actinobacteria	-1	0
Actinomycetales	+1	0
Bifidobacterium spp.	-1	0

Bacteroidetes	Result	Reference Interval
Alistipes spp.	0	0
Alistipes onderdonkii	0	0
Bacteroides fragilis	0	0
Bacteroides spp. & *Prevotella* spp.	0	0
Bacteroides spp.	0	0
Bacteroides pectinophilus	0	0
Bacteroides stercoris	+1	0
Bacteroides zoogleoformans	0	0
Parabacteroides johnsonii	0	0
Parabacteroides spp.	0	0

Firmicutes	Result	Reference Interval
Firmicutes	0	0
Bacilli Class	-2	0
Catenibacterium mitsuokai	0	0

Notes:
The gray-shaded area of the bar graph represents reference values outside the reporting limits for this test.
*This test was developed and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements. The U. S. Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA clearance is not currently required for clinical use. The results are not intended to be used as a sole means for clinical diagnosis or patient management decisions.
Methodology: Multiplex PCR

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Results reported as -3 to +3 standard deviations from the normobiotic reference population.

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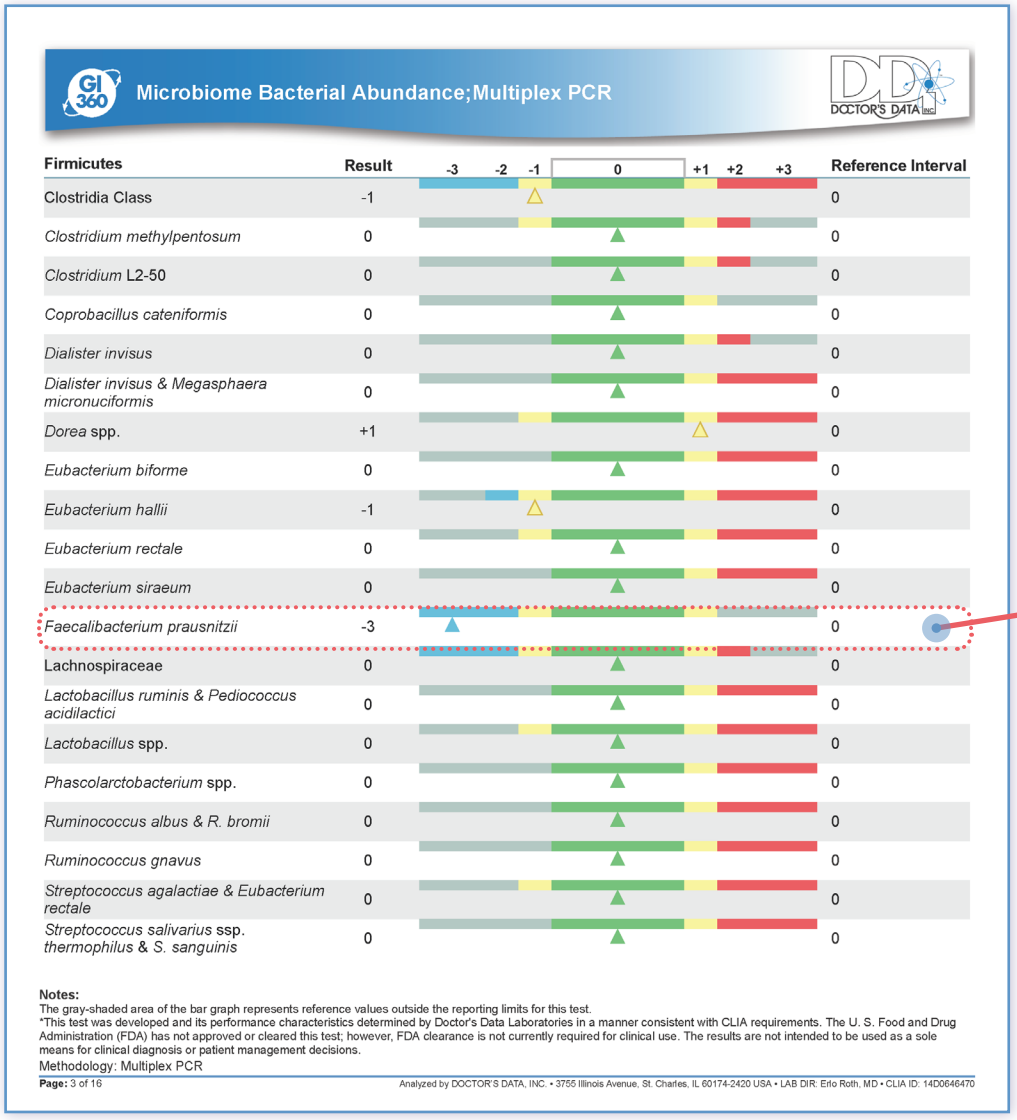

Microbiome Bacterial Abundance;Multiplex PCR

Firmicutes	Result	-3 -2 -1 0 +1 +2 +3	Reference Interval
Clostridia Class	-1		0
Clostridium methylpentosum	0		0
Clostridium L2-50	0		0
Coprobacillus cateniformis	0		0
Dialister invisus	0		0
Dialister invisus & Megasphaera micronuciformis	0		0
Dorea spp.	+1		0
Eubacterium biforme	0		0
Eubacterium hallii	-1		0
Eubacterium rectale	0		0
Eubacterium siraeum	0		0
Faecalibacterium prausnitzii	-3		0
Lachnospiraceae	0		0
Lactobacillus ruminis & Pediococcus acidilactici	0		0
Lactobacillus spp.	0		0
Phascolarctobacterium spp.	0		0
Ruminococcus albus & R. bromii	0		0
Ruminococcus gnavus	0		0
Streptococcus agalactiae & Eubacterium rectale	0		0
Streptococcus salivarius ssp. *thermophilus & S. sanguinis*	0		0

Notes:
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Methodology: Multiplex PCR

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Predominant butyrate producer

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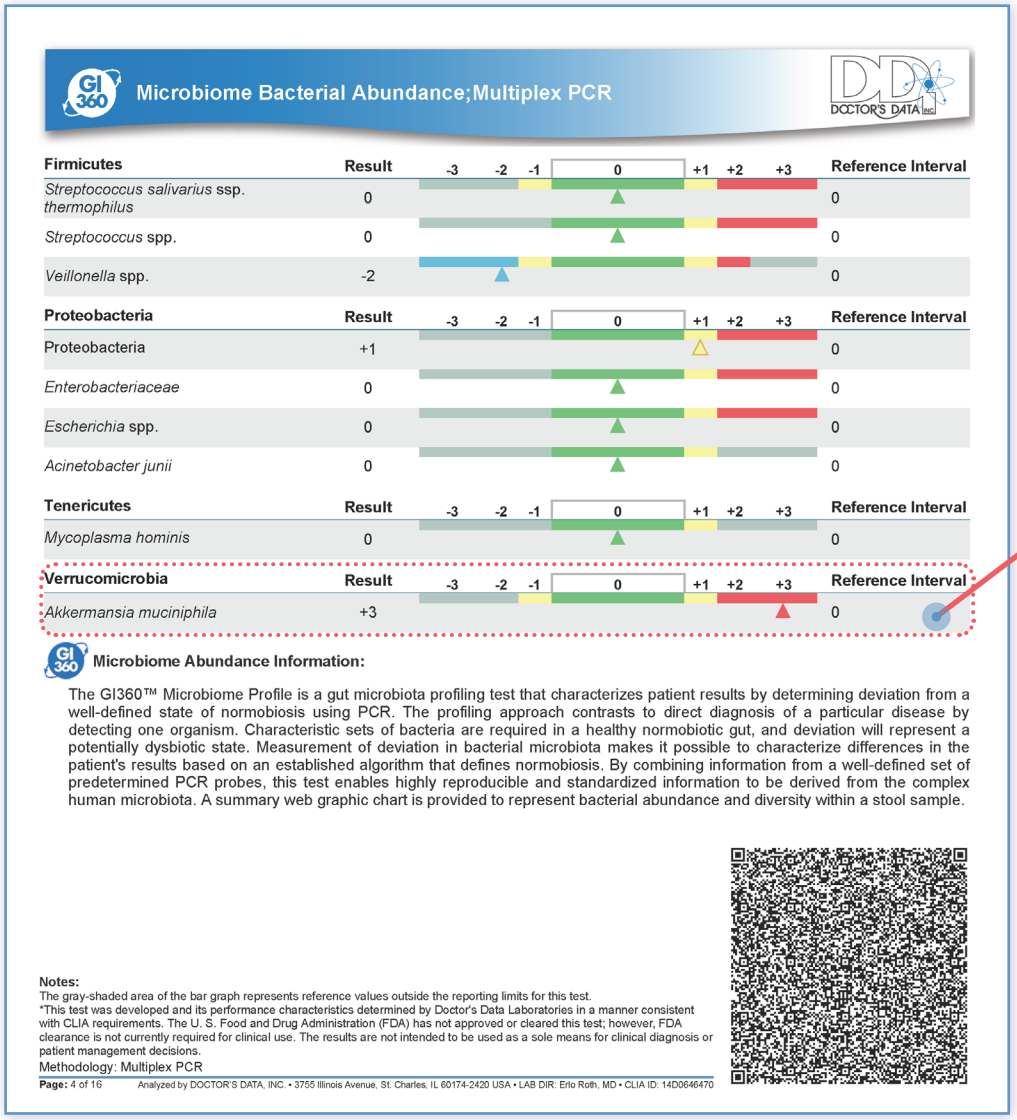

Microbiome Bacterial Abundance;Multiplex PCR

Firmicutes	Result	-3 -2 -1 0 +1 +2 +3	Reference Interval
Streptococcus salivarius ssp. *thermophilus*	0		0
Streptococcus spp.	0		0
Veillonella spp.	-2		0

Proteobacteria	Result	-3 -2 -1 0 +1 +2 +3	Reference Interval
Proteobacteria	+1		0
Enterobacteriaceae	0		0
Escherichia spp.	0		0
Acinetobacter junii	0		0

Tenericutes	Result	-3 -2 -1 0 +1 +2 +3	Reference Interval
Mycoplasma hominis	0		0

Verrucomicrobia	Result	-3 -2 -1 0 +1 +2 +3	Reference Interval
Akkermansia muciniphila	+3		0

Microbiome Abundance Information:

The GI360™ Microbiome Profile is a gut microbiota profiling test that characterizes patient results by determining deviation from a well-defined state of normobiosis using PCR. The profiling approach contrasts to direct diagnosis of a particular disease by detecting one organism. Characteristic sets of bacteria are required in a healthy normobiotic gut, and deviation will represent a potentially dysbiotic state. Measurement of deviation in bacterial microbiota makes it possible to characterize differences in the patient's results based on an established algorithm that defines normobiosis. By combining information from a well-defined set of predetermined PCR probes, this test enables highly reproducible and standardized information to be derived from the complex human microbiota. A summary web graphic chart is provided to represent bacterial abundance and diversity within a stool sample.

Notes:
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Methodology: Multiplex PCR

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Although *A. muciniphila* has important anti-inflammatory functions, too much of it's mucolytic activity may be associated with increased intestinal permeability.

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GI Pathogens;Multiplex PCR

Viruses	Result
Adenovirus F40/41	Negative
Norovirus GI/GII	Negative
Rotavirus A	Negative

Pathogenic Bacteria	Result
Campylobacter (*C. jejuni*, *C. coli* and *C. lari*)	Negative
Clostridioides difficile (Toxin A/B)	Positive
Escherichia coli O157	Negative
Enterotoxigenic *Escherichia coli* (ETEC) lt/st	Negative
Salmonella spp.	Negative
Shiga-like toxin-producing *Escherichia coli* (STEC) stx1/stx2	Negative
Shigella (*S. boydii*, *S. sonnei*, *S. flexneri* & *S. dysenteriae*)	Negative
Vibrio cholerae	Negative

Parasites	Result
Cryptosporidium (*C. parvum* and *C. hominis*)	Negative
Entamoeba histolytica	Negative
Giardia duodenalis (AKA *intestinalis & lamblia*)	Negative

PCR testing is very sensitive and allows for the detection of extremely low levels of pathogens. Decisions regarding clinical intervention should take the patient's complete clinical history and presentation into account.

Parasitology; Microscopy

Protozoa	Result
Balantidium coli	Not Detected
Blastocystis spp.	Not Detected
Chilomastix mesnili	Not Detected
Dientamoeba fragilis	Not Detected
Endolimax nana	Not Detected
Entamoeba coli	Not Detected
Entamoeba hartmanni	Not Detected
Entamoeba histolytica/*Entamoeba dispar*	Not Detected
Entamoeba polecki	Not Detected
Enteromonas hominis	Not Detected
Giardia duodenalis	Not Detected
Iodamoeba bütschlii	Not Detected
Isospora belli	Not Detected
Pentatrichomonas hominis	Not Detected
Retortamonas intestinalis	Not Detected

Cestodes - Tapeworms	Result
Diphyllobothrium latum	Not Detected
Dipylidium caninum	Not Detected
Hymenolepis diminuta	Not Detected
Hymenolepis nana	Not Detected
Taenia	Not Detected

Trematodes - Flukes	Result
Clonorchis sinensis	Not Detected
Fasciola hepatica/*Fasciolopsis buski*	Not Detected
Heterophyes heterophyes	Not Detected
Paragonimus westermani	Not Detected

Nematodes - Roundworms	Result
Ascaris lumbricoides	Not Detected

Notes:
Methodology: Microscopy

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Microscopy (O&P) permits detection of many additional parasites not detected using PCR.

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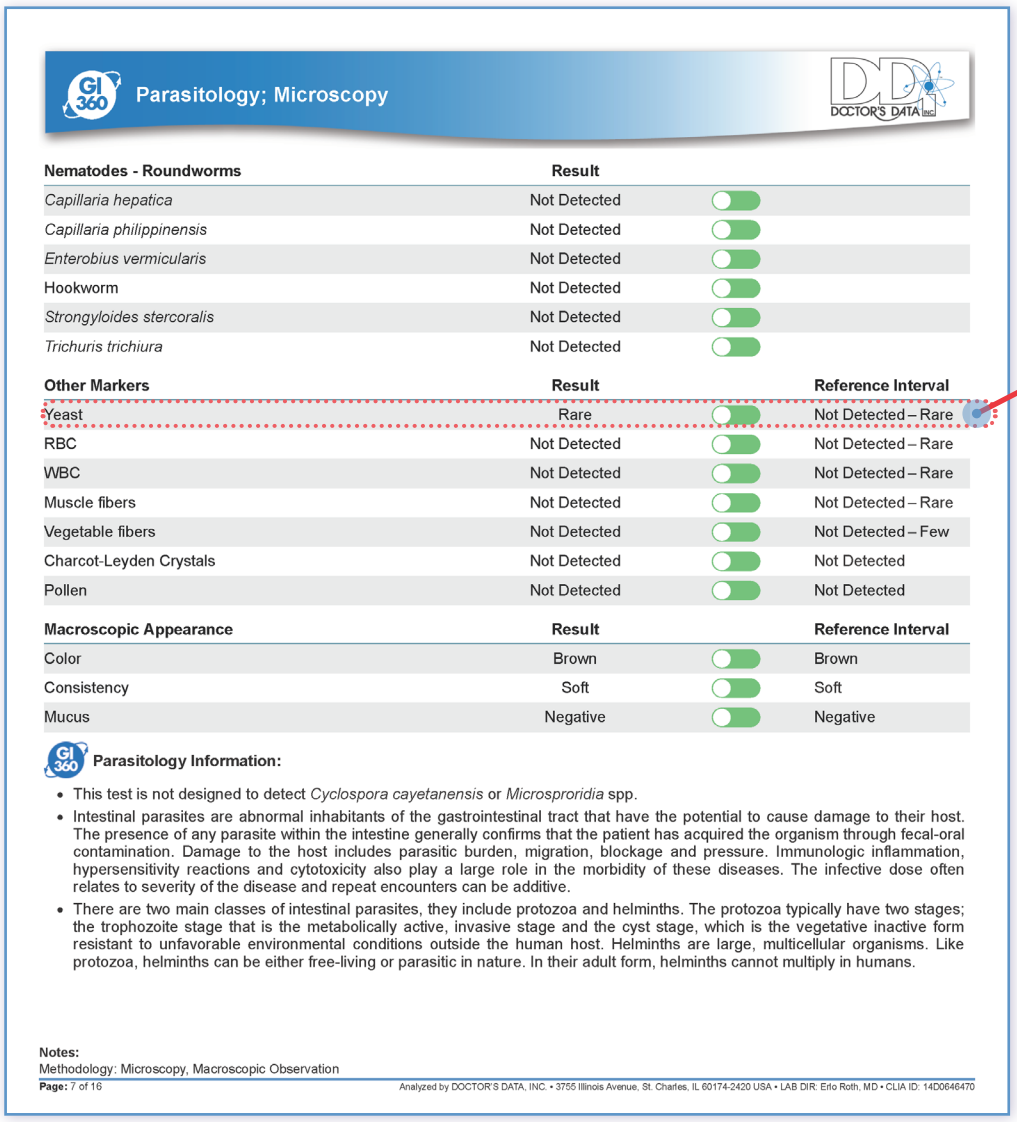

Parasitology; Microscopy

Nematodes - Roundworms	Result
Capillaria hepatica	Not Detected
Capillaria philippinensis	Not Detected
Enterobius vermicularis	Not Detected
Hookworm	Not Detected
Strongyloides stercoralis	Not Detected
Trichuris trichiura	Not Detected

Other Markers	Result	Reference Interval
Yeast	Rare	Not Detected – Rare
RBC	Not Detected	Not Detected – Rare
WBC	Not Detected	Not Detected – Rare
Muscle fibers	Not Detected	Not Detected – Rare
Vegetable fibers	Not Detected	Not Detected – Few
Charcot-Leyden Crystals	Not Detected	Not Detected
Pollen	Not Detected	Not Detected

Macroscopic Appearance	Result	Reference Interval
Color	Brown	Brown
Consistency	Soft	Soft
Mucus	Negative	Negative

Parasitology Information:

- This test is not designed to detect *Cyclospora cayetanensis* or *Microsproridia* spp.
- Intestinal parasites are abnormal inhabitants of the gastrointestinal tract that have the potential to cause damage to their host. The presence of any parasite within the intestine generally confirms that the patient has acquired the organism through fecal-oral contamination. Damage to the host includes parasitic burden, migration, blockage and pressure. Immunologic inflammation, hypersensitivity reactions and cytotoxicity also play a large role in the morbidity of these diseases. The infective dose often relates to severity of the disease and repeat encounters can be additive.
- There are two main classes of intestinal parasites, they include protozoa and helminths. The protozoa typically have two stages; the trophozoite stage that is the metabolically active, invasive stage and the cyst stage, which is the vegetative inactive form resistant to unfavorable environmental conditions outside the human host. Helminths are large, multicellular organisms. Like protozoa, helminths can be either free-living or parasitic in nature. In their adult form, helminths cannot multiply in humans.

Notes:
Methodology: Microscopy, Macroscopic Observation

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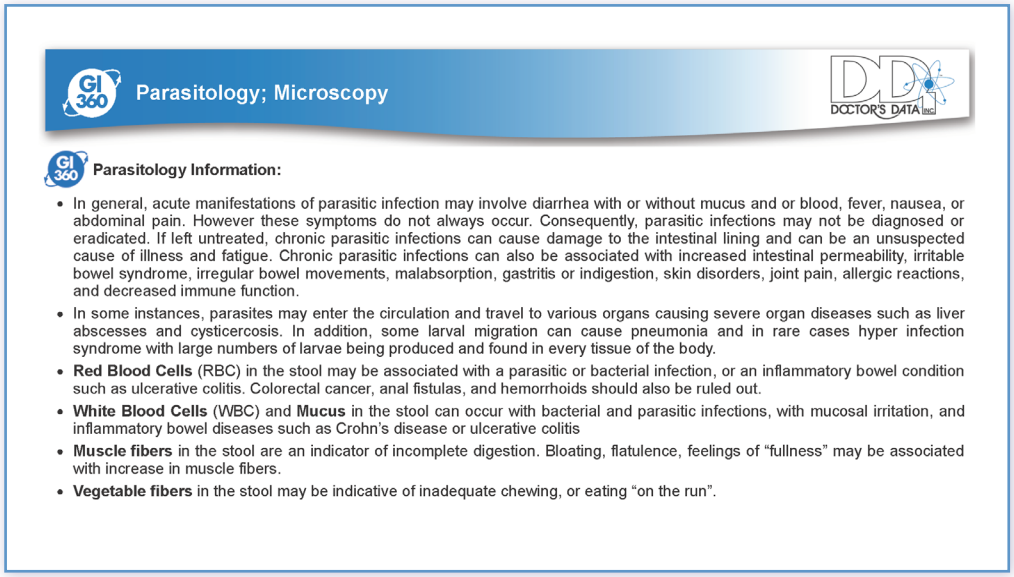
Visualization of moderate to many yeast microsopically in the absence of cultured yeast may be consistent with small intestinal fungal overgrowth. Consider symptomology for the patient.



Parasitology; Microscopy

Parasitology Information:

- In general, acute manifestations of parasitic infection may involve diarrhea with or without mucus and or blood, fever, nausea, or abdominal pain. However these symptoms do not always occur. Consequently, parasitic infections may not be diagnosed or eradicated. If left untreated, chronic parasitic infections can cause damage to the intestinal lining and can be an unsuspected cause of illness and fatigue. Chronic parasitic infections can also be associated with increased intestinal permeability, irritable bowel syndrome, irregular bowel movements, malabsorption, gastritis or indigestion, skin disorders, joint pain, allergic reactions, and decreased immune function.
- In some instances, parasites may enter the circulation and travel to various organs causing severe organ diseases such as liver abscesses and cysticercosis. In addition, some larval migration can cause pneumonia and in rare cases hyper infection syndrome with large numbers of larvae being produced and found in every tissue of the body.
- **Red Blood Cells** (RBC) in the stool may be associated with a parasitic or bacterial infection, or an inflammatory bowel condition such as ulcerative colitis. Colorectal cancer, anal fistulas, and hemorrhoids should also be ruled out.
- **White Blood Cells** (WBC) and **Mucus** in the stool can occur with bacterial and parasitic infections, with mucosal irritation, and inflammatory bowel diseases such as Crohn's disease or ulcerative colitis
- **Muscle fibers** in the stool are an indicator of incomplete digestion. Bloating, flatulence, feelings of "fullness" may be associated with increase in muscle fibers.
- **Vegetable fibers** in the stool may be indicative of inadequate chewing, or eating "on the run".

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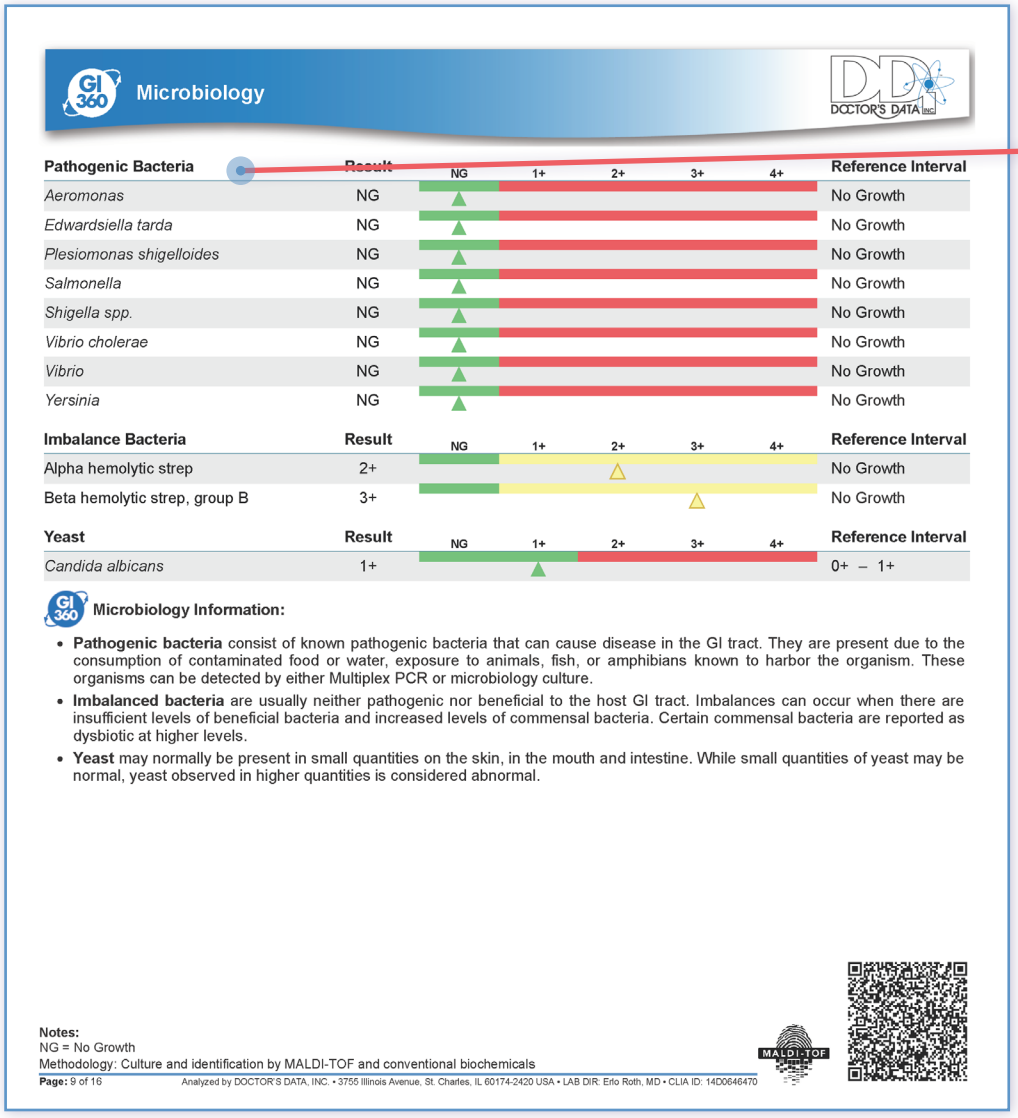

Microbiology

Pathogenic Bacteria	Result	NG	1+	2+	3+	4+	Reference Interval
Aeromonas	NG						No Growth
Edwardsiella tarda	NG						No Growth
Plesiomonas shigelloides	NG						No Growth
Salmonella	NG						No Growth
Shigella spp.	NG						No Growth
Vibrio cholerae	NG						No Growth
Vibrio	NG						No Growth
Yersinia	NG						No Growth

Imbalance Bacteria	Result	NG	1+	2+	3+	4+	Reference Interval
Alpha hemolytic strep	2+						No Growth
Beta hemolytic strep, group B	3+						No Growth

Yeast	Result	NG	1+	2+	3+	4+	Reference Interval
Candida albicans	1+						0+ – 1+

Microbiology Information:

- **Pathogenic bacteria** consist of known pathogenic bacteria that can cause disease in the GI tract. They are present due to the consumption of contaminated food or water, exposure to animals, fish, or amphibians known to harbor the organism. These organisms can be detected by either Multiplex PCR or microbiology culture.
- **Imbalanced bacteria** are usually neither pathogenic nor beneficial to the host GI tract. Imbalances can occur when there are insufficient levels of beneficial bacteria and increased levels of commensal bacteria. Certain commensal bacteria are reported as dysbiotic at higher levels.
- **Yeast** may normally be present in small quantities on the skin, in the mouth and intestine. While small quantities of yeast may be normal, yeast observed in higher quantities is considered abnormal.

Notes:
NG = No Growth
Methodology: Culture and identification by MALDI-TOF and conventional biochemicals

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Culture complements PCR detection of dysbiotic and pathogenic bacteria and yeast. All sections within Microbiology are expandable fields. The greater the number of detected bacteria and yeast, the more expansive the reporting.

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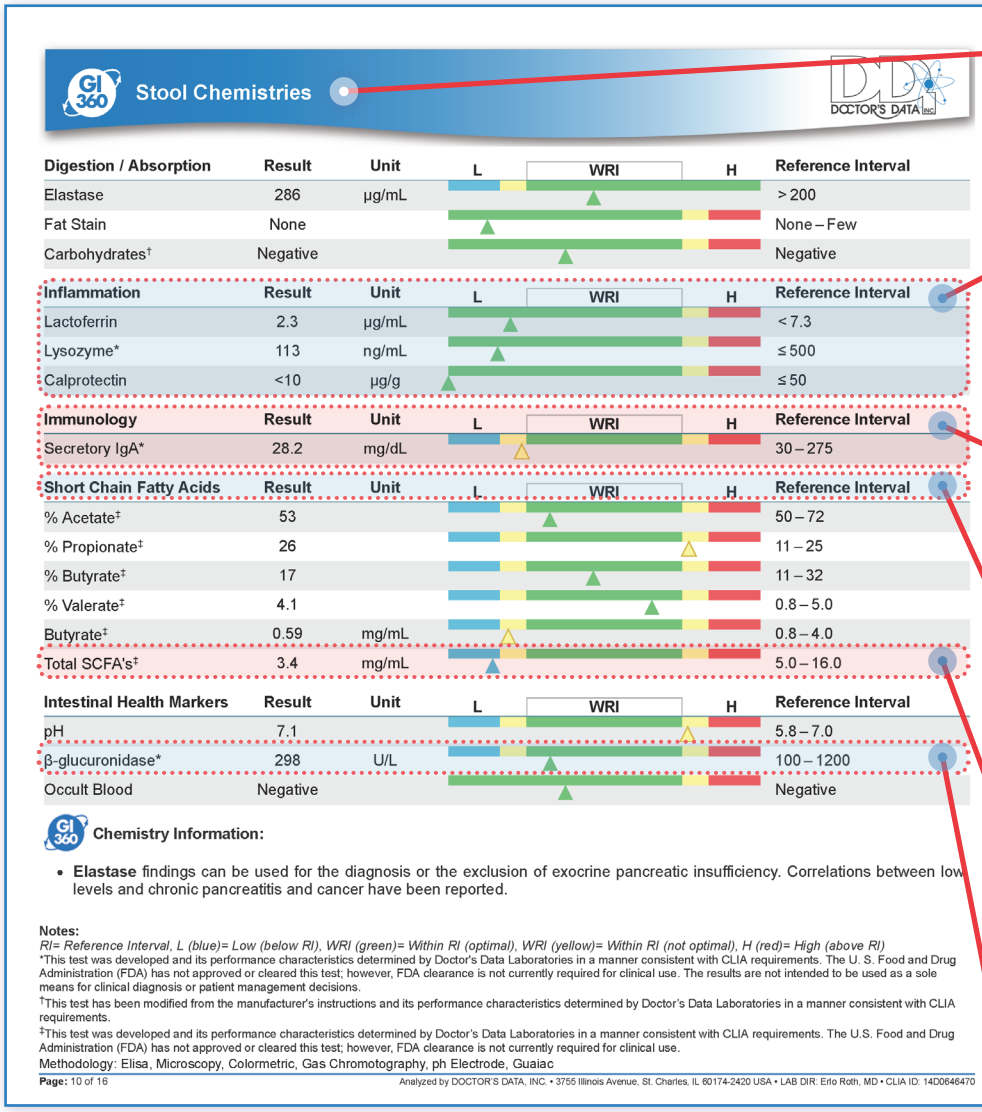

Stool Chemistries

Digestion / Absorption	Result	Unit	L	WRI	H	Reference Interval
Elastase	286	µg/mL				> 200
Fat Stain	None					None – Few
Carbohydrates†	Negative					Negative
Inflammation	**Result**	**Unit**	**L**	**WRI**	**H**	**Reference Interval**
Lactoferrin	2.3	µg/mL				< 7.3
Lysozyme*	113	ng/mL				≤ 500
Calprotectin	<10	µg/g				≤ 50
Immunology	**Result**	**Unit**	**L**	**WRI**	**H**	**Reference Interval**
Secretory IgA*	28.2	mg/dL				30 – 275
Short Chain Fatty Acids	**Result**	**Unit**	**L**	**WRI**	**H**	**Reference Interval**
% Acetate‡	53					50 – 72
% Propionate‡	26					11 – 25
% Butyrate‡	17					11 – 32
% Valerate‡	4.1					0.8 – 5.0
Butyrate‡	0.59	mg/mL				0.8 – 4.0
Total SCFA's‡	3.4	mg/mL				5.0 – 16.0
Intestinal Health Markers	**Result**	**Unit**	**L**	**WRI**	**H**	**Reference Interval**
pH	7.1					5.8 – 7.0
β-glucuronidase*	298	U/L				100 – 1200
Occult Blood	Negative					Negative

Chemistry Information:

- **Elastase** findings can be used for the diagnosis or the exclusion of exocrine pancreatic insufficiency. Correlations between low levels and chronic pancreatitis and cancer have been reported.

Notes:
RI= Reference Interval, L (blue)= Low (below RI), WRI (green)= Within RI (optimal), WRI (yellow)= Within RI (not optimal), H (red)= High (above RI)
*This test was developed and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements. The U. S. Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA clearance is not currently required for clinical use. The results are not intended to be used as a sole means for clinical diagnosis or patient management decisions.
†This test has been modified from the manufacturer's instructions and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements.
‡This test was developed and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements. The U.S. Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA clearance is not currently required for clinical use.
Methodology: Elisa, Microscopy, Colormetric, Gas Chromotography, ph Electrode, Guaiac

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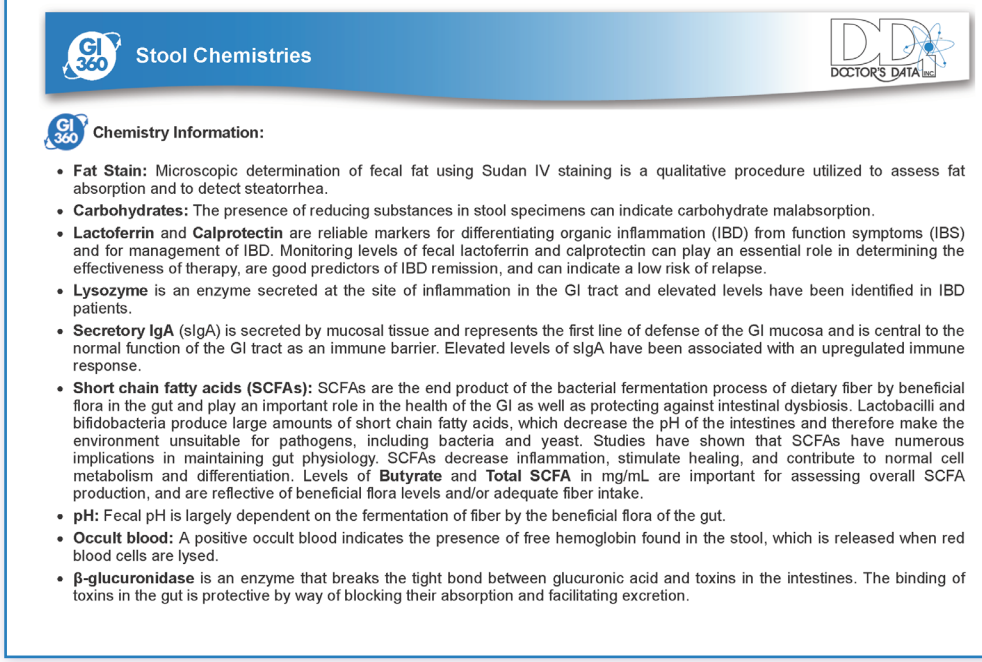

Stool Chemistries

Chemistry Information:

- **Fat Stain:** Microscopic determination of fecal fat using Sudan IV staining is a qualitative procedure utilized to assess fat absorption and to detect steatorrhea.
- **Carbohydrates:** The presence of reducing substances in stool specimens can indicate carbohydrate malabsorption.
- **Lactoferrin** and **Calprotectin** are reliable markers for differentiating organic inflammation (IBD) from function symptoms (IBS) and for management of IBD. Monitoring levels of fecal lactoferrin and calprotectin can play an essential role in determining the effectiveness of therapy, are good predictors of IBD remission, and can indicate a low risk of relapse.
- **Lysozyme** is an enzyme secreted at the site of inflammation in the GI tract and elevated levels have been identified in IBD patients.
- **Secretory IgA** (sIgA) is secreted by mucosal tissue and represents the first line of defense of the GI mucosa and is central to the normal function of the GI tract as an immune barrier. Elevated levels of sIgA have been associated with an upregulated immune response.
- **Short chain fatty acids (SCFAs):** SCFAs are the end product of the bacterial fermentation process of dietary fiber by beneficial flora in the gut and play an important role in the health of the GI as well as protecting against intestinal dysbiosis. Lactobacilli and bifidobacteria produce large amounts of short chain fatty acids, which decrease the pH of the intestines and therefore make the environment unsuitable for pathogens, including bacteria and yeast. Studies have shown that SCFAs have numerous implications in maintaining gut physiology. SCFAs decrease inflammation, stimulate healing, and contribute to normal cell metabolism and differentiation. Levels of **Butyrate** and **Total SCFA** in mg/mL are important for assessing overall SCFA production, and are reflective of beneficial flora levels and/or adequate fiber intake.
- **pH:** Fecal pH is largely dependent on the fermentation of fiber by the beneficial flora of the gut.
- **Occult blood:** A positive occult blood indicates the presence of free hemoglobin found in the stool, which is released when red blood cells are lysed.
- **β-glucuronidase** is an enzyme that breaks the tight bond between glucuronic acid and toxins in the intestines. The binding of toxins in the gut is protective by way of blocking their absorption and facilitating excretion.

Stool chemistries provide important clinical information beyond assessment of microbes.

Evaluation of the three inflammatory proteins permits comprehensive differentiation between potential Inflammatory bowel disease and non-organic inflammation.

Levels of sIgA provide important information regarding gastrointestinal immune responsiveness. Chronic stress is associated with low sIgA.

Altered percentages of SCFAs is consistent with deviation from normobiosis, and perhaps a very restricted diet.

Low absolute concentrations of total SCFAs and butyrate indicate compromised status of key commensal bacterial guilds, and/or insufficient soluble fiber.

High ß-glucuronidase activity may be associated with increased enterohepatic uptake of toxins that the body has worked hard to eliminate.

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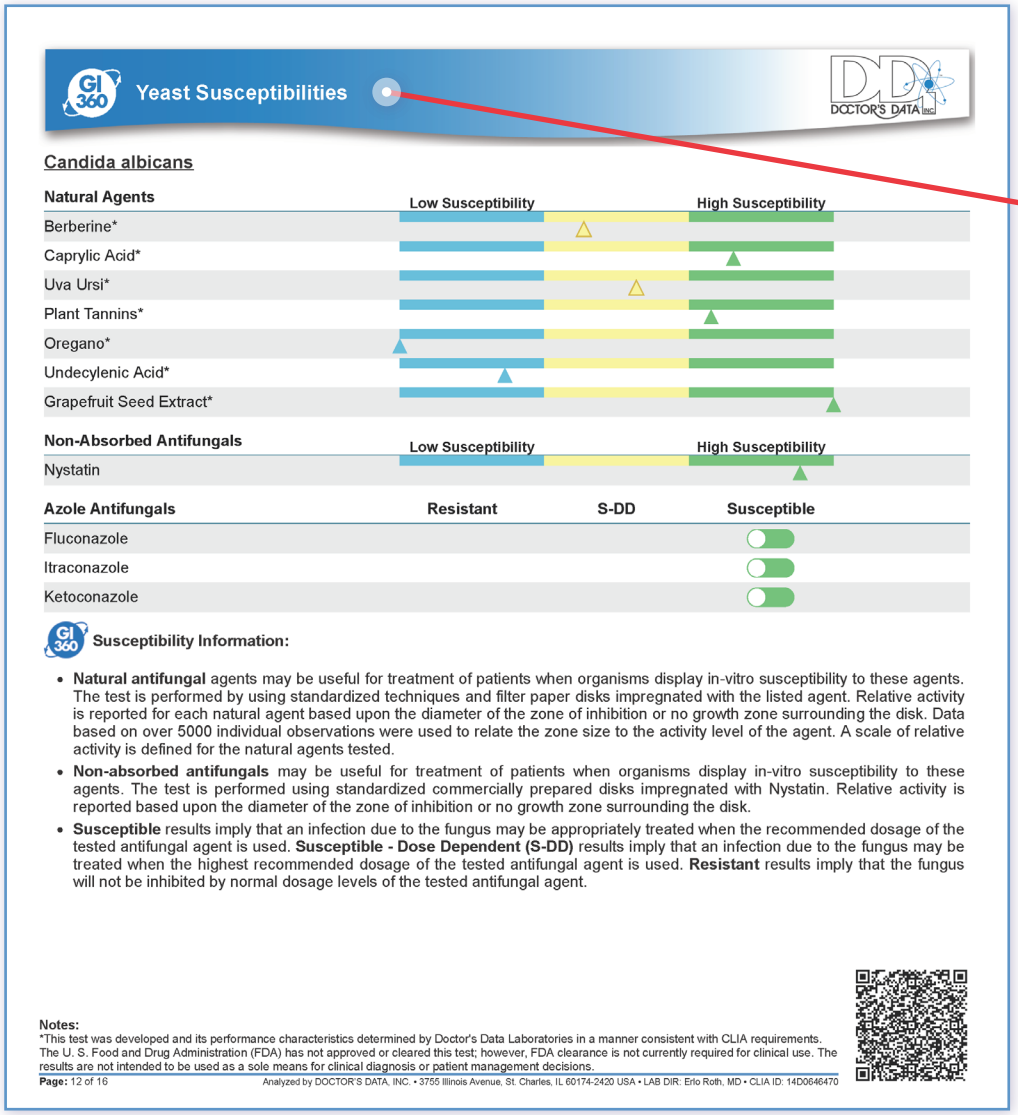

Yeast Susceptibilities

Candida albicans

Natural Agents	Low Susceptibility	High Susceptibility
Berberine*		
Caprylic Acid*		
Uva Ursi*		
Plant Tannins*		
Oregano*		
Undecylenic Acid*		
Grapefruit Seed Extract*		

Non-Absorbed Antifungals	Low Susceptibility	High Susceptibility
Nystatin		

Azole Antifungals	Resistant	S-DD	Susceptible
Fluconazole			
Itraconazole			
Ketoconazole			

Susceptibility Information:

- **Natural antifungal** agents may be useful for treatment of patients when organisms display in-vitro susceptibility to these agents. The test is performed by using standardized techniques and filter paper disks impregnated with the listed agent. Relative activity is reported for each natural agent based upon the diameter of the zone of inhibition or no growth zone surrounding the disk. Data based on over 5000 individual observations were used to relate the zone size to the activity level of the agent. A scale of relative activity is defined for the natural agents tested.
- **Non-absorbed antifungals** may be useful for treatment of patients when organisms display in-vitro susceptibility to these agents. The test is performed using standardized commercially prepared disks impregnated with Nystatin. Relative activity is reported based upon the diameter of the zone of inhibition or no growth zone surrounding the disk.
- **Susceptible** results imply that an infection due to the fungus may be appropriately treated when the recommended dosage of the tested antifungal agent is used. **Susceptible - Dose Dependent (S-DD)** results imply that an infection due to the fungus may be treated when the highest recommended dosage of the tested antifungal agent is used. **Resistant** results imply that the fungus will not be inhibited by normal dosage levels of the tested antifungal agent.

Notes:
*This test was developed and its performance characteristics determined by Doctor's Data Laboratories in a manner consistent with CLIA requirements. The U. S. Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA clearance is not currently required for clinical use. The results are not intended to be used as a sole means for clinical diagnosis or patient management decisions.

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Direct susceptibility testing requires pure isolates of yeast and bacteria. Susceptibility testing provides guidance for potential clinical intervention for each individual patient.

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Comprehensive commentaries are provided for all abnormal results.

Commentary

Introduction

This analysis of the stool specimen provides fundamental information about the overall gastrointestinal health of the patient. When abnormal microflora or significant aberrations in intestinal health markers are detected, specific commentaries are presented. If no significant abnormalities are found, commentaries are not presented.

Microbiome Abundance Information

Actinobacteria (phylum)

Actinobacteria is one of the largest bacterial phyla, comprised of Gram-positive bacteria. This phylum includes a wide range of species, with different morphological and physiological characteristics. Significant groups in the human colon include Actinomycetales and Bifidobacteriales. Actinomycetales were inversely associated with clinically significant depression in IBS patients, suggesting these bacteria may be depleted in depressed IBS patients. A strict vegetarian diet may increase the total count of Actinomyces spp. compared to following a Western diet.

Actinomycetales (order)

Actinomycetales are considered low abundance colonizers of the gastrointestinal tract with primary residence on the skin. Intake of proton-pump inhibitor drugs has been shown to increase the abundance of Actinomycetales in the gut, possibly by reducing gastric acidity and enabling intestinal colonization by oral microbes. Actinomycetales may be depleted in depressed irritable bowel syndrome patients. The abundance of Actinomyces spp. was shown to be higher with a strict vegetarian diet compared to a common Western diet.

Bifidobacterium (genus)

Considered amongst the most beneficial commensal bacteria in the human gut, Bifidobacterium spp. are able to degrade monosaccharides, galacto-, manno-, and fructo-oligosaccharides, as well as some complex carbohydrates. Many of the non-digestible oligosaccharides, found as natural components in mother's milk, select for colonization of these species which dominate the infant gut shortly after birth. Bifidobacteria may provide health benefits directly through interactions with the host, and indirectly through interactions with other microorganisms. Bifidobacterium spp. take part in production and adsorption of vitamins, such as vitamins K and B12, biotin, folate, thiamine, riboflavin, and pyridoxine. They are also involved in lipid absorption and metabolism, glucose and energy homeostasis, and regulating intestinal barrier function. Although Bifidobacterium produce acetate over butyrate, healthy levels of Bifidobacterium spp. facilitate colonization of F. prausnitzii.

Multiple published studies have suggested that there is an association between obesity and a lower abundance of Bifidobacteria. They may also be less abundant in elderly populations, patients with rheumatoid arthritis, and in individuals diagnosed with Alzheimer's disease. Patients with active inflammatory bowel disease (IBD) have a lower abundance of Bifidobacterium spp. than patients whose IBD is in remission. Taking a probiotic containing Bifidobacteria, Lactobacillus, and Streptococcus spp. might help in controlling ulcerative colitis symptoms and preventing their recurrence. Some Bifidobacterium strains have been shown to have beneficial effects in irritable bowel syndrome (IBS). Bifidobacterium spp. abundance has been shown to be diminished with IBD and with long term use of macrolide antibiotics. Luminal Bifidobacteria is reduced with restriction of fermentable carbohydrates, i.e. a low FODMAP diet. High fat dietary feeding is also associated with reduced abundance of Bifidobacteria. Consumption of maize and barley-based whole grain products and red berries, which are comprised of anthocyans, are known to increase levels of Bifidobacteria.

Bacteriodetes (phylum)

Bacteroidetes make up approximately 28% of the gut microbiota in healthy human adults. They are early colonizers of the infant gut and are amongst the most stable, at a species and strain level, in the healthy host. A low preponderance of Bacteriodetes relation to Firmicutes has been associated with obesity, though this can increase with weight loss and restricted calorie intake.

Bacteroides (genus)

Species in the genus Bacteroides carry out broad metabolic functions, including degradation of complex plant polysaccharides, proteolytic activities, de-conjugation of bile acids, mucosal barrier integrity, short chain fatty acid production, fatty acid storage and glucose metabolism. Bacteroides spp. are maintained at a higher abundance in breastfed individuals into adulthood. Bacteroides fragilis plays an important role in the prevention of intestinal inflammation. An energy-restricted diet has been shown to increase B. fragilis in overweight adolescents. An increase in B. stercoris has been associated with higher risk of colon cancer. Decreased levels of Bacteroides species have been reported in association with multiple sclerosis, rheumatoid arthritis and Parkinson's disease.

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Higher levels of Veillonella were found in formula-fed infants compared to breast-fed infants.

Proteobacteria (phylum)

Proteobacteria include a wide variety of pathogens, including species within the Escherichia, Shigella Salmonella, Vibrio, and Helicobacter genera. The phylum includes a number of species that are permanent residents of the microbiota and capable of inducing nonspecific inflammation and diarrhea when their presence is increased. Proteobacteria make up approximately 2% of the gut microbiota in healthy adults.

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Imbalanced Flora

Imbalanced flora are those bacteria that reside in the host gastrointestinal tract and neither injure nor benefit the host. Certain dysbiotic bacteria may appear under the imbalanced category if found at low levels because they are not likely pathogenic at the levels detected. When imbalanced flora appear, it is not uncommon to find inadequate levels of one or more of the beneficial bacteria and/or a fecal pH more towards the alkaline end of the reference range (6 - 7.8). It is also not uncommon to find hemolytic or mucoid E. coli with a concomitant deficiency of beneficial E. coli and alkaline pH, secondary to a mutation of beneficial E. coli in alkaline conditions (DDI observations). Treatment with antimicrobial agents is unnecessary unless bacteria appear under the dysbiotic category.

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The pH of this stool sample was higher than expected (>7.8, alkaline). Ideally, the colonic (stool) pH stool is slightly acidic. Predominant beneficial bacteria normally produce large amounts of short chain fatty acids (butyrate, acetate, propionate), which contribute to lower colonic pH. Check short chain fatty acid status (SCFA).

Many G.I. pathogens, including bacteria and yeast, thrive in an alkaline pH. When fecal pH is more towards the alkaline end of the reference range, it is not uncommon to find low levels of organisms in the Microbiome of the GI360™ or expected bacteria. There may also possibly be an increase in the imbalanced flora, dysbiotic bacteria and/or yeast present. Insufficient daily intake of fermentable soluble fiber is often associated with insufficiency dysbiosis, low absolute levels of butyrate and total SCFAs and high pH.

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Microbiome Abundance and Diversity

Introduction

The GI360™ Microbiome Profile is a focused gut microbiota DNA analysis tool that identifies more than 45 targeted analytes across six phyla using a CE-marked multiplex PCR system. Patient results are compared to a highly defined normobiotic reference population (n > 1,100). The white shadowed web plot within the hexagonal diagram illustrates the degree to which an individual's microbiome profile deviates from normobiosis. The center of the diagram represents less bacterial abundance while the outer edges represent greater than normobiosis. Deviation from a hexagon-shaped plot indicates variant diversity of the microbial community. Key findings for patient's microbiome profile are summarized in the table below the diagram, and detailed results for all of the analytes are presented on the next 3 pages of the report. Detailed results for the specific bacteria are reported as -3 to +3 standard deviations, as compared to the normobiotic reference population.

The Dysbiosis Index the (DI) is calculated strictly from the results of the Microbiome Profile, with scores from 1 to 5. A DI score above 2 indicates dysbiosis; a microbiota profile that differs from the defined normobiotic reference population. The higher the DI above 2, the more the sample deviates from the normobiotic profile. The dysbiosis test and DI does not include consideration of dysbiotic and pathogenic bacteria, yeast, parasites and viruses that may be reported in subsequent sections of the GI360™ test.

Actinobacteria (phylum)

Actinobacteria is one of the largest bacterial phyla, comprised of Gram-positive bacteria. This phylum includes a wide range of species, with different morphological and physiological characteristics. Significant groups in the human colon include Actinomycetales and Bifidobacteriales. Actinomycetales were inversely associated with clinically significant depression in IBS patients, suggesting these bacteria may be depleted in depressed IBS patients. A strict vegetarian diet may increase the total count of *Actinomyces* spp. compared to following a Western diet.

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Bifidobacterium (genus)

Considered amongst the most beneficial commensal bacteria in the human gut, *Bifidobacterium* spp. are able to degrade monosaccharides, galacto-, manno-, and fructo-oligosaccharides, as well as some complex carbohydrates. Many of the non-digestible oligosaccharides, found as natural components in mother's milk, select for colonization of these species which dominate the infant gut shortly after birth. Bifidobacteria may provide health benefits directly through interactions with the host, and indirectly through interactions with other microorganisms. *Bifidobacterium* spp. take part in production and adsorption of vitamins, such as vitamins K and B12, biotin, folate, thiamine, riboflavin, and pyridoxine. They are also involved in lipid absorption and metabolism, glucose and energy homeostasis, and regulating intestinal barrier function. Although *Bifidobacterium* produce acetate over butyrate, healthy levels of *Bifidobacterium* spp. facilitate colonization of *Faecalibacterium. prausnitzii*. Polyphenols derived from chocolate, green tea, blackcurrant, red wine and grape seed extracts have been shown to increase *Bifidobacterium* species. The increased abundance of *Bifidobacterium* species has been associated with amelioration of inflammation.

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Multiple published studies have suggested that there is an association between obesity and a lower abundance of bifidobacteria. They may also be less abundant in elderly populations, patients with rheumatoid arthritis, and in individuals diagnosed with Alzheimer's disease. Patients with active inflammatory bowel disease (IBD) have a lower abundance of *Bifidobacterium* spp. than patients whose IBD is in remission. Taking a probiotic containing bifidobacteria, lactobacilli, and streptococci might help in controlling ulcerative colitis symptoms and preventing their recurrence. Some *Bifidobacterium* strains have been shown to have beneficial effects in irritable bowel syndrome (IBS). *Bifidobacterium* spp. abundance has been shown to be diminished with IBD and with long term use of macrolide antibiotics. Luminal bifidobacteria is reduced with restriction of fermentable carbohydrates, i.e. a low FODMAP diet. High fat dietary feeding is also associated with reduced abundance of bifidobacteria. Consumption of maize and barley-based whole grain products and red berries, which are comprised of anthocyans, are known to increase levels of bifidobacteria.

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Bacteroidetes make up approximately 28% of the gut microbiota in healthy human adults. They are early colonizers of the infant gut and are amongst the most stable, at a species and strain level, in the host. A low preponderance of Bacteroidetes in relation to Firmicutes has been associated with obesity, though this can increase with weight loss and restricted calorie intake.

Alistipes (genus)

Alistipes does not contribute significantly to short chain fatty acid production. A diet rich in animal protein and fat increases the abundance of *Alistipes.* High abundance of *Alistipes* was identified as a possible predictor of successful weight loss. Increased abundance of *Alistipes* has been correlated with a greater frequency of pain in pediatric irritable bowel syndrome patients. In contrast, *Alistipes onderdonkii* was shown to be decreased in patients diagnosed with ulcerative colitis. Lower abundance of the *Alistipes* genus has been observed in patients with psoriatic arthritis and pediatric Crohn's disease. *Alistipes* may positively correlate with depression.

Bacteroides (species)

Species in the genus *Bacteroides* carry out broad metabolic functions, including degradation of complex plant polysaccharides, proteolytic activities, de-conjugation of bile acids, mucosal barrier integrity, short chain fatty acid production, fatty acid storage and glucose metabolism. *Bacteroides* spp. are maintained at a higher abundance in breastfed individuals into adulthood. *Bacteroides fragilis* plays an important role in the prevention of intestinal inflammation. An energy-restricted diet has been shown to increase *B. fragilis* in overweight adolescents. An increase in *B. stercoris* has been associated with higher risk of colon cancer. Decreased levels of *Bacteroides* spp. have been reported in association with multiple sclerosis, rheumatoid arthritis and Parkinson's disease.

Bacteroides pectinophilus (species)

Bacteroides pectinophilus contributes to breakdown of dietary pectins which are prebiotics. Pectins are complex, plant-derived carbohydrates that are indigestible by human enzymes, but can be easily degraded by certain commensal bacteria in the gut. Subsequent microbial fermentation of constituent sugar moieties yields important short chain fatty acids and other metabolites. The pectin-derived microbial fermentation products have important functions including reduction of ammonia, delay of gastric emptying and postprandial glucose regulation, induction of gut immunity, and maintenance of the mucosal barrier. Adequate intake and microbial metabolism of pectins appears to stimulate growth of various beneficial bacteria, including *Lachnospiraceae*, *Dorea* species, *Bifidobacterium*, *Lactobacillus* species, *Faecalibacterium prausnitzii*, and *Eubacterium rectale*.

The abundance of *B. pectinophilus* has been positively correlated with a healthy fasting serum lipid profile, and negatively correlated with biomarkers of for insulin resistance and dyslipidemia. *B. pectinophilus* was less abundant for IBS patients compared to healthy controls. High consumption of kimchi (fermented cabbage) may be associated with lower than normal levels of *B. pectinophilus*.

Prevotella (genus)

Prevotella-rich dysbiosis has been associated with insulin-resistance, obesity and hypertension. *Prevotella* have been shown to be significantly decreased in Crohn's disease and Parkinson's disease. High levels of fiber and carbohydrates from fruits and vegetables in a Mediterranean diet have been shown to increase the relative abundance of *Prevotella*.

Parabacteroides (genus)

The abundance of *Parabacteroides* spp., major anaerobic producers of acetate and succinate is increased with a high fat diet and is positively correlated with body weight. *Parabacteroides* spp., along with certain *Bacteroides* spp., have been shown to distinguish healthy adults from patients with irritable bowel syndrome or ulcerative colitis. Reduced abundance of this group of bacteria has also been linked to Crohn's disease in children. *Parabacteroides* spp. has been found to be less abundant in patients with multiple sclerosis.

Firmicutes (phylum)

The phylum Firmicutes constitutes the most diverse and abundant group of gastrointestinal microbiota which are grouped into four classes, Bacilli, Clostridia, Erysipelotrichia, and Negativicutes. They constitute about 39% of gut bacteria in healthy adults, but may increase to as high as 80% in an imbalanced microbial community.

Firmicutes

High Firmicutes and low Bacteroidetes abundances have been equivocally associated with obesity. A high-fat diet is associated higher abundance of both Firmicutes and Proteobacteria, and lower abundance of Bacteroidetes in mice. Low abundance of Firmicutes and greater abundance of *Akkermansia muciniphila* have been reported in lean individuals. Increased levels of Firmicutes have been associated with Crohn's disease and ulcerative colitis.

Catenibacterium mitsuokai (species)

Consumption of a Western diet has been shown to increase *C. mitsuokai* in the human gut microbiota. *C. mitsuokai* ferments glucose, mannose, galactose, fructose, sucrose, maltose, cellobiose, lactose and salicin in the production of lactic acid, acetic acid as well as iso-butyric acid. The presence of *C. mitsuokai* has been positively associated with obesity-related insulin resistance.

Clostridium (genus)

Clostridium spp. represents an extremely heterogeneous class of organisms that are still actively undergoing taxonomic revision. *Clostridium* spp. are strict anaerobic, spore-forming bacteria. Decreased abundance of the genus *Clostridium* was found to be associated with prediabetes. Some *Clostridium* spp. are transferred to infants from breast milk within the first months of life. Increased levels of some *Clostridium* spp. were observed in irritable bowel syndrome patients. Many species, some of them related to diarrhea, were decreased after consumption of inulin combined with maltodextrin.

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Clostridium methylpentosum (species)

Appropriate digestion and metabolism of complex dietary carbohydrates from plants drives healthy diversity in the gut microbiota. *Clostridium methylpentosum* ferments the naturally occurring sugar L-rhamnose that is released by microbial breakdown of plant-derived pectin. Rhamnose is fermented to propionate and acetate which are short chain fatty acids that have pivotal regulatory roles in the maintenance of mucosal barrier integrity, gut microbiota balance, post-prandial appetite suppression and normoglycemia. Lower levels of *C. methylpentosum* were reported for children with autism and pervasive developmental disorder compared to neurotypicals controls. Consumption of probiotic yogurt LKM512 containing *Bifidobacterium animalis* (subspecies lactis LKM512) increased the levels of *C. methylpentosum*.

Coprobacillus cateniformis (species)

Coprobacillus cateniformis ferment glucose and other common sugars primarily to acetic and lactic acid, and to a lesser extent butyrate and valerate. Higher levels of this species were found in patients with relapsing polychondritis compared to a control group. A study reported elevated levels of *C. cateniformis* following a Specific Carbohydrate Diet (SCD) in patients with Primary sclerosing cholangitis and Ulcerative colitis. Chitosan (prebiotic) supplementation increased the anti-obesity-related species, such as *Coprobacillus cateniformis*, in high-fat fed mice. Correlation analysis indicated that levels of *C. cateniformis* were positively associated with serum leptin levels.

Dialister (genus)

Dialister invisus is generally considered an endodontic pathogen associated with periodontitis, caries, halitosis, and endodontic infections. *Dialister invisus* has been found to be decreased with Crohn's disease. Abundance of *Dialister* was found to be positively associated with spondyloarthritis, whereas depletion of this genus has been related with systemic lupus erythematosus. A negative correlation between *Dialister* and autism spectrum disorders has been reported.

Dorea (genus)

Dorea is a genus within the *Lachnospiraceae* family that is in the Firmicutes phylum. *Dorea* species are known to produce hydrogen and carbon dioxide as end-products of glucose fermentation and may be associated with bloating. Decreased levels of *Dorea* spp. were observed in patients with Parkinson's disease. Recent studies have identified increased levels of *Dorea* spp. in patients diagnosed with IBS, nonalcoholic fatty liver disease and non-alcoholic steatohepatitis, multiple sclerosis and colorectal cancer.

Eubacterium hallii (species)

Eubacterium hallii and *Eubacterium rectale* are both part of the *Lachnospiraceae* family that is in the Firmicutes phylum. *E. hallii* and *E. rectale* produce butyrate that is a key regulator of mucosal barrier integrity and function. Decreased levels of *Eubacterium* spp. have been associated with very high protein diets. *Eubacterium hallii* is capable of metabolizing glucose into products with antimicrobial properties.

Eubacterium rectale (species)

Eubacterium rectale is part of the *Lachnospiraceae* family and produces butyrate. *Eubacterium rectale* was found to be in lower abundance in patients with type 2 diabetes, colorectal cancer, and chronic idiopathic diarrhea. There is a negative correlation between *Eubacterium rectale* levels and the symptomatology of irritable bowel syndrome (IBS). Decreased levels of *Eubacterium* spp. have been associated with very high protein diets.

Faecalibacterium prausnitzii (species)

Faecalibacterium prausnitzii is one of the most abundant butyrate producing bacteria in a healthy gastrointestinal tract. As such, *F. prausnitzii* is a protective factor for the intestinal mucosa and supports very important intestinal barrier functions. *F. prausnitzii* exerts anti-inflammatory effects via metabolites

such as short-chain fatty acids. *F. prausnitzii* is reduced in inflammatory bowel disease, irritable bowel syndrome, celiac disease and gastrointestinal inflammation in general. It is reduced in patients diagnosed with Parkinson's disease, bipolar disorder, colorectal cancer, diabetes and chronic idiopathic diarrhea. Diminished levels of *F. prausnitzii* were found in patients with major depressive disorder. The abundance of *F. prausnitzii* together with *E. coli* has been proposed as a discrimination tool between ulcerative colitis and Crohn's disease. *F. prausnitzii* has been correlated with pediatric obesity in instances of high consumption of foods that are rich in unabsorbed carbohydrate (banana, maize, rice). The prebiotic inulin has been shown to increase the proportion of *F. prausnitzii* in the human intestinal microbiota. Low FODMAP diets are associated with diminished *F. prausnitzii* and butyrate production.

Lachnospiraceae (family)

The *Lachnospiraceae* family is a diverse group of butyric acid producers, which have been associated with beneficial microbial and epithelial cell growth. Consumption of a Mediterranean diet decreased levels of species belonging to *Lachnospiraceae*. *Lachnospiraceae* are known to increase with intake of cruciferous vegetables and wheat bran, and decrease with a resistant starch diet.

Lactobacillus (genus)

Decreased and normal levels of *Lactobacillus* spp. have been reported in patients with irritable bowel syndrome. *Lactobacillus* spp. abundance was shown to be lower in the active phase of ulcerative colitis. *Lactobacillus* levels were shown to be increased after inulin consumption, but decreased after consumption of maltodextrin. Polyphenols derived from chocolate, green tea, blackcurrant, red wine and grape seed extracts have been shown to increase *Lactobacillus* species. The increased abundance of *Lactobacillus* species has been associated with amelioration of inflammation.

Phascolarctobacterium (genus)

Phascolarctobacterium are in the Firmicutes phylum. *Phascolarctobacterium* can produce short chain fatty acids, including acetate and propionate, and may be associated with metabolic effects and mental state of the host. Patients diagnosed with major depressive disorder had increased levels of these species. Decreased levels of *Phascolarctobacterium* were found to be associated with Crohn's disease, ulcerative colitis and Alzheimer's disease. Consumption of cruciferous vegetables, such as broccoli, increases the abundance of *Phascolarctobacterium* in the gut.

Ruminococcus (genus)

Members of *Ruminococcus* sensu produce acetate, but not butyrate. *Ruminococcus gnavus*, like *Akkermansia muciniphila* is a mucin degrading specialist. Higher levels of *Ruminococcus* spp. were associated with non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Reduced levels of *R. bromii* were observed in patients with primary biliary cirrhosis. Increased abundance of *Ruminococcus* spp. has been reported in irritable bowel syndrome (IBS), whereas *Ruminococcus* spp. are reportedly decreased in abundance with Crohn's disease and ulcerative colitis. *Ruminococcus gnavus* has been found to be in higher abundance in diarrhea predominant IBS. Intake of resistant starch has been associated with increased levels of *R. bromii*, whereas a diet rich in animal protein and fat was found to reduce the abundance of this species in human gut.

Streptococcus (genus)

Higher abundance of *S. salivarius* and *S. thermophilus* (Firmicutes phylum) have been associated with a moderate to severe disease course in newly diagnosed ulcerative colitis (UC) patients. These findings are in accordance with a study that showed that UC patients have significantly increased *Streptococcus* spp. and depletion of *Bifidobacterium* spp. Higher levels of *Streptococcus* spp. were also observed in patients with colorectal cancer compared to healthy controls. Administration of *S. salivarius* together with *Bifidobacterium bifidum* was shown to reduce the incidence of acute diarrhea and rotavirus shedding in infants. *S. salivarius* and S. *thermophilus* are also widely used in dairy products like yogurt and cheese.

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Veillonella (genus)

Veillonella (Firmicutes phylum) are known for their ability to ferment lactate, producing the short chain fatty acids propionate and acetate. *Veillonella* spp. were shown to be significantly increased in patients with Crohn's disease, type 1 diabetes, and patients diagnosed with liver cirrhosis. Increased amounts of *Veillonella* have been found in patients with constipation dominant irritable bowel syndrome (IBS-C). It is hypothesized that the relationship between *Veillonella* strains and IBS stems from its robust production of organic acids (propionate and acetate) which contribute to bloating, anxiety and abdominal pain. Higher levels of *Veillonella* were found in formula-fed infants compared to breast-fed infants.

Proteobacteria (phylum)

Proteobacteria include a wide variety of pathogens, including species within the *Escherichia*, *Shigella Salmonella*, *Vibrio*, and *Helicobacter* genera. The phylum includes a number of species that are permanent residents of the microbiota and capable of inducing nonspecific inflammation and diarrhea when their presence is increased. Proteobacteria make up approximately 2% of the gut microbiota in healthy adults.

Proteobacteria

A high-fat diet is positively associated with an abundance of Proteobacteria. Slightly increased abundance of Proteobacteria may be associated with low-grade inflammation. Proteobacteria are increased in inflammatory bowel disease and irritable bowel syndrome. Higher abundance of Proteobacteria has been associated with a moderate to severe disease course in newly discovered ulcerative colitis patients. They are associated with diarrhea in IBS.

Enterobacteriaceae (family)

Enterobacteriaceae is a large family of bacteria within the Proteobacteria phyla. *Enterobacteriaceae* is inclusive of normal commensal species, harmless opportunists, and many of the more familiar pathogens, such as *Salmonella*, *Escherichia coli*, *Klebsiella*, *Shigella* and *Proteus*. Other potential disease-causing bacteria in this family include *Enterobacter* and *Citrobacter* species. The abundance of Proteobacteria, which are generally pro-inflammatory, is presented on the white shadowed web plot within the hexagonal diagram. The presence of specific dysbiotic and pathogenic *Enterobacteriaceae* bacteria, if detected by PCR or culture, are reported in the Gastrointestinal Pathogens and Microbiology sections of this report.

Overall, *Enterobacteriaceae* were found at higher levels in patients with NAFLD and PD. Diets rich in in complex carbohydrates are associated with lower levels of *Enterobacteriaceae*, in comparison to diets rich in fat and/or protein.

Escherichia (genus)

Clinically, *Escherichia* has been reported to contribute to irritable bowel syndrome. *Escherichia* spp. are commonly recovered from inflamed tissues of both Crohn's disease and ulcerative colitis patients. Untreated inflammatory bowel disease patients were shown to have higher abundance of *Escherichia* and lower abundance of *Faecalibacterium prausnitzii*. Increased levels of *Escherichia* were observed in colorectal cancer patients. Patients diagnosed with nonalcoholic steatohepatitis have higher abundance of *Escherichia*. Consumption of a Western diet is positively associated with *Escherichia* levels. Increased levels of *E. coli* were observed in people on a gluten-free diet. A non-pathogenic strain of *Escherichia*, *Escherichia nissle*, is a widely used probiotic for treating gut related diseases such as chronic constipation.

Acinetobacter junii (species)

Acinetobacter junii is rarely a cause of disease in humans. *A. junii* has mainly been associated with bacteremia in preterm infants and pediatric oncologic patients. *Acinetobacter junii* is one of more than 50 different species belonging to the genus *Acinetobacter*, most of which are nonpathogenic environmental organisms. They may cause opportunistic infections only in people with compromised immune status or

with an indwelling device, or both. *Acinetobacter* species are ubiquitous and can be isolated from many sources including soil, water, sewage, and food. *Acinetobacter* species can colonize skin, wounds, the oral mucosa, and respiratory and gastrointestinal tracts. Elevated levels of *A. junii* were found in patients with Crohn's disease. Patients with chronic IBS-diarrhea that respond favorably to a short-term low FODMAP diet tend to have higher levels of *A. junii* compared to non-responders.

Verrucomicrobia (phylum)

Verrucomicrobia is a less common phylum in the human gut microbiota, but one with increasing recognition with regards to health. Verrucomicrobia includes *Akkermansia muciniphila*. The obligate anaerobe *A. muciniphila* constitutes 3-5% of total bacteria in a healthy microbiome, and has a protective or anti-inflammatory role in the intestinal mucosa.

Akkermansia muciniphila (genus)

Higher abundance of *Akkermansia muciniphila* has been associated with a milder disease course in newly discovered ulcerative colitis patients. Archaea and *Akkermansia* were significantly more prevalent after weight reduction. A Low FODMAP diet has been shown to decrease the abundance of *A. muciniphila* leading to recommendations against long-term use of such a diet. *A. muciniphila* is a mucolytic specialist that has potent anti-inflammatory effects in part associated with a specific surface coat protein (Amuc-1100).

Tenericutes (phylum)

Tenericutes are cell wall-less bacteria that do not synthesize precursors of peptidoglycan. Tenericutes consist of four main clades designated as the *Acholeplasma, Spiroplasma, Pneumoniae* and *Hominis clusters*. Tenericutes are typically parasites or commensals of eukaryotic hosts.

GI Pathogens

Introduction

The GI Pathogen profile is performed using an FDA-cleared multiplex PCR system. It should be noted that PCR testing is much more sensitive than traditional techniques and allows for the detection of extremely low numbers of pathogens. PCR testing does not differentiate between viable and non-viable pathogens and should not be repeated until 21 days after completion of treatment or resolution to prevent false positives due to lingering traces of DNA. PCR testing can detect multiple pathogens in the patient's stool but does not differentiate the causative pathogen. All decisions regarding the need for treatment should take the patient's complete clinical history and presentation into account.

Adenoviruses

Adenoviruses are non-enveloped DNA viruses. Adenovirus is a cause of acute gastroenteritis in infants, young children, the elderly and immuno-compromised patients. The Adenovirus serotypes most frequently associated with gastroenteritis are Adenovirus 40 and 41. Adenovirus gastroenteritis generally causes watery diarrhea lasting one to two weeks. Usual symptoms include onset of fever and vomiting followed by diarrhea and abdominal pain with occasional respiratory symptoms. Asymptomatic carriage may occur in children who may shed the virus. Adenoviral infection occurs throughout the year and primarily affects children four years of age and younger. Route of infection is via fecal-oral route or aerosol droplets from respiratory infection. Prevent spread of virus by cleaning environs with 1:5 bleach dilution or ultraviolet light (serotype F40). The scientific literature does not currently support any specific herbal or nutritional antiviral therapies for this virus type. Small studies indicate that zinc may reduce severity of illness. Oral rehydration therapy and symptomatic treatment is indicated.

Campylobacter

Most *Campylobacter* infections in industrialized countries are caused by *C. jejuni, C. coli*, and *C. lari* with an estimated 1.5 million cases of foodborne illness due to *Campylobacter* per year in the US. *Campylobacter* spp. are responsible for approximately 15% of hospitalizations resulting from foodborne infections. Generally, campylobacteriosis presents as one to three days of fever, vomiting, and headaches followed by three to seven days of watery or bloody diarrhea and may include abdominal pain, cramping, nausea, headache, and/ or muscle pain within 2-5 days of infection. Contaminated water, pets, food, unpasteurized milk and undercooked poultry, are sources of infection. Use of antibiotics is controversial but may benefit children whom have had symptoms for less than 7 days, and immunocompromised individuals. Recommendations potentially include Azithromycin 500 mg daily for 3 days or Fluoroquinolone for 3 days, but infection may resist fluoroquinolones. Extracts of *Acacia nilotiac* show in vitro antibacterial activities against *Campylobacter* spp. isolated from sheep. Oral rehydration therapy is recommended to prevent dehydration, along with symptomatic treatment of fever and muscle aches.

Clostridioides difficile

C. difficile may cause diarrhea following the production of two toxins, enterotoxin A and cytotoxin B. *C. difficile* is the most common cause of nosocomial infectious diarrhea in developed countries and is the major cause of antibiotic-associated pseudo-membranous colitis. *C. difficile* infection (CDI) symptoms vary from asymptomatic carriage (30% of young children) to mild/moderate watery diarrhea with fever and malaise to pseudomembranous colitis with bloody diarrhea, severe abdominal pain and fever. CDI occurs almost exclusively after broad-spectrum antibiotic use. No treatment is necessary for asymptomatic carriers. Anti-motility agents are contraindicated. CDI can be treated with vancomycin 125 mg given 4 times daily for 10 days, administered orally, and fidaxomicin 200 mg given twice daily for 10 days, as first-line options for both non-severe and severe initial CDI. Patients with fulminant CDI should receive vancomycin 500 mg 4 times per day in combination with IV metronidazole. In second or subsequent recurrences, patients can be treated with oral vancomycin, fidaxomicin, or a fecal transplant. Co-administration of *Saccharomyces boulardii* and *Lactobacillus rhamnosus* during antibiotic therapy may reduce the risk of infection relapse. Oral rehydration therapy is recommended to prevent dehydration.

Cryptosporidium

The *Cryptosporidium* parasite causes disease in humans through ingestion of infectious oocysts in contaminated water or food, and by direct contact with fecal material from individuals or animals actively shedding oocysts. Two species of *Cryptosporidium* cause disease in humans, *Cryptosporidium hominis* and *Cryptosporidium parvum*. *C. hominis* is more prevalent in the US, South America, Australia, and Africa, while *C. parvum* accounts for most cases in Europe. Along with *Giardia*, *Cryptosporidium* is the most common parasitic cause of diarrheal illness in the US and other developed nations. The main symptom of *Cryptosporidium* infection is voluminous diarrhea lasting one to 14 days, often accompanied by abdominal cramps, fatigue, vomiting, fever, and malaise. Immuno-compromised individuals can develop severe cases of *C. parvum* infection with profuse, cholera-like diarrhea. Routes of infection are via contaminated water (recreational or drinking), or by contact with infected animals (mammals, birds, reptiles). Antibiotics may be considered for prolonged illness or immunocompromised. Antimotility agents and/or nitazoxanide 500mg twice daily for 3 days may be utilized for immunocompetent individuals. Animal studies indicate that the probiotics *Lactobacillus reuteri* or *L. acidophilus* reduced oocyte shedding. No specific herbal parasiticides are listed in scientific literature. *Allium sativum* (garlic), *Berberis vulgaris* (barberry), *Berberis aquifolium* (Oregon grape), *Pimpinella anisum* (anise), *Artemisia annua* (wormwood), *Mentha crispa* (curly mint), and *Juglans nigra* (black walnut) may be considered or used adjunctively, based on historical uses of these herbs. Nutritional support may include oral rehydration therapy and a lactose-free diet.

Entamoeba histolytica

Entamoeba histolytica is a protozoan parasite that infects an estimated 34 to 50 million people per year worldwide, and kills 100,000 individuals annually. The disease caused by *E. histolytica*, amebiasis, is common in tropical areas with poor sanitary conditions but is also endemic in the US with the prevalence of *E. histolytica* infection estimated to be 4%. The most common clinical manifestation of infection with *E. histolytica* is amoebic diarrhea without dysentery; however, more severe cases can result in amoebic dysentery, which is diarrhea with mucous and visible or microscopic blood, severe abdominal pain, fever, and elevated fecal lysozyme. Occasional asymptomatic carriage is possible. Route of infection is fecal oral via contaminated food or water, especially related to international travel (Mexico, China, and South East Asia). Steroids are contraindicated and may exacerbate symptoms. Treatment recommended; Metronidazole 500 mg three times daily for 7-10 days, Tinidazole 2 g daily for 3 days, or Nitazoxanide 500 mg twice daily for 3 days followed by paromomycin 25 mg/kg/day in 3 divided doses for 7 days, oral rehydration therapy and symptomatic treatment of fever. No specific herbal parasiticides are listed in scientific literature. *Allium sativum* (garlic), *Berberis vulgaris* (barberry), *Berberis aquifolium* (Oregon grape), *Pimpinella anisum* (anise), *Artemisia annua* (wormwood), *Mentha crispa* (curly mint), and *Juglans nigra* (black walnut) may be considered or used adjunctively, based on historical uses of these herbs.

Escherichia coli O157

E. coli O157 is a member of the pathogenic enterohemorrhagic *E. coli* strains (also known as verocytotoxin producing or Shiga-toxin producing *E. coli* (STEC)), and is an uncommon but serious cause of gastroenteritis. Infection with *E. coli* O157 often causes hemorrhagic colitis which involves severe abdominal cramps with watery non-hemorrhagic diarrhea which can become grossly bloody after two or three days. Symptoms may include fever or vomiting. Common sources of infection include handling of ruminants (cattle, goats, sheep, deer, elk, etc.), consumption of raw or unpasteurized milk, untreated water and fecal-oral transmission. Almost half of the *E. coli* O157 outbreaks worldwide are food-derived. Incubation period is typically 2-8 days. Antibiotics, natural antimicrobial agents and anti-motility agents are contraindicated and increase the risk of disease progression to hemolytic uremic syndrome. Approximately 5% of infected individuals develop hemolytic uremic syndrome, which can be life threatening. Children under five and the elderly are more susceptible to this complication. Oral rehydration therapy is indicated to prevent dehydration.

Enterotoxigenic *Escherichia coli* (ETEC)

ETEC is a major cause of traveler's diarrhea in adults in industrialized countries and a leading cause of infant diarrhea in developing countries. ETEC is estimated to cause 200 million episodes of diarrhea and approximately 380,000 deaths in children in the developing world and travelers to those areas. In the US, ETEC is estimated to cause approximately 17,800 foodborne illnesses annually. Enterotoxins produced by ETEC strains include heat-labile LT toxin and heat-stable ST toxin. ETEC illnesses are usually associated with acute watery diarrhea and sometimes nausea, headache, vomiting, or fever. Typical symptoms include profuse, watery diarrhea free of polymorphonuclear leukocytes, and abdominal cramping; occasional fever, nausea or vomiting, chills, anorexia, headache, muscle aches and bloating. Severe symptoms may resemble cholera with approximately 7 days of "rice-water" stools and dehydration. International travel is a risk factor and transmission is via fecal-oral route, contaminated food or water. Average incubation period is 40 hours. Anti-motility agents are contraindicated. Antibiotics may be considered in immunocompromised or if > 4 stools daily, pus in stool, or fever, and may shorten the duration of the diarrhea by 24-36 hours. Appropriate therapies include Levofloxacin 500 mg daily for 3 days, Ciprofloxacin 500 mg twice daily for 3 days, Rifaximin 200 mg three times daily for 3 days, or Azithromycin 1 g for 1 dose or 500 mg daily for 3 days, oral rehydration therapy and symptomatic treatment for fever or muscle aches. Essential oils of *Pinus sylvestris* (pine), *Thymus officinalis* (thyme), *Melaleuca alternifolia* (tea tree), *Coriandrum sativum* (coriander seed), *Cymbopogon citrates* (lemon grass), *Mentha piperita* (peppermint), and *Melissa officinalis* (lemon balm) have been shown to have antimicrobial effects against *E. coli* in vitro. Aqueous, ethanol and methanol extracts of *Triphala churna*; ethanol extract of *Mahsudarshan churna*; and methanol extract of *Sukshsarak churna* have all been shown to have antimicrobial activity against *E. coli* in vitro.

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Giardia

Giardia duodenalis (also known as *G. intestinalis* and *G. lamblia*) is the most common intestinal parasite of humans identified in the United States. *Giardia* infection occurs following consumption of *Giardia* cysts through contaminated (fecal) water or food, or through person-person contact. *Giardia* is found worldwide and is commonly found in travelers to disease-endemic areas and children in day-care facilities, but it can also be found in the general population associated with sexual activity. *Giardia* infection can result in acute self-limited diarrhea, or a chronic syndrome of diarrhea, abdominal cramps, malabsorption, and weight loss. Asymptomatic carrier status is also possible. Incubation period is typically 7 days. Adult treatment recommendations; Tinidazole 2 g x 1 dose, Nitazoxanide 500 mg twice daily for 3 days, Metronidazole 500 mg three times daily for 5-7 days, and oral rehydration therapy to prevent dehydration. Avoid dairy and remain dairy-free for several months after symptoms abate. *Saccharomyces boulardii* may enhance eradication when used with metronidazole. *Lactobacillus johnsonii* (LA1) inhibits *Giardia* growth in vitro. Animal studies indicate that *Lactobacillus casei* MTCC 1423 eradicated *Giardia.* No specific herbal parasiticides are listed in scientific literature. *Allium sativum* (garlic), *Berberis vulgaris* (barberry), *Berberis aquifolium* (Oregon grape), *Pimpinella anisum* (anise), *Artemisia annua* (wormwood), *Mentha crispa* (curly mint), and *Juglans nigra* (black walnut) may be considered or used adjunctively, based on historical uses of these herbs.

Norovirus

Norovirus affects people of all ages causing 19 to 21 million illnesses in the US per year. It is the leading contributor to acute gastroenteritis (AGE) across all age groups. While infections can occur year-round, Norovirus outbreaks tend to peak in cold weather. Norovirus infection symptoms include vomiting with watery, non-bloody diarrhea and abdominal cramps; occasionally fever, headache, muscle aches, or fatigue. Norovirus disease is usually self-limiting and rarely causes severe illness. Route of transmission is direct contact or fecal-oral via contaminated objects, food or water (drinking or recreational). Incubation period is typically 12-48 hours and virus may shed prior to presentation of symptoms. Oral rehydration therapy may be warranted along with symptomatic treatment, including anti-emetics (contraindicated in young children). The scientific literature does not currently support any specific herbal or nutritional antiviral therapies for this virus type. Small studies indicate that zinc may reduce severity of illness. *Lactobacillus casei* GG and *Saccharomyces boulardii* may provide moderate clinical benefit in the treatment of watery diarrhea.

Rotavirus

Rotaviruses are classified into seven serogroups (A-G); however, only groups A, B, and C are human pathogens. The Group A Rotaviruses are responsible for the majority of infections. Globally, Rotavirus is estimated to cause more than 125 million cases of gastroenteritis in children each year. Rotavirus symptoms can include non-bloody watery diarrhea, loss of appetite, low-grade fever, vomiting and abdominal cramping. Symptoms may be severe in infants, young children and virus may shed after resolution. While Rotavirus predominately infects children, it can also affect adults, and produces a more severe disease in immuno-compromised hosts. Transmission is via direct contact or fecal-oral via contaminated objects, food or water (drinking or recreational). Incubation period is typically two days and virus may shed prior to symptom presentation. Anti-emetics may be considered for children > 6 months old. Studies indicate that zinc may reduce severity of illness. The scientific literature does not currently support any specific herbal or nutritional antiviral therapies for this virus type. *Lactobacillus casei* GG and *Saccharomyces boulardii* may provide moderate clinical benefit in the treatment of watery diarrhea.

Salmonella

Salmonella are facultative anaerobic bacteria in the family of *Enterobacteriaceae*. There are two species of *Salmonella, Salmonella enterica* and *Salmonella bongori* that include over 2,600 different serotypes. The majority of the pathogenic serotypes of *Salmonella* that affect humans are within the species of *Salmonella enterica* (*S. enterica*). Worldwide, *Salmonella* spp. causes an estimated 93.8 million cases of gastroenteritis

each year. The majority of laboratory-confirmed cases in the U.S. were in children under five years of age. Symptoms of salmonellosis based on the two types of infection include: Typhoidal — debilitating, sustained high fever and headache, and Non-typhoidal — enterocolitis, bacteremia, endovascular infections, septic arthritis or osteomyelitis. Sources of infection include eggs, meats, dairy products, shellfish and produce; processed foods and pet foods. Handling of chicks, ducklings, reptiles, kittens and hedgehogs is a source of infection as well. Incubation period is typically between 6-72 hours. Antibiotics for uncomplicated non-typhoidal *Salmonella* infection are not indicated and may increase the risk of asymptomatic carriage up to one year. Treatment for adults may include Levofloxacin 500 mg daily for 7 days, Ciprofloxacin 500 mg twice daily for 7 days, Azithromycin 500 mg daily for 7 days, or Trimethoprim/ sulfamethoxazole twice daily for 7 days. Relapsing or immunocompromised patients require 14 days treatment. *Calpurnia aurea*; *Salivia schimperi*; *Azadirachta indica* (neem) methanol extract; *Allium sativa* aqueous extract have shown anti-microbial effects in vitro.

Shiga Toxin-producing *Escherichia coli* (STEC)

Shiga Toxin 1 (stx 1) and Shiga Toxin 2 (stx 2) producing *E. coli* (STEC) (*E. coli* O157 and non-O157 STEC) cause approximately 100,000 illnesses, 3,000 hospitalizations, and 90 deaths annually in the United States. The ability of non-O157 strains to cause disease is related to production of toxins, stx 1 and stx 2. Non-O157 stx 1 and stx 2 toxins are very similar to those produced by the O157 STEC; therefore, non-O157 STEC strains cause a similar range of illness severity to O157 STEC, and can result in the same complications. Symptoms usually include severe abdominal cramps, diarrhea (progressing to bloody), vomiting, and moderate (< 101* F/38.5* C) fever. Sources of infection include handling of ruminants (cattle, goats, sheep, deer, elk, etc.), consumption of raw or unpasteurized milk, soft unpasteurized cheeses, unpasteurized apple cider, undercooked meat, and contaminated water. Incubation period varies with serotypes from 10 hours-6 days. Oral rehydration therapy is recommended to prevent dehydration. Antibiotics, natural antimicrobial agents and anti-motility agents are contraindicated and increase the risk of disease progression to hemolytic uremic syndrome (HUS).

Shigella

Shigella spp. are non-sporulating bacteria that belong to the family *Enterobacteriaceae*. *Shigella* infections account for 5% to 20% of all diarrheal episodes throughout the world, and although these infections are commonly seen in children younger than five years old, they can be found in adults of all ages. Shigellosis often begins with fever, watery diarrhea, and abdominal cramps, and can progress to bloody diarrhea. Shigellosis is usually self- limiting, but can become life-threatening if patients are immuno-compromised. Contaminated food or water (recreation or drinking) is a source of exposure and fecal-oral transmission at daycare or nursing homes facilities is common. Incubation period is typically 3-4 days. Antimotility agents are contraindicated and antibiotics may decrease the course of illness by two days which may be considered in immunocompromised or to prevent shedding (public health precaution). Recommendations include Trimethoprim/ sulfamethoxazole 160-800 mg twice daily for 3 days, Levofloxacin 500 mg daily for 3 days, Ciprofloxacin 500 mg twice daily for 3 days and clear liquid, lactose (dairy)-free diet may be used until symptoms resolve. Essential oils of *Pinus sylvestris* (pine), *Thymus officinalis* (thyme), *Melaleuca alternifolia* (tea tree), *Coriandrum sativum* (coriander seed), *Cymbopogon citrates* (lemon grass), *Mentha piperita* (peppermint), and *Melissa officinalis* (lemon balm) have been shown to have antimicrobial effects against *E. coli* spp. in vitro. Aqueous, ethanol and methanol extracts of *Triphala churna*; ethanol extract of *Mahsudarshan churna*; methanol extract of *Sukshsarak churna* have all been shown to have antimicrobial activity against *E. coli* in vitro.

Vibrio cholerae

Vibrio cholerae is one of the most common causes of diarrhea worldwide. While diarrhea associated with *V. cholerae* is predominately seen in the Indian subcontinent, South East Asia, Africa, and South America, sporadic cases of *V. cholerae*-induced diarrhea have been reported in the United States. Gastrointestinal disease caused by *V. cholerae* is due to production of the cholera toxin. Two types of infection occur;

cholera, which is a severe illness presents with profuse, "rice-water" diarrhea, vomiting, tachycardia, dehydration, muscle cramps, restlessness or irritability, and vibriosis, which is characterized by abdominal cramps, nausea, vomiting, fever and chills which is a self-limited illness of 3-4 days. Consumption of raw or undercooked seafood and contaminated food or water are potential sources of infection. Symptoms usually occur within 24 hours of ingestion. Cholera treatment includes Azithromycin 1 g x 1 dose and Doxycycline 300 mg x 1 dose. Erythromycin may be considered for pediatric and pregnant patients. Oral rehydration therapy is recommended to prevent dehydration. Vibriosis illness treatment recommendations advise against antibiotics unless patient is immunocompromised, in which case treatments above may be used. Fresh *Citrus aurantifolia* (lime) juice; *Clitoria ternatea* methanol extract and *Limonia acidissima* ethanol extract have shown anti-microbial activity in vitro.

Parasitology, Microscopy

Blastocystis spp

Blastocystis spp was identified in this specimen. *Blastocystis* is a common protozoan found throughout the world. *Blastocystis* is transmitted via the fecal-oral route or from contaminated food or water. Whether *Blastocystis* infection can cause symptoms is still considered controversial. Symptoms may be compounded by concomitant infection with other parasitic organisms, bacteria, or viruses. Often, *Blastocystis* is found along with other such organisms. Nausea, diarrhea, abdominal pain, anal itching, weight loss, and excess gas have been reported in some persons with *Blastocystis* infection.

Metronidazole has been the traditionally considered the most effective drug (recommended adult dosage varies from 250 mg bid for 5-7 days to 750 mg tid x 10 days). Iodoquinol is also an effective medication (650 mg tid x 20 days). Recommended therapy can also eliminate *G. lamblia, E. histolytica* and *D. fragilis*. Various herbs may be effective, including oil of oregano. Limit refined carbohydrates in diet.

Dientamoeba fragilis

Dientamoeba fragilis, an ameboflagellate, was detected in this specimen. *D. fragilis* infects the large intestine. This parasite does not have a cyst stage, and cannot survive long outside the body alone. It may be spread in pinworm (*Enterobius vermicularis*) eggs. Infection is common worldwide, including in the United States. *D. fragilis* is known to cause non-invasive diarrheal illness in humans. 90% of children are symptomatic, whereas only 15-20% of adults are. The most common symptoms include diarrhea, stomach pain, and stomach cramping. Loss of appetite and weight, nausea, and fatigue are also common.

Recommended treatment is iodoquinol (650 mg tid x 20 days, adult dose). Alternatives include tetracycline (500 mg qid x 10 days, adult dose) and metronidazole (500-750 mg tid x 10 days, adult dose). Natural agents include berberine, wormwood, black walnut, grapefruit seed extract, and oil of oregano.

Endolimax nana

Endolimax nana, an amoeba, was identified in this specimen. *E. nana* is generally considered nonpathogenic or commensal. It lives in the large intestine of humans, mainly at the level of the cecum and feeds on bacteria. Infection occurs via fecal-oral route, and indicates increased risk of exposure to potential pathogens. Some research indicates that infection with *Endolimax nana* may be associated with diarrhea, urticaria, or reactive arthritis, possibly due to prolonged antigenic stimulation with formation of circulating antigen antibody complexes.

As *E. nana* is generally considered nonpathogenic there is no treatment suggested in the Sanford Guide or Medical Letter. Natural agents include oil of oregano and quassia.

Entamoeba coli

Entamoeba coli, an amoeba, was identified in this specimen. *E. coli* is generally considered nonpathogenic or commensal. It lives in the large intestine of humans, mainly at the level of the cecum and feeds on bacteria. Infection occurs via fecal-oral route, and indicates increased risk of exposure to potential pathogens.

Natural treatment agents include quassia, berberine, and hydrastis.

Entamoeba hartmanni

Entamoeba hartmanni, an amoeba, was identified in this specimen. *E. hartmanni* is generally considered nonpathogenic or commensal. It lives in the large intestine of humans, mainly at the level of the cecum and feeds on bacteria. Infection occurs via fecal-oral route, and indicates increased risk of exposure to potential pathogens.

Natural treatment agents include garlic, berberine, and quassia.

Entamoeba dispar/histolytica/moshkovskii/bangladeshi

Entamoeba dispar/histolytica/moshkovskii/bangladeshi, an amoeba, was detected in this specimen. The World Health Organization (WHO) defines amebiasis as infection with *Entamoeba histolytica* regardless of the symptomology. It is one of the most common parasitic diseases worldwide, infecting about 50 million people. Humans can be infected with three other species of *Entamoeba, E. dispar, E. moshkovskii and E. bangladeshi*, which are microscopically indistinguishable from *E. histolytica*. Among the 4 species that infect humans, *Entamoeba histolytica* unequivocally causes disease; *Entamoeba dispar* is a harmless commensal; *Entamoeba moshkovskii* seems to be an emerging pathogen; and the pathogenicity of *Entamoeba bangladeshi* remains to be investigated. This parasite normally infects the lumen of the large intestine, where it feeds on bacteria. In some cases *E. histolytica* can invade the intestinal mucosa. Migration to the liver, lung, brain, skin, or other tissues can also occur. Infection occurs when cysts are ingested in food or water contaminated with feces. There is a high prevalence of *E. histolytica* in Mexico, China, and South East Asia.

Entamoeba histolytica infection is asymptomatic in about 90% of patients. Acute symptoms most commonly occur 1 to 4 weeks after exposure. Symptoms often are quite mild and can include loose stools and abdominal discomfort. Mucosal invasion and ulceration results in amebic dysentery, associated with severe abdominal pain, bloody stools, and fever. Elevated fecal lysozyme, a biomarker of GI inflammation, can indicate more invasive infection. Rarely, *E. histolytica* invades the liver and forms an abscess. Even less commonly, it spreads to other parts of the body, such as the lung or brain.

For asymptomatic infection paromomycin (500 mg tid x 7 days, adult dose) or iodoquinol (650 mg tid x 20 days, adult dose) is recommended. For mild/moderate disease metronidazole (500-750 mg tid x 10 days, adult dose) or tinidazole (2 gm qd x 3 days, adult dose), followed by paromomycin or iodoquinol as described above. For severe disease or extraintestinal infection intravenous antiparasitic therapy may be warranted. Anti-diarrheal medications should not be used. Natural agents include berberine, grapefruit seed extract, *Saccharomyces boulardii*, quassia, and curcumin. Limiting refined carbohydrates in the diet, repairing injured intestinal mucosa, and preventing constipation can also be beneficial.

Giardia duodenalis (*intestinalis, lamblia*)

Giardia duodenalis was detected in this specimen. *G. duodenalis*, a single celled protozoa, is the most frequent cause of non-bacterial diarrhea in the United States. The Centers for Disease Control and Prevention (CDC) estimates as many as 2.5 million cases of *Giardia* infection occur annually in the U.S. Symptomatic individuals may experience diarrhea, abdominal cramps, dehydration, malabsorption, loss of appetite, anemia, and weight loss 1-2 weeks following the ingestion of cysts. Typically symptoms will last 1-2 weeks and infections are self-limiting. Most individuals will be completely asymptomatic. Prevalence of giardiasis in adults has been estimated to be 4-7%. Higher prevalence rates have been reported in children.

According to the Food and Drug administration, the higher prevalence of giardiasis in children versus adults suggests that many individuals have a lasting immunity following infection. Approximately 40% of patients diagnosed with giardiasis will demonstrate disaccharide (particularly lactose) intolerance that may last up to six months. Chronic cases of giardiasis may last months to years and are difficult to treat. Chronic giardiasis may lead to a malabsorption syndrome, weight loss, and general weakness and fatigue.

Giardia lives in the intestines of infected humans or animals. Contamination with *Giardia* from soil, food, water, or surfaces can occur from contact with feces from infected sources. Person to person transmission is common in day-care centers where diapering is done, as well as in institutions for persons with special needs. Resistance to drug treatment is common; however, Metronidazole (Flagyl) is effective. Paromomycin is the alternative for treating *Giardia* during pregnancy. Other therapeutic alternatives include nitazoxanide, furazolidone, and quinacrine. Natural substances include berberine, grapefruit seed extract, and quassia. Fatty foods should be avoided, as *Giardia* feeds on bile salts.

Iodamoeba büetschlii

Iodamoeba buetschlii, an amoeba, was identified in this specimen. *Iodamoeba buetschlii* is considered nonpathogenic or commensal. Infection occurs via fecal-oral route, and indicates increased risk of exposure to potential pathogens.

Pentatrichomonas (Trichomonas) hominis

Pentatrichomonas hominis, a flagellated protozoan, was identified in this specimen. *P. hominis* is considered nonpathogenic or commensal. It lives in the large intestine. Exposure occurs via fecal-oral route, and indicates increased risk of exposure to potential pathogens.

Taenia spp. (tapeworm)

Analysis of this specimen indicates *Taenia solium* (pork tapeworm) or *Taenia saginata* (cattle tapeworm) infection. These cestodes usually infect the intestinal tract, but *Taenia solium* may also invade the CNS. Transmission is by ingesting cysts in raw or undercooked meat. *Taenia solium* transmission may also occur via the fecal-oral route, which increases chance of CNS involvement. *T. saginata* is prevalent in Ethiopia, Kenya, the Middle East, Yugoslavia, Mexico, and parts of South America and Eastern Europe. *T. solium* is found in Europe, Latin America, India, and China. Infection in the USA is rare. Taeniasis is usually asymptomatic. Mild abdominal symptoms may include epigastric discomfort, increased hunger, diarrhea, nausea, and weight loss. Death of cysts can elicit an intense inflammatory tissue response, with symptoms appearing 4 to 5 years after infection. Infection of the brain or CNS (neurocysticercosis) can cause severe symptoms including seizures, altered mental status, focal neurological signs, and aseptic meningitis.

For treatment of intestinal infection praziquantel (5-10 mg/kg x 1 dose, adult dose) is recommended. An alternative is niclosamide (2 g x 1 dose, adult dose). Not all patients with neurocysticercosis should be treated, as the inflammatory response to treatment may be more damaging than the disease.

Clonorchis sinensis (Oriental liver fluke)

Clonorchis sinensis ova were identified in this specimen. This trematode infects the bile ducts. Infection occurs through consumption of encysted metacercariae in raw, dried, or pickled fish imported from endemic areas, which include Japan, Korea, China, Taiwan, and Vietnam. Light infection is usually asymptomatic. Most pathologic manifestations result from inflammation and intermittent obstruction of the biliary ducts. In the acute phase, abdominal pain, nausea, diarrhea, and eosinophilia can occur. In long-standing infections cholangitis, cholelithiasis, pancreatitis, biliary obstruction, portal fibrosis, and cholangiocarcinoma can develop.

Praziquantel (25 mg/kg tid x 1 day, adult dose) is considered the most effective drug. Natural agents include elecampane, costus, and quassia.

Schistosoma mansoni (bilharzia)

Ova of *Schistosoma mansoni*, a trematode, were identified in this specimen. These blood flukes inhabit the mesenteric veins. *S. mansoni* is widespread in Africa, and is also seen in Brazil, Surinam, Venezuela, and some Caribbean islands. Infection occurs when *cercariae* in fresh water penetrate the skin. Eggs appear in feces 1-3 months later. Transmission of schistosomiasis cannot occur in the United States or Canada because the snails that serve as an intermediary host are absent.

Schistosoma dermatitis may occur in previously sensitized persons, causing a pruritic papular rash where the cercariae penetrated the skin. Acute schistosomiasis (Katayama fever) may occur 2 to 4 wk after heavy exposure. Symptoms include fever, chills, nausea, abdominal pain, malaise, myalgia, urticarial rashes, and marked eosinophilia. Occasionally, eggs lodge aberrantly in the CNS and cause transverse myelitis or seizures. Chronic schistosomiasis, often resulting from repeated exposures, results mostly from host responses to eggs retained in tissues. Early on, abscesses in the intestinal mucosa may ulcerate and produce bloody diarrhea. As the lesions progress, focal fibrosis, strictures, fistulas, and papillomatous growths may develop. Granulomatous reactions to eggs of *S. mansoni* in the liver produce a diffuse nodular periportal cirrhosis (pipestem fibrosis). Typically, liver cell function is not seriously compromised, but damage to the circulation may result in severe portal hypertension. Hematemesis is common and can be fatal. Portal hypertension also shunts the eggs to the lungs, where they produce focal obliterative arteritis and granulomas, which may cause pulmonary hypertension and cor pulmonale.

Praziquantel (20 mg/kg bid x 1 day, adult dose) or Oxamniquine- not commercially available in US (15 mg/kg x 1 dose/ for African strains 20 mg/kg qd x 3 days, adult doses) are recommended drug therapies. Resistance to each drug has been identified in some strains. Serologic tests are used to monitor treatment efficacy. Natural agents include quassia and pau d'arco.

Ascaris lumbricoides (round worm)

Ascaris lumbricoides ova were detected in this specimen. *Ascaris* is a worm that infects the small intestine, but its life cycle includes migration through the circulation to the heart and lungs, and into the oropharynx. Infection is acquired through the ingestion of embryonated eggs in contaminated soil or water. Ascariasis is the most prevalent intestinal helminth infection in the world; current estimates suggest that more than 1 billion persons are infected. In the US, infection is more common in rural parts of the southeast. Most individuals have no noticeable symptoms. Migrating larvae may produce "verminous pneumonia". However, heavy infection, especially in children, may produce abdominal cramps, and a mass of tangled worms may cause intestinal obstruction. Aberrant migration of individual adult worms occasionally leads to obstructions resulting in cholangitis, cholecystitis, liver abscess, pancreatitis, appendicitis, or peritonitis. Passage of an adult worm by mouth or rectum is possible.

All Ascaris infections should be treated. If other intestinal helminths are present, *Ascaris* must be treated first to prevent aberrant migration of worms. Treat adults with mebendazole (200 mg bid x 20 days) or albendazole (400 mg x 1 dose). Repeat stool exam 1-2 weeks after therapy. Garlic, onion, wormwood, black walnut, quassia, and pumpkin seeds may be effective natural agents. Minimize meat, dairy, and refined carbohydrates while increasing fiber in the diet.

Enterobius vermicularis (pinworm)

Enterobius vermicularis ova were detected in this specimen. This nematode inhabits the large intestine and migrates to the anus at night, where it deposits eggs on the perianal surface. Pinworm is the most common worm infection in the United States. School-age children, followed by preschoolers, have the highest rates of infection. Perianal pruritus, especially at night, is a characteristic symptom. Disturbed sleep, loss of appetite, restlessness and irritability may be experienced with heavy infection. Very rarely, *E. vermicularis* will migrate to the urinary bladder, vagina, or peritoneal cavity.

All infected individuals in a communal group should be treated simultaneously. Eggs can survive up to 2 weeks on clothing or bedding, so laundering these items is imperative during treatment. Mebendazole (100 mg x 1 dose, repeat in 2 weeks) or pyrantel pamoate (11 mg/kg <1.0 gm qd x 1 dose, repeat in 2 weeks, adult dose) are effective drug treatments. Natural agents include quassia, black walnut, and garlic.

Hookworm species

Eggs of hookworm spp. *Ancylostoma duodenale* or *Necator americanus* were detected in this specimen. The adult nematodes attach to the wall of the small intestine and suck blood. The hookworm life cycle also includes migration through the circulation to the heart and lungs, and into the oropharynx. Infection occurs when larvae from fecally-contaminated soil penetrate human skin. *A. duodenale* is widely distributed in the Mediterranean basin, India, China, Japan, and the Pacific coastal areas of South America but is rare in the USA and equatorial Africa. *N. americanus* is the predominant hookworm of Central and South Africa, southern Asia, Melanesia, and Polynesia. It is widely distributed in the southern USA, on islands of the Caribbean, and on the Atlantic side of Central and South America. About 25% of the world's population is infected with hookworms.

Hookworm infection is asymptomatic in most cases. Nutritional status, anemia, and iron stores should be evaluated in infected persons. During the acute phase, adult worms in the intestine may cause colicky epigastric pain, anorexia, flatulence, diarrhea, and weight loss. A pruritic papulovesicular rash (ground itch) may develop at the site of larval penetration. Migration of large numbers of larvae though the lungs occasionally causes Löffler's syndrome. Chronic infection may lead to iron-deficiency anemia and hypoproteinemia, causing pallor, dyspnea, weakness, tachycardia, lassitude, impotence, and edema. A low grade eosinophilia often persists. Severe chronic blood loss may lead to growth retardation, cardiac failure, and anasarca.

Mebendazole is the drug of choice in the USA. A cure rate of > 99% has been reported after a course of 100 mg bid x 3 days. Natural agents include quassia, black walnut, garlic, and thyme.

Strongyloides stercoralis (threadworm)

Strongyloides stercoralis, a nematode, was identified in this specimen. *Strongyloides* infects the duodenum and jejunum, but its life cycle also includes migration through the lungs. Infection occurs when larvae penetrate the skin of humans or are passed via the fecal-oral route. Strongyloidiasis is endemic throughout the tropics and subtropics, including rural areas of the southern USA. More than half of infected persons are asymptomatic. Acute symptoms of strongyloidiasis may include epigastric pain and tenderness, diarrhea, nausea, vomiting, constipation, and weight loss. Chronic infection may lead to glucose malabsorption and protein-losing enteropathy. Maculopapular or urticarial eruptions may occur. Occasionally, larvae mature in the bronchial submucosa and produce chronic bronchitis and asthma. Immunosuppression may lead to an overwhelming hyperinfection that can be potentially fatal.

In uncomplicated infection, treatment with ivermectin (200 ug/kg/d x 2 days, adult dose) or thiabendazole (25 mg/kg bid, <3 g/day, for 2 days, adult dose) results in 80 to 90% cure. Side effects of thiabendazole are frequent and occasionally disabling. Patients with a history of *Strongyloides stercoralis* exposure should undergo several stool examinations and, if necessary, a string test or duodenal aspiration before receiving corticosteroids or other immunosuppressive therapy.

Trichuris trichiura (whipworm)

Trichuris trichiura eggs were identified in this specimen. *T. trichiura* is a nematode that infects the large intestine. Infection is spread via the fecal-oral route. The parasite is found principally in the tropics and subtropics. Mild asymptomatic infections are common in rural parts of the southeastern USA. Light infections are often asymptomatic. Heavy infections cause abdominal pain, anorexia, and diarrhea and may retard growth. Very heavy infections may cause weight loss, anemia, and rectal prolapse in children and parturient women.

No drug treatment is required for asymptomatic or light infections. Mebendazole (100 mg bid x 3 days, adult dose) or albendazole (400 mg qd x 3 days, adult dose) are used for more severe infections. These drugs should not be used during pregnancy. Natural agents include quassia and black walnut.

Other Markers, Microscopy

Microscopic yeast

Microscopic examination has revealed more yeast in this sample than normal. While small quantities of yeast (reported as rare) may be normal, yeast observed in higher amounts (moderate to many) is considered abnormal. Yeast does not appear to be dispersed uniformly throughout the stool. Yeast may therefore be observed microscopically, but not grow out on culture even when collected from the same bowel movement. Further, some yeast may not survive transit through the intestines rendering it unviable for culturing. Therefore, both microscopic examination and culture are helpful in determining if abnormally high levels of yeast are present. If significant yeast are reported by microscopy, but not by culture, consider the presentation of patient symptoms.

Red Blood Cells (RBCs)

The number of RBCs in this specimen is higher than expected. This indicates active bleeding lower in the intestinal tract and may be associated with parasitic or bacterial infection. Microbial analysis including PCR and culture can identify enteroinvasive pathogens such as *Shigella, Salmonella, Campylobacter,* and *Yersinia* which can cause mucosal ulceration. Bleeding may also occur with an active inflammatory bowel condition such as ulcerative colitis (check fecal calprotectin and lactoferrin). Hemorrhoids (very common), colorectal cancer, and anal fistulas, may also be a factor in the finding of RBCs and may require further evaluation.

White Blood Cells (WBCs)

The number of WBCs in this specimen is higher than expected. Elevated levels of WBCs in the stool are an indication of an inflammatory process resulting in the infiltration of leukocytes within the intestinal lumen. This could be the result of an inflammatory bowel conditions including ulcerative colitis (UC) or Crohn's disease (check fecal calprotectin and lactoferrin). Enteroinvasive bacteria such as *Campylobacter, Shigella, Salmonella*, and Enteropathogenic parasites such as *Giardia, Blastocystis, Cryptosporidium*, and *Entamoeba* can be a cause of inflammation to the mucosal lining. WBCs are often accompanied by mucus in the stool (macroscopic examination). Other conditions that may be associated with WBCs in the stool include localized abscesses and anal fistulas. A positive WBC result may warrant identification and eradication of the cause of inflammation and possible anti-inflammatory therapy.

Muscle fibers

The amount of undigested muscle fibers is abnormally high in this specimen. The presence of muscle fibers in the stool is an indicator of incomplete digestion. This may be due to a number of factors including excessive meat intake and insufficient mastication. Other factors may include insufficient hydrochloric acid secretion within the stomach and/or insufficient output of pancreatic enzymes. Bloating and flatulence often accommodate hypochlorhydria and insufficient pancreatic enzyme output.

Vegetable fibers

Excessive amounts of vegetable fibers were found in this stool specimen. The presence of vegetable fibers must be considered in conjunction with other parameters such as muscle fibers and Elastase for a proper assessment of maldigestion. Elevated levels of vegetable fibers may be indicative of inadequate chewing or rapid transit time.

Charcot-Leyden Crystals

Charcot-Leyden crystals were identified in this specimen. Charcot-Leyden crystals are formed from the breakdown of immune cells, especially eosinophils, and may be seen in the stool of patients with parasitic diseases. The crystals are indicative of immune response and can sometimes be seen in association with non-parasitic infection or inflammation.

Mucus

Mucus was detected in this specimen. The presence of mucus in the stool may be due to prolonged irritation to the intestinal mucosa and may be secondary to a proliferation of gastrointestinal enteropathogens such as bacteria, yeast, or parasites. It can also be associated with an inflammatory bowel condition. Mucus is also secreted by the intestinal mucosa in response to parasympathetic excitability such as spastic constipation, mucus colitis, neoplasm of the rectum, or villous adenoma of the colon. A positive mucus result requires treatment of the cause of inflammation and possibly anti-inflammatory therapy. Microbial analysis, including PCR and culture along with microscopic studies of the stool are useful in the detection of dysbiotic bacteria, viruses, yeast, or parasites. Localized abscesses and inflammatory disorders should also be ruled out.

Microbiology

Imbalanced Flora

Imbalanced flora are those bacteria that reside in the host gastrointestinal tract and neither injure nor benefit the host. Certain dysbiotic bacteria may appear under the imbalanced category if found at low levels because they are not likely pathogenic at the levels detected. Imbalanced bacteria are commonly more abundant in association with insufficiency dysbiosis, and/or a fecal pH more towards the alkaline end of the reference range (6 - 7.8). Treatment with antimicrobial agents is unnecessary unless bacteria appear under the dysbiotic category.

Pathogenic/Dysbiotic Flora

In a healthy balanced state of intestinal flora, the beneficial bacteria make up a significant proportion of the total microflora. However, in many individuals there is an imbalance or deficiency of beneficial flora (insufficiency dysbiosis) and an overgrowth of non-beneficial (imbalance) or even pathogenic microorganisms. This can be due to a number of factors including: consumption of contaminated water or food; daily exposure of chemicals that are toxic to beneficial bacteria; the use of antibiotics, oral contraceptives or other medications; poor fiber intake and high stress levels.

A number of toxic substances can be produced by the dysbiotic bacteria including amines, ammonia, hydrogen sulfide, phenols, and secondary bile acids which may cause inflammation or damage to the brush border of the intestinal lining. If left unchecked, long-term damage to the intestinal lining may result in leaky gut syndrome, allergies, autoimmune disease (e.g. rheumatoid arthritis), irritable bowel syndrome, fatigue, chronic headaches, and sensitivities to a variety of foods. In addition, pathogenic bacteria can cause acute symptoms such as abdominal pain, nausea, diarrhea, vomiting, and fever in cases of food poisoning.

Bacterial sensitivities to a variety of prescriptive and natural agents have been provided for the pathogenic bacteria that were cultured from this patient's specimen. This provides the practitioner with useful information to help plan an appropriate treatment regimen. Supplementation with probiotics or consumption of foods (yogurt, kefir, miso, tempeh, tamari sauce) containing strains of lactobacilli, bifidobacteria, and enterococci may help restore healthy flora levels. Soluble fiber and polyphenols derived from chocolate, green tea, blackcurrant, red wine and grape seed extracts have been found to increase the

numbers of beneficial bacteria. Hypochlorhydria may also predispose an individual to bacterial overgrowth, particularly in the small intestine. Nutritional anti-inflammatories can aid in reversing irritation to the GI lining. These include quercetin, vitamin C, curcumin, gamma-linoleic acid, omega-3 fatty acids (EPA, DHA), and aloe vera. Other nutrients such as zinc, beta-carotene, pantothenic acid, and L-glutamine provide support for regeneration of the GI mucosa. A comprehensive program may be helpful in individuals in whom a dysbiotic condition has caused extensive GI damage.

Arcobacter butzleri

Arcobacter butzleri was detected in this specimen. *A. butzleri* is a gram-negative campylobacter-like organism that is an underestimated enteric pathogen. The suspected mode of transmission of *A. butzleri* is untreated water or contaminated food. *A. butzleri* has been isolated in different animals and is present in a variety of retail meats, including chicken, beef, pork, and lamb with a high prevalence in poultry. Two species, *A. butzleri* and, more rarely, *A. cryaerophilus*, have been associated with enteritis and occasionally bacteremia. Patients with *A. butzleri* infections report diarrhea associated with abdominal pain; nausea and vomiting or fever may also occur. *A. butzleri* are more frequently associated with a persistent and watery diarrhea and less associated with bloody diarrhea.

Similar to *Campylobacter*, the majority of the cases of enteritis appear to be self-limiting and do not require antibiotic treatment. However, the severity of prolongation of symptoms may justify antibiotic use. *A. butzleri* are resistant to ampicillin, clindamycin, azithromycin, metronidazole, carbenicillin and cefoperazone. Fluoroquinolones and tetracycline have been suggested for the treatment of infections; however, strains resistant to naladixic acid and ciprofloxacin have been detected.

Aeromonas spp

Aeromonas is a gram-negative bacterium belonging to the *Vibrionaceae* family that is considered pathogenic in any amount. It is most frequently observed in pediatrics (6 months to 2 years of age) and in adults over 60 years of age. This particular bacterium is present in all freshwater environments and in brackish water. Most *Aeromonas* spp., particularly those associated with human infection, are found in a wide variety of fresh produce, meat (beef, poultry, pork), and dairy products (raw milk, ice cream). It may also be isolated from raw fish and shellfish, especially oysters.

Two distinct types of gastroenteritis have been associated with *Aeromonas* spp. The first is a cholera-like illness with an acute watery diarrhea, mild or absent fever, possible abdominal pain, and possible vomiting in pediatrics. The second is a dysenteric illness characterized by loose stools containing blood and mucus. The symptoms are usually mild and self-limiting. Individuals with impaired immune systems or underlying malignancy are susceptible to more severe, sometimes fatal, infection. Complications from *Aeromonas* diarrheal disease include hemolytic uremic syndrome or kidney disease requiring kidney transplantation. Also, non-resolvable, intermittent diarrhea can occur months after initial infection and may persist for months or several years. Most cases of *Aeromonas* gastroenteritis have been sporadic rather than associated with large outbreaks, but increased reports have been noted from several clinical centers. On rare occasions the dysentery-like syndrome is severe and may last for several weeks. Extraintestinal symptoms, typically in immunocompromised individuals, include septicemia, meningitis, hypotension, skin disturbances, eye or urinary tract infection, pneumonia and endocarditis.

Antibiotics may be indicated if symptoms are prolonged and in systemic infections Refer to the antimicrobial susceptibilities to identify the most appropriate agent.

Bacillus cereus or *Bacillus anthracis*

Bacillus is a facultatively aerobic spore forming gram-positive bacillus. This bacterium is considered dysbiotic in the amount of 3 - 4+. Five species of *Bacillus* are now recognized as causative organisms of food poisoning with *B. cereus* being the most common.

Bacillus spp. are extremely widespread in nature, found in soil, air, dust, water and decaying matter. *B. cereus* has been isolated from the stool in 15% of healthy individuals. *B. cereus* is most commonly associated with large-scale food preparation; *B. anthracis* is acquired from animal handling. Food poisoning in heat-treated foods consists of two types: the diarrheal syndrome, associated with a wide variety of foods including meats, fish, milk, vegetables, casseroles and salads, and the emetic syndrome, associated with rice or other starchy foods (mashed potato, pasta, cheese, sauces, creams, custards, puddings, soups, casseroles, and pastries). The diarrheal syndrome may present with abdominal cramps, watery stools and rectal tenesmus occurring 6-15 hours after consumption of contaminated food. Nausea may accompany diarrhea, but vomiting rarely occurs. The emetic syndrome typically presents with nausea, followed by vomiting and malaise with onset within 0.5 to 6 h after consumption of contaminated foods. Occasionally abdominal cramps and/or diarrhea may also occur. Symptoms usually resolve within 24 hours.

Food poisoning is almost always self-limiting and does not require treatment. However, if treatment is necessary, *B. cereus* is always susceptible to clindamycin, erythromycin, chloramphenicol, vancomycin and the aminoglycosides.

B. *anthracis* is susceptible to penicillin.

Butyrichimonas virosa

Butyrichimonas virosa was detected in this stool specimen. *B. virosa* is an anaerobic gram-negative organism. It has been recovered in the feces of rats and humans. Three cases of *B. virosa* bacteremia have been reported, two patients with adenocarcinoma of the colon and one with diverticulitis.

There is no recommendation for antibiotic treatment for patients with this organism in their stool. Additional research is warranted to ascertain the etiologic association between *B. virosa* and diverticulitis or adenocarcinoma.

Campylobacter spp

Campylobacter spp. was detected in this specimen. *Campylobacter* is a gram-negative microaerophilic bacterium that is recognized as an enteric pathogen. The usual mode of transmission is consumption of contaminated food or water. Most cases of *Campylobacter* infection (campylobacteriosis) are isolated cases and occur as a result of handling or consuming undercooked poultry. Larger, less frequent outbreaks of *Campylobacter* have been related to consumption of unpasteurized milk from infected cow udders or contaminated with manure or water consumed from contaminated mountain streams or rivers. Household pets can also carry *Campylobacter* and can pass the bacteria to their owners. Campylobacteriosis occurs more often in the summer and early fall than in the winter months.

Campylobacter is a leading cause of diarrheal disease in the United States. The infective dose of *Campylobacter* is not clearly defined, but as few as 1,000 organisms may be capable of causing illness. Infection may mimic appendicitis, particularly in children. It has been estimated that in 5 to 10% of campylobacteriosis cases relapse has occurred. Complications, although rare, include reactive arthritis, hemolytic uremic syndrome, and infection of organs following septicemia. *Campylobacter* is microaerophilic, which means that sensitivities cannot be performed.

Most illnesses typically last one week and are self-limiting. Use of antibiotics is controversial and may benefit children whom have had symptoms for less than 7 days, and immunocompromised individuals. Recommendations potentially include Azithromycin 500 mg daily for 3 days or Fluoroquinolone for 3 days, but infection may resist fluoroquinolones. Extracts of *Acacia nilotiac* show in vitro antibacterial activities against *Campylobacter* spp. isolated from sheep. Oral rehydration therapy is recommended to prevent dehydration, along with symptomatic treatment of fever, muscle aches.

Campylobacter jejuni

The infectious organism *Campylobacter jejuni* was detected in this specimen. *Campylobacter* is a leading cause of diarrheal disease in the United States. The species *Campylobacter jejuni* is responsible for 99% of all cases of campylobacteriosis which causes mild to moderate, often bloody, diarrhea. Other symptoms may include fever, cramping, nausea, headache, and/or muscle pain within 2 to 5 days following exposure. In immunocompromised individuals, antibiotics may be used to prevent septicemia as well as to shorten the duration of symptoms. However, resistance to the antibiotics has been observed. Serological and fecal studies have also indicated prior infection with *C. jejuni* in 20-40% of patients with Guillain-Barre syndrome, a rare acute peripheral neuropathy lasting up to four weeks. However, the expected incidence rate of Guillain-Barre syndrome following *C. jejuni* infection is only 0.3 per 100,000 campylobacteriosis cases.

Extended Spectrum Beta-lactamase (ESBL)-producing *Citrobacter* spp

This bacterium produces an extended spectrum beta-lactamase enzyme, which confers resistance to penicillins, cephalosporins, and monobactams. Plasmid transfer of the genes coding for this enzyme increases prevalence of drug-resistance. Promotion of gene exchange allows different genera of bacteria to adopt resistance. The reservoir for these drug-resistant opportunistic pathogens is the gastrointestinal tract of healthy individuals. If treatment is required, carbapenems and cephamycins are commonly used.

Citrobacter spp

Citrobacter spp., a gram-negative bacterium and member of the *Enterobacteriaceae* family, is considered dysbiotic at 3+ or greater. *Citrobacter freundii* complex (including *C. freundii, C. braakii, C. gullenii, C. murliniae, rodentium, C. wermanii, C. youngae*, *C. koseri* and *C. farmeri*, can cause diarrheal disease. Symptoms are the result of an *E.* coli-like heat-stable enterotoxin and hydrogen sulfide. *Citrobacter freundii* complex has been implicated as a cause of gastrointestinal infection and inflammation, acute dysentery, and dyspepsia. Acute symptoms can include profuse, watery diarrhea without abdominal pain, fecal blood, or white blood cells.

Citrobacter spp. thrive on fructooligosaccharides (FOS), a common ingredient in artificial or alternative sweetener.

Antibiotics may be indicated if symptoms are prolonged. Refer to the antimicrobial susceptibilities to identify the most appropriate agent.

Edwardsiella tarda

The bacterium *Edwardsiella tarda* is a gram-negative enteric pathogen belonging to the *Enterobacteriaceae* family. The chief reservoirs in nature are reptiles (especially snakes, toads, and turtles) and freshwater fish. *E. tarda* causes diarrhea, gastroenteritis and wound infections in humans. Most reports of enteric illness describe a mild gastroenteritis that improves without therapy in 2 to 3 days. Iron availability has been thought to regulate the seriousness of *E. tarda* infection. Iron overload, caused by such conditions as red cell sickling, leukemia, and cirrhosis, is associated with E. tarda septicemia.

Antibiotics may be indicated if symptoms are prolonged. Refer to the antimicrobial susceptibilities for treatment.

Extended Spectrum Beta-lactamase (ESBL)-producing *Enterobacter cloacae*

This bacterium produces an extended spectrum beta-lactamase enzyme (ESBL), which confers resistance to penicillins, third generation cephalosporins, and monobactams. Plasmid transfer of the genes coding for the ESBL enzyme increases prevalence of drug-resistance. Promotion of gene exchange allows different genera of bacteria to adopt resistance. The reservoir for these drug-resistant opportunistic pathogens is the gastrointestinal tract of healthy individuals. If treatment is required, carbapenems and cephamycins are commonly used.

Enterobacter cloacae complex

Enterobacter cloacae complex is part of the *Enterobacteriaceae* family. *E cloacae* complex is a group of six closely related species with similar resistance patterns: *E. cloacae, E. asburiae, E. hormaechei, E. kobei, E. ludwigii,* and *E. nimipressuralis*. This gram-negative bacterium is considered dysbiotic at levels of 3+ or greater. *E. cloacae* complex is considered an opportunistic pathogen associated with diarrhea in children. A Shiga-like toxin-producing *E. cloacae* was isolated from the feces of an infant with hemolytic-uremic syndrome. However, *E. cloacae* complex is most often involved in extraintestinal infections including the urinary tract, respiratory tract, and cutaneous wounds.

Widely distributed in the environment, *Enterobacter* spp. is commonly isolated from both human and animal feces. Environmental strains of *Enterobacter* spp. are capable of growth in foods at refrigeration temperature.

E. cloacae complex is known to possess inducible ß-lactamases. Isolates may become resistant to all cephalosporins after initiation of therapy. Avoid ß-lactam-inhibitor drugs such as amoxicillin/ clavulanate, ampicillin/sulbactam, and piperacillin/tazobactam.

Antibiotics may be indicated in systemic infections if symptoms are prolonged. Refer to the antimicrobial susceptibilities for treatment.

Extended Spectrum Beta-lactamase (ESBL)-producing *Escherichia coli*

This bacterium produces an extended spectrum beta-lactamase enzyme (ESBL), which confers resistance to penicillins, third generation cephalosporins, and monobactams. Plasmid transfer of the genes coding for the ESBL enzyme increases prevalence of drug-resistance. Promotion of gene exchange allows different genera of bacteria to adopt resistance. The reservoir for these drug-resistant opportunistic pathogens is the gastrointestinal tract of healthy individuals. Normally, the presence of *Escherichia coli* in stool is considered beneficial; however, colonization of an ESBL-producing bacteria could result in subsequent positive clinical cultures. If treatment is required, carbapenem or cephamycins are recommended. Refer to the antimicrobial susceptibilities for treatment.

Enterohemorrhagic *Escherichia coli* (*E. coli* O157:H7)

Escherichia coli O157:H7, a gram-negative bacterium and member of the *Enterobacteriaceae* family, is a highly contagious enteropathogen. This bacterium is listed as dysbiotic in any amount. These organisms are known to produce one or more Shiga toxins and are the most frequently identified diarrheagenic *E. coli* serotypes in North America and Europe.

Infection with *E. coli* O157:H7 can develop into a range of symptoms. These symptoms may include mild diarrhea or no symptoms at all. Most identified cases develop severe diarrhea and abdominal cramps. Blood is often seen in the stool. Fever and vomiting may also occur. Symptoms usually subside within 5 - 7 days. About 8% of those who are diagnosed with *E. coli* O157:H7 infection develop hemolytic uremic syndrome (HUS). Children and the elderly are more likely to develop this serious complication. HUS is characterized by acute renal failure, hemolytic anemia and thrombocytopenia. The incubation period is usually 3-4 days after the exposure, but ranges from 1 - 10 days. The symptoms often begin slowly with mild abdominal pain or non-bloody diarrhea that worsens over several days. HUS, if it occurs, develops an average of 7 days after the first symptoms, when the diarrhea is improving. *E. coli* O157:H7 typically disappear from the feces by the time the illness is resolved, but may be shed for several weeks. Young children carry *E. coli* O157:H7 longer than adults.

E. coli O157:H7 live in the guts of ruminant animals, including cattle, goats, sheep, deer, and elk. The major source for human illnesses is cattle. Exposure can occur by consumption of contaminated food, unpasteurized (raw) milk, unchlorinated water, contact with cattle, or contact with the feces of infected people.

Non-specific supportive therapy, including hydration, is important. Susceptibility testing of *E. coli* O157:H7

is contraindicated since treatment of disease with antimicrobial agents can induce bacterial cell lysis and release of toxin which is more likely to lead to hemolytic uremic syndrome. Antidiarrheal agents like Imodium (R) may also increase that risk.

Helicobacter canadensis

Enterohepatic *Helicobacter* spp. are recognized microbial pathogens in humans and animals. Infection with *H. canadensis* typically presents with symptoms of diarrhea. There are now six *Helicobacter* spp. that have been isolated from humans, the other five being *H. pullorum, H. canis, H. rappini, H. fennelliae,* and *H. cinaedi*. *H. canadensis* has been isolated from *H. pullorum* cultures. *H. pullorum* is a common contaminant of chicken carcasses. Other *H. canadensis* strains have been isolated from pig feces. It is possible that the handling of raw chicken or pork, or consumption of undercooked meats, may be a vector for infection. Water contamination may be possible, as the bacterium has been found in wild rodent and geese populations.

H. canadensis is microaerophilic, which means that sensitivities cannot be performed. The bacterium is known to be resistant to first-generation cephalosporins (cephalothin) and naladixic acid (quinolone). Oral rehydration therapy may be employed to prevent dehydration during diarrhea, and symptomatic treatment of fever and muscle aches may provide patient relief. Severe, persistent cases may require antibiotic treatment.

Helicobacter canis

Helicobacter canis was detected in this stool specimen. *H. canis* is an enterohepatic *Helicobacter* spp. It is a gram negative, spiral shaped bacteria that colonizes the gastrointestinal tract and/or liver of mammals, including humans. It has been recovered from the feces of dogs with and without diarrhea. It has also been isolated from feces of a 5 ½ year old boy with gastroenteritis, from the liver of a 2-month-old puppy suffering from multifocal necrotizing hepatitis and from bloodstream of both immunocompromised and immunocompetent patients. *H. canis* has also been detected in duodenal ulcers from a patient with Crohn's disease.

H. canis are colonizers of certain animals, and human infection is zoonotic in most cases. This organism may be associated with diarrhea, bacteremia with or without multifocal cellulitis and visceral disease, including cholecystitis, hepatitis, and possible hepatic cancer. *H. canis* has also been detected in livers of patients with ulcerative colitis and non-concomitant liver disease, as well as in children with other liver diseases. Close contact with either dogs or cats was reported in all cases.

H. canis is microaerophilic, which means that sensitivities cannot be performed. The recommendation for antibiotic treatment is to reserve use for those who are immunosuppressed or have severe disease. Additional research is warranted to ascertain the etiologic association between *H. canis* and diarrhea.

Helicobacter pullorum

H. pullorum is an enterohepatic Helicobacter spp. A documented pathogen, *H. pullorum* usually causes acute gastroenteritis. Cases of chronic diarrhea have also been reported. *H. pullorum*-associated gastroenteritis has been increasingly recognized in both Europe and North America.

H. pullorum has pro-inflammatory effects on intestinal mucosal cells in vitro. The organism is able to colonize both the gastrointestinal and hepatobiliary tracts. *H. pullorum* DNA has been isolated from human livers, gallbladders and biliary vessels of patients suffering from cholecystitis or liver disease. There is a high risk of exposure via chicken or guinea fowl droppings; chicken meat, eggs, and eggshells may also be contaminated. Consumption of undercooked poultry or egg products is a likely source of infection.

The chronic use of flavomycin, furazolidone, nitrovin, tetracycline, tylosin, sulphaquinoxaline, virginiamycin and zinc bacitracin in poultry feeds and the discovery of ciprofloxacin residues in feather meal in the United States may impact antibiotic susceptibilities.

H. pullorum is microaerophilic, which means that sensitivities cannot be performed. The recommendation for antibiotic treatment is to reserve use for those who are immunosuppressed or have severe disease.

Extended Spectrum Beta-lactamase (ESBL)-producing *Klebsiella*

This bacterium produces an extended spectrum beta-lactamase enzyme (ESBL), which confers resistance to penicillins, third generation cephalosporins, and monobactams. Plasmid transfer of the genes coding for the ESBL enzyme increases prevalence of drug-resistance. Promotion of gene exchange allows different genera of bacteria to adopt resistance. The reservoir for these drug-resistant opportunistic pathogens is the gastrointestinal tract of healthy individuals. If treatment is required, carbapenems and cephamycins are commonly used.

Klebsiella spp

Klebsiella spp. are gram-negative bacilli belonging to the *Enterobacteriaceae* family and closely related to the genera *Enterobacter* and *Serratia*. *Klebsiella* spp. are considered dysbiotic in the amount of 3 - 4 +. *Klebsiella* spp. are widely distributed in nature and in the gastrointestinal tract of humans. In humans, they may colonize the skin, oral cavity, pharynx, or gastrointestinal tract. Regarded as normal flora in many parts of the colon, intestinal tract and biliary tract, the gut is the main reservoir of opportunistic strains. This bacteria has the potential to cause intestinal, lung, urinary tract, and wound infections, but overgrowth of *Klebsiella* spp. is commonly asymptomatic. *K. pneumoniae*, in particular, may cause diarrhea and some strains are enterotoxigenic. Infection has been linked to ankylosing spondylitis as well as myasthenia gravis (antigenic cross-reactivity), and these patients usually carry larger numbers of the organism in their intestines than healthy individuals. *Klebsiella* oxytoca causes antibiotic associated hemorrhagic colitis. These strains have been shown to produce a cytotoxin that is capable of inducing cell death in various epithelial-cell cultures.

Klebsiella is a significant nosocomial infectious agent, partially due to the ability of organisms to spread rapidly. *Klebsiella* accounts for approximately 3-7% of all hospital-acquired infections, placing it among the top eight pathogens in hospitals. Extraintestinal infection typically involves the respiratory or urinary tracts, but may infect other areas such as the biliary tract and surgical wound sites. *K. pneumoniae* and *K. oxytoca* are the two members of this genus responsible for most extraintestinal human infections.

Treatment of these organisms has become a major problem because of resistance to multiple antibiotics and potential transfer of plasmids to other organisms. Proper hand washing is crucial to prevent transmission from patient to patient via medical personnel. Contact isolation should be used for patients colonized or infected with highly antibiotic-resistant *Klebsiella* strains. *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis* are infrequent isolates that are subspecies of *K. pneumoniae*; however, each is associated with at unique spectrum of disease. *K. ozaenae* is associated with atrophic rhinitis, a condition called ozena, and purulent infections of the nasal mucous membranes. *K. rhinoscleromatis* causes the granulomatous disease rhinoscleroma, an infection of the respiratory mucosa, oropharynx, nose, and paranasal sinuses.

Antibiotics may be indicated if symptoms are prolonged and in systemic infections. Refer to the antimicrobial susceptibilities for treatment.

Laribacter hongkongensis

Laribacter hongkongensis was detected in this stool specimen. *L. hongkongensis* is a gram-negative bacillus believed to be associated with community-acquired gastroenteritis and traveler's diarrhea. The gastroenteritis attributed to *L. hongkongensis* is similar to that of *Salmonella* and *Campylobacter jejuni* (excessive watery diarrhea), except that some patients also have bloody diarrhea. The isolation of *L. hongkongensis* from patients who reside in Hong Kong or who have recently traveled to Asia, Europe, North or South America, and Africa implies that the bacterium is likely to be globally dispersed. Antibiotic treatment is reserved for those who are immunosuppressed or have severe disease. Additional research is warranted to ascertain the etiologic association between *L. hongkongensis* and diarrhea and to identify the host and the routes of transmission of the bacterium.

Listeria spp

Listeria is a gram-positive, non-spore forming, facultative anaerobic bacillus that is able to invade and survive within mammalian cells. *Listeria* spp. are widely distributed in the environment, primarily in the soil and decaying vegetable matter. Because *Listeria* is so widespread and is able to grow at low temperatures (4 degrees C), it easily contaminates food production and causes disease in persons ingesting the tainted food.

Two species of *Listeria* are considered pathogenic; *L. monocytogenes* and *L. ivanovii*. *L. monocytogenes* is well known to infect humans and animals and is associated with food borne outbreaks. *L. ivanovii* is generally considered a pathogen only of ruminate animals; however, there are case reports associating this organism including gastroenteritis and bacteremia in immunocompromised humans.

Listeria is usually susceptible to penicillin, ampicillin, the aminoglycosides, erythromycin, tetracycline, and trimethoprim-sulfamethoxazole. Cephalosporins and fluoroquinolones are not active.

Listeria ivanovii

Listeria ivanovii has been identified in this specimen. *L. ivanovii* is considered an opportunistic pathogen in humans. Symptoms of infection may include non-bloody diarrhea, vomiting, and low-grade fever. Infection may inflame the liver and elevate liver glutamyltranferase (GGT) in some patients. Infection may be more likely or progress to systemic infection in immunocompromised individuals. Vertical transmission from mother to fetus may be possible. While listeriosis is not common in humans, the disease may be clinically significant; a related organism *Listeria monocytogenes* is known for its severity and high mortality.

L. ivanovii is commonly found in ruminant animals (cattle, sheep, goats, deer, elk, etc.). *Listeria* spp. are susceptible to amoxicillin and gentamicin; resistant to third-generation cephalosporins, clindamycin, and aztreonam; *L. ivanovii* has been reported susceptible to fosfomycin as well.

Listeria monocytogenes

Listeria monocytogenes has been identified in this specimen. This organism is able to invade and survive within mammalian cells. *L. monocytogenes* is associated with a spectrum of clinical syndromes. The most common result of infection with *Listeria* is the transient asymptomatic gastrointestinal carrier state that usually develops from ingestion of contaminated food. Acute symptomatic infection often occurs during pregnancy, usually during the last half of pregnancy, and the illness presents with influenza-like symptoms of fever, sore throat, myalgia, malaise, lower abdominal pain, and back pain. Occasionally, a vaginal discharge, diarrhea, and urinary tract symptoms are noted. During maternal infection, bacteremia and transplacental transmission of the organism may occur, leading to intrauterine infection of the fetus. Infection in utero may induce labor, resulting in premature birth of an infected or stillborn fetus. Spontaneous abortion may occur if the infection is acquired early in pregnancy.

Nonpregnant adults infected with *L. monocytogenes* may present with acute sepsis, subacute meningitis, meningoencephalitis, or rhombencephalitis. Adults with acute listerial sepsis usually have underlying malignancies or are immunocompromised. Gastrointestinal symptoms such as diarrhea have been observed in some individuals with systemic listeriosis. In the past decade, several outbreaks of febrile gastroenteritis caused by *L. monocytogenes* have been documented. Symptoms appear within several hours (18 to 72 h) of exposure and consist of nonbloody diarrhea, nausea, and vomiting accompanied by fever, fatigue, chills, and myalgia. Febrile gastroenteritis is usually self-limiting, but invasive disease may occur in immunocompromised patients and those with other underlying bacterial or viral gastrointestinal infections.

Moellerella wisconsensis

Moellerella wisconsensis, a gram-negative bacillus and member of the *Enterobacteriaceae* family, is identified as a potential pathogen when cultured at 3 - 4+. It was originally found in human stool specimens from patients with diarrhea or gastroenteritis. Water is the most common source for fecal isolates; one strain was isolated in large numbers from a city's non-chlorinated water supply. It has on rare occasions been associated with acute cholecystitis, peritonitis and bacteremia.

Due to the self-limiting nature of typical infection, treatment is often unnecessary. If treatment is necessary, refer to the antimicrobial susceptibilities to identify the most appropriate therapy.

Morganella morganii

The bacterial genus *Morganella* includes only one species, *M. morganii*, recognized in 1906 as an organism isolated from infant diarrheal samples. This gram-negative bacterium is considered dysbiotic in the amount of 3 - 4+. *Morganella morganii* is a gram-negative rod commonly found in the environment and in the intestinal tracts of humans, mammals, and reptiles as normal flora. It is the cause of both urinary tract and wound infections and has been implicated as a cause of diarrhea. Enterotoxigenic strains of *M. morganii* have been implicated in two cases of gastrointestinal disease, of which one of the two individuals presented with loose stools only while the second person had clinically defined gastroenteritis.

Antibiotics may be indicated if symptoms are prolonged and in systemic infections. Refer to the antimicrobial susceptibilities for treatment.

Methicillin-resistant Staphylococcus aureus (MRSA)

Methicillin-resistant *Staphylococcus aureus* is a major cause of hospital acquired infections. Colonization of MRSA on the skin, mucous membranes, and in the gastrointestinal tract may represent important reservoirs of the bacteria. To minimize the risk of spreading MRSA, inpatients should be isolated and proper protective equipment utilized. Outpatients should also be isolated to minimize the spread of MRSA.

Staphylococcus aureus

S. aureus is well documented as an opportunistic human pathogen. *S. aureus* is considered as dysbiotic in the amount of 3 - 4+. Staphylococci are widespread in nature and have been cultured from skin, mucous membranes, hair, blood, stool, mammary glands, intestinal tract, and genitourinary tract of healthy individuals. Staphylococcal bacterial are one of the most common causes of skin infections in the United States. Toxic shock syndrome is also attributable to *S. aureus*. Toxic shock syndrome is associated with exotoxins produced by *S. aureus.*

S. aureus can cause acute food poisoning. Due to the high rate of human carriage of *S. aureus*, food handlers play a significant role in its spread. The presence of enterotoxigenic strains of *S. aureus* in various food products is regarded as a public health hazard. Foods commonly associated with staphylococcal food poisoning are meat, meat products, salads, cream-filled bakery products, and dairy products. The toxin produced is heat-stable and not easily destroyed by cooking. Even if the organism is destroyed, the toxin can remain. Symptoms of staphylococcal food poisoning usually appear within 2-4 hours of consumption of contaminated food and typically last for only 24 hours. Common symptoms include nausea, vomiting, dizziness, weakness, subnormal temperatures and occasional headaches and diarrhea. Symptoms may be confused with those of *Bacillus cereus* food poisoning. Symptoms usually resolve within 2 days, but may take 3 days and sometimes longer to resolve.

Due to the self-limiting nature of typical infection, treatment is often unnecessary. If treatment is necessary, refer to the antimicrobial susceptibilities to identify the most appropriate therapy.

Plesiomonas shigelloides

Plesiomonas shigelloides, a gram-negative bacillus of the *Enterobacteriaceae* family, is considered dysbiotic in any amount. *P. shigelloides* can be isolated from freshwater, freshwater fish, shellfish and many animals. This bacterium is found in individuals with a history of travel to tropical or subtropical countries, exposure to contaminated freshwater, seafood consumption, or exposure to amphibians or reptiles. Occasional outbreaks have been reported. The highest incidence of disease caused by *P. shigelloides* has been among persons returning from Japan with 'traveler's diarrhea.' The most common disease associated with *P. shigelloides* is a self-limiting diarrhea which usually begins 20 to 24 hours after ingestion of contaminated food or water. Typically, the stool is watery without mucus or blood. In severe cases, however, the stool can be greenish-yellow and blood-tinged. Accompanying symptoms vary and may include nausea, vomiting, abdominal pain, chills, headaches, and dehydration. The illness usually lasts for 1 to 7 days. In about 30% of cases, persistent diarrhea and abdominal pain may develop lasting up to three weeks. High fever and chills associated with the infection may occur in children and infants. Non-intestinal infections, particularly septicemia, can occur in immunocompromised persons or those with underlying diseases such as cancer, leukemia and liver disease.

Plesiomonas infection is often self-limiting. Antibiotics may be indicated if symptoms are prolonged or in systemic infections. Refer to the antimicrobial susceptibilities for treatment.

Extended Spectrum Beta-lactamase (ESBL)-producing *Proteus*

This bacterium produces an extended spectrum beta-lactamase enzyme (ESBL), which confers resistance to penicillins, third generation cephalosporins, and monobactams. Plasmid transfer of the genes coding for the ESBL enzyme increases prevalence of drug-resistance. Promotion of gene exchange allows different genera of bacteria to adopt resistance. The reservoir for these drug-resistant opportunistic pathogens is the gastrointestinal tract of healthy individuals. If treatment is required, carbapenems and cephamycins are commonly used.

Proteus spp

Proteus spp. are gram-negative bacilli of the *Enterobacteriaceae* family. *Proteus* spp. are normal flora of the gut but are listed as dysbiotic flora at 3 - 4+. *P. vulgaris* and *P. mirabilis* are the best-known species of the genus *Proteus*, with *P. mirabilis* being the most common infectious species. *Proteus* is commonly found in contaminated meat, soil, polluted water, sewage, manure, and in the intestinal contents of wild and domestic animals. This bacterium is often found in higher numbers in those who have received antibiotics.

Some *Proteus* isolates can produce an enterotoxin. Organisms often localize in already damaged tissues in various parts of the body where they may produce an exudative inflammatory reaction. *Proteus* has been associated with diarrheal disease in susceptible individuals and has been implicated as a possible cause of epidemic diarrhea in infants. In addition, *Proteus mirabilis* is a urease-producing organism that contributes to the formation of kidney stones. Urinary tract infections, most common in patients with long-term catheterizations, may lead to kidney failure and may be the source of bacteremia. Infection has been linked to rheumatoid arthritis (antigenic cross-reactivity). There is also evidence for osteomyelitis caused by *Proteus* bacteria.

For the otherwise healthy individual, antimicrobial therapy is often unnecessary. Antibiotics may be indicated if symptoms are prolonged and in systemic infections. Refer to the antimicrobial susceptibilities to identify the most appropriate therapy.

Providencia spp

Providencia spp. belong to the *Enterobacteriaceae* family. This gram-negative bacilli is considered dysbiotic in the amount of 3 - 4+. *P. alcalifaciens* has been described as a possible diarrhea-causing pathogen in travelers and children in developing countries. In 1996, *P. alcalifaciens* caused a large food-borne outbreak involving 290 patients in Japan. *P. rettgeri* and *P. stuartii* also have been implicated as causes of

travelers' diarrhea with symptoms including diarrhea, vomiting, fever and abdominal pain. Vomiting was a characteristic complaint among patients with diarrhea caused by *P. rettgeri*. The routes of exposure to *Providencia* have not been firmly established, but contaminated beef is a suspected source.Like *Proteus*, *Providencia* has the ability to alkalinize urine and to promote the formation of crystals and calculi.

Antibiotics may be indicated if symptoms are prolonged. Refer to the bacterial sensitivities to identify the most appropriate antimicrobial therapy.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative aerobic bacillus belonging to the bacterial family *Pseudomonadaceae*. This opportunistic pathogen is considered dysbiotic in the amount of 3+ - 4+. *P. aeruginosa* is tolerant to a wide variety of physical conditions and temperatures. The exact source and mode of transmission is not known due to its ubiquitous presence in the environment. This bacterium has minimal nutritional requirements and is very resistant to disinfectants and most antibiotics. It survives in moist environments and can therefore grow in bottled non-carbonated mineral water, distilled water, tap water, and food. *Pseudomonas* can cause urinary tract infections, dermatitis, soft tissue infections, bacteremia, endocarditis, and osteochondritis. Skin and soft tissue infections can also proliferate in high moisture conditions (swimmer's ear, toe webs of athletes, skin of hot tub users).

Gastrointestinal colonization of immunocompromised patients with *P. aeruginosa* may lead to bacteremia. Gastrointestinal infections are found primarily in these immunosuppressed patients; however, a number of cases of diarrhea have been reported in otherwise healthy individuals. *Pseudomonas* is often asymptomatic in the gastrointestinal tract, but is considered a potential cause of antibiotic associated diarrhea.

For the otherwise healthy individual, antimicrobial therapy is often unnecessary. Antibiotics may be indicated if symptoms are prolonged and in systemic infections. Refer to the antimicrobial sensitivities to identify appropriate therapy.

Raoultella ornithinolytica

Raoultella ornithinolytica, formally *Klebsiella ornithinolytica*, is a gram negative bacillus in the *Enterobacteriaceae* family. This bacteria is considered dysbiotic in the amount of 3 - 4+. *Raoultella* are widely distributed in nature and in the gastrointestinal tract of humans. *R. ornithinolytica* has been isolated from dentin of infected root canals. Human infections caused by bacteria of this genus are infrequent. Bacteremia cases had not been reported until a case of enteric fever-like syndrome was identified. The patient had both blood and fecal samples that were positive for *R. ornithinolytica*. This organism has also been associated with histamine poisoning. Histamine poisoning occurs when persons ingest fish in which bacteria have converted histidine to histamine. The incubation period for histamine poisoning is 1 minute to 3 hours after eating and manifests with facial flushing, dizziness, vomiting, diarrhea, other gastrointestinal symptoms, dyspnea, headache, burning of the mouth, urticaria, and pruritus. Histamine poisoning is usually a mild illness; however, serious issues, such as cardiac and respiratory complications can occur in individuals with preexisting conditions.

Antibiotics may be indicated if symptoms are prolonged and in systemic infections. Refer to the bacterial sensitivities to identify the most appropriate antimicrobial therapy.

Salmonella group

Salmonella, a gram-negative bacilli of the *Enterobacteriaceae* family, is listed as pathogenic in any amount. The bacteria may be isolated from a wide range of mammals, birds, reptiles, human or animal excrement and contaminated water. *Salmonella* is the most diverse of all the *Enterobacteriaceae*, with more than 2,500 serotypes. *Salmonella* is responsible for foodborne toxic infections due to contaminated raw or undercooked meats, eggs, milk products, and plants used as food. This bacteria may also be found in fish, chocolate, dried pasta, and mayonnaise. Prevention of *Salmonella* infection relies on avoiding contamination by improving hygiene and food handling practices. *Salmonella* infections have more severe effects on infants, the elderly and immunocompromised individuals.

Salmonella can cause either acute gastroenteritis or typhoid fever (typhus). Typhus results from bacterial invasion of the bloodstream. Typically, symptoms of enteritis appear about 12-72 hours following ingestion of contaminated food and last 4-7 days. Symptoms include abdominal pain, diarrhea, vomiting, fever, and possible bloody stools. Most persons recover without treatment. However, in some persons, the diarrhea may be so severe that the patient needs to be hospitalized. In these patients, the infection may cause localized infections (e.g., osteomyelitis or urinary tract infection) or bacteremia. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness. *Salmonella* has also been associated with reactive arthritis. Typhoid fever is a serious bloodstream infection and typically presents with a sustained debilitating high fever and headache, without diarrhea. About 5% of patients clinically cured of *S. typhi* remain carriers for up to one year.

Treatment of enteritis with antibiotics is not advised because it is ineffective, increases the chance of a carrier state developing, and may promulgate the further spread of antibiotic resistance. Antibiotics may be indicated if symptoms are prolonged and in systemic infections. Refer to the bacterial sensitivities to identify the most appropriate antimicrobial therapy.

Shigella spp

Shigella, a gram-negative bacilli and member of the *Enterobacteriaceae* family, is a highly contagious enteric pathogen. This bacterium is listed as pathogenic in any amount. The genus *Shigella* consists of four subgroups: Group A, *Shigella dysenteriae*; Group B, *S. flexneri*; Group C, *S. boydii*; and Group D, *S. sonnei*. All species are pathogenic to humans. In general, *S. dysenteriae*, *S. flexneri*, and *S. boydii* are the most isolated Shigella spp. in developing countries. Conversely, *S. sonnei* is most common *Shigella* species isolated in the U.S., accounting for over two-thirds of shigellosis in the U.S. *S. dysenteriae* is the least common *Shigella* species isolated in developed countries. Only a small inoculum (10-200 organisms) is needed to cause infection. Transmission is via the fecal-oral route and occurs in areas where hygiene is poor. Epidemics may be foodborne or waterborne. Examples of transmission are food by contaminated food handlers, vegetables by contaminated sewage, and parents by children in daycare settings.

Shigella is the second most common cause of traveler's diarrhea. Symptoms appear within 1-3 days after exposure to *Shigella*, or within one week for *S. dysenteriae*. Symptoms include diarrhea that may be mild or severe, watery or bloody, with or without mucus and/or white blood cells. Associated symptoms include vomiting, fever, abdominal cramps, mucosal ulceration (similar to enteroinvasive *E. coli*), and electrolyte loss. Some infected people are asymptomatic; however, others, especially young children and the elderly, may need to be hospitalized due to dehydration. Convulsions and/or bacteremia may occur in children. Infection has been associated with rheumatoid diseases including Reiter's syndrome and reactive arthritis.

The spread of *Shigella* from an infected person to other persons can be stopped by frequent and careful hand washing with soap. When possible, young children with *Shigella* infections should not be in contact with uninfected children. People with shigellosis should not prepare food or pour water for others until they have been shown to no longer be a carrier. For most people, this will be for one to two weeks, rarely exceeding four weeks. Food handlers, children or staff in daycare, and health care workers must have two negative stool cultures collected 24 hours apart before they can return to work or daycare. If they were on antibiotics for their illness, the first specimen must be collected 24 hours after completion of antibiotic therapy.

Shigella infection is usually a self-limiting disease, running a course of 4-7 days. Persons with diarrhea usually recover completely, although it may be several months before their bowel habits are entirely normal. If symptoms are severe, antibiotics and fluid replacement are indicated. Multiresistant strains are common. Antidiarrheal agents should be avoided. Once someone has had shigellosis, they are not likely to be infected with that specific type again for at least several years. However, they can still be infected with other types of *Shigella*.

If treatment is necessary, refer to the antimicrobial susceptibilities to identify the most appropriate therapy.

Yersinia spp

Yersinia, a gram-negative bacillus and member of the *Enterobacteriaceae* family, is listed as pathogenic in any amount. Of the 10 known species, three are well known human pathogens. They are *Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis*. Of these, *Y. enterocolitica* is the most important cause of foodborne illness and is an invasive enteric pathogen causing enterocolitis, terminal ileitis, lymphadenitis, and septicemia. *Y. pestis* is responsible for human plague. The other eight species *Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. aldovae, Y. bercovieri, Y. mollaretti*, and *Y. rohdei* are found in both intestinal and extraintestinal specimens; however, the pathogenicity of these species has not yet been determined. *Yersinia* species are often isolated from animals and have been detected in environmental and food sources, such as ponds, lakes, meats, oysters, fish, ice cream, and milk. Improper food handling and unsanitary conditions increase risk of infection. Yersiniosis is an infectious disease caused by a bacterium of the genus Yersinia. The CDC estimates about 17,000 cases of yersiniosis occur annually in the U.S. Yersiniosis is far more common in Northern Europe, Scandinavia, and Japan. The most susceptible are pediatric, geriatric, and immunocompromised populations

In the United States, most human illness is caused by one species, *Y. enterocolitica*. Enterocolitis caused by *Y. enterocolitica* is characterized by diarrhea, fever, and abdominal pain. Leukocytes and blood may be present in the stool. This condition may progress to mesenteric lymphadenitis and terminal ileitis, often misdiagnosed as appendicitis. Infection can be a predisposing factor for development of rheumatoid arthritis, Sjogren's syndrome, arterial inflammation, inflammatory dermatitis, hardening of connective tissue, and autoimmune thyroid disease (antigenic cross-reactivity). Sore throat or other symptoms may appear without GI symptoms. Since iron is vital for the survival and proliferation of microorganisms, persons with iron overload are more susceptible to *Y. enterocolitica* infections.

Y. pseudotuberculosis is responsible for mesenteric lymphadenitis, particularly in children exhibiting clinical disease similar to appendicitis. The transmission is unknown, but it is thought to be a possible foodborne pathogen. This organism rarely causes diarrhea.

Uncomplicated cases of diarrhea due to *Yersinia* usually resolve on their own without antibiotic treatment. Antibiotics may be indicated if symptoms are prolonged and in systemic infections. Refer to the antimicrobial susceptibilities to identify the most appropriate therapy.

Vibrio spp

The bacterial genus *Vibrio* is in the family *Vibrionaceae* and is pathogenic in any amount. There are more than ninety species of this gram-negative bacillus. *Vibrio* spp. are primarily found in aquatic habitats. Their distribution and abundance depends on water temperature, sodium concentration, nutrient content, and the presence of certain plant and animal species. In temperate climates, *Vibrio* spp. are predominately found during the summer months. Species that require only low sodium concentrations (e.g. *V. cholerae, V. mimicus*) can be found in freshwater. *V. parahaemolyticus* is frequently found in codfish, mackerel, flounder, clam, octopus, shrimp, crab, lobster, crawfish, scallop, and oyster.

V. parahaemolyticus is the species most commonly isolated from clinical specimens in the U.S, and the leading cause of food-borne intestinal infections in Asia. It is almost always associated with consumption of raw fish or shellfish. The most common clinical presentation of *V. parahaemolyticus* infections is watery, occasionally bloody, diarrhea that is often accompanied with nausea, vomiting, abdominal cramps, low- grade fever, and chills. Incubation periods range from 4 to 96 hours with a mean of 15 hours. The disease is often self-limiting lasting an average of 3 days, but can occasionally be life threatening in cases of severe dehydration or in patients with liver disease or immunocompromised states that predispose to septicemia. Extraintestinal infections may occur and mainly present as wound infections and septicemia. *V. cholerae* causes life-threatening illness characterized by watery diarrhea (sometimes bloody), emesis, and rapid onset of dehydration. Other strains associated with diarrhea following seafood consumption are *V. mimicus, V. hollisae*, and *V. vulnificus*. *V. fluvialis* is another strain associated with diarrhea but not necessarily seafood consumption. Infections due to *V. alginolyticus* are typically extra intestinal (wounds, ears) and not associated with diarrhea

Antimicrobial resistance is less common in *Vibrio* than in *Enterobacteriaceae* but can be acquired through plasmid transfer or through exposure to antimicrobials. Most *Vibrio* infections are self-limiting. Rehydration is imperative. Refer to the antimicrobial susceptibilities to identify the most appropriate therapy.

Cultured Yeast

Small amounts of yeast (+1) may be present in a healthy GI tract. However higher levels of yeast (> +1) are considered to be dysbiotic. A positive yeast culture and sensitivity to prescriptive and natural agents may help guide decisions regarding potential therapeutic intervention for yeast overgrowth. When investigating the presence of yeast, disparity may exist between culturing and microscopic examination. Yeast grows in colonies and is typically not uniformly dispersed throughout the stool. Further, some yeast may not survive transit through the intestines rendering it unviable for culturing. This may lead to undetectable or low levels of yeast identified by culture, despite a significant amount of yeast visualized microscopically. Therefore, both microscopic examination and culture are helpful in determining if abnormally high levels of yeast are present.

Dysbiotic Yeast

Yeast was cultured from this stool specimen at a level that is considered to be dysbiotic. A positive yeast culture and sensitivity to prescriptive and natural agents may help guide decisions regarding potential therapeutic intervention for chronic yeast syndrome. When investigating the presence of yeast, disparity may exist between culturing and microscopic examination. Yeast grows in colonies and is typically not uniformly dispersed throughout the stool. This may lead to undetectable or low levels of yeast identified by culture, despite a significant amount of yeast visualized microscopically.

Exophiala spp

Exophiala is a genus of saprophytic fungi; they are sometimes referred to as "black yeasts". Saprophytic fungi grow upon decaying organic matter, including body tissues. Species of *Exophiala* include *E. oligosperma, E. jeanselmei, E. lecanii-corni, E. bergeri, E. cancerae, E. dermatitidis, E. hongkongensis* and *E. xenobiotica*.

A European study recovered *E. dermatitidis* from stool with a frequency of 5.2% in the sample population. The majority of donors positive for *E. dermatitidis* reported diarrhea during the stool collection period. *Exophiala* may be found in wastewater, dishwashers, steam baths and bathrooms. Colonization of the gastrointestinal tract by *E. cancerae* and *E. dermatitidis* has been reported. Most patients with *Exophiala* present with nail, skin or subcutaneous infections. The fungi may cause systemic infections in immunocompromised patients. There is a reported pulmonary infection by *E. dermatitidis* in a multiple myeloma patient, and a high rate of *E. dermatitidis* recovery from the airways of cystic fibrosis patients. An *E. dermatitidis* infection of the bile ducts has also been reported in an immunocompetent patient.

Antifungal therapy may be clinically indicated if symptoms are present or persistent. Refer to the antimicrobial susceptibilities to identify the most appropriate therapy.

Stool Chemistries

Elastase

Elastase is a pancreatic enzyme that digests and degrades a number of proteins. A finding of low elastase in a formed stool specimen is an indicator of pancreatic exocrine insufficiency. Moderate pancreatic insufficiency is defined at 100-200 µg/mL, and severe pancreatic insufficiency as <100 µg/mL. Fecal elastase can be artefactually low due to fluid dilution effects in a loose/watery stool sample. Check the reported consistency of the stool specimen.

Fecal elastase measured by a sensitive immunoassay is a specific marker for pancreatic function and maintains a high diagnostic accuracy among patients with small intestinal diseases. This elastase marker allows for the diagnosis or exclusion of pancreatic exocrine insufficiency and degree of severity, which can be caused by chronic pancreatitis, cystic fibrosis, pancreatic tumor, cholelithiasis or diabetes mellitus. This test does not differentiate between pancreatic insufficiency due to chronic pancreatitis and that due to pancreatic cancer. Immunoreactive elastase concentrations are similar for children and adults.

In cases of severe exocrine pancreatic insufficiencies, fecal fat stain may also be elevated. Supplementation with pancreatic enzymes, minerals, and vitamins may be warranted.

Fat Stain

The amount of total fat is higher than normal in this specimen. Individuals who have pancreatic insufficiency secondary to pancreatic or biliary tract disease may be unable to efficiently digest and absorb fat normally. The microscopic fecal fat test is a reliable marker for fat malabsorption, and evaluation enzyme therapy in patients with pancreatic exocrine insufficiency. When assessing the root cause of fat malabsorption the following should be considered: dietary fat intake, gastric surgery, pancreatic disease, biliary obstruction, liver disease, mucosal integrity, and problems with chylomicron formation. Supplementation with pancreatic enzymes, HCL, and/or bile salts may be indicated. Steatorrhea is associated with a particularly foul odor of the stool.

Carbohydrates

The level of carbohydrates in this specimen is abnormal and represents the presence of sugars in the stool. Simple sugars are absorbed in the small intestine and should not be appreciably present in the colon because they are a primary energy source for pathogenic or dysbiotic bacteria and yeast. Dietary carbohydrates are broken down by salivary and pancreatic enzymes. Enzyme insufficiency is typically associated with flattening or disruption of the intestinal brush border cells as occurs with gastrointestinal infections (including viruses), Celiac or Crohn's diseases or short bowel syndrome. Rapid transit through the bowels is commonly associated with elevated levels of sugar in the stool.

Lactoferrin

The level of fecal lactoferrin is elevated in this sample. Lactoferrin is a biomarker of serious gastrointestinal inflammation which may be associated is with inflammatory bowel disease (IBD) such as Ulcerative colitis (UC) or Crohn's disease (CD), but NOT Irritable bowel syndrome (IBS). Such distinction is critical because, although both IBD and IBS may share some common symptoms such as diarrhea, abdominal cramping and weight loss, the diseases are treated quite differently. IBD may become life threatening, requires lifelong treatment and possibly surgery. Very elevated lactoferrin should be reassessed in about four weeks, and if confirmed referral to a gastroenterologists should be considered. Lactoferrin is commonly high in breast-fed infants due to the high content in breast milk.

Patients with IBD oscillate between active and inactive disease states, and fecal lactoferrin levels increase 2-3 weeks prior to onset of clinical symptoms. During remission and effective treatment, fecal lactoferrin decreases significantly. Therefore disease activity and efficacy of treatment can be monitored by following fecal lactoferrin levels. The test can be ordered separately to track disease activity in patients with IBD.

Moderately elevated levels of fecal lactoferrin can occur, often with fecal red and/or white blood cells, in association with invasive enteropathogens. Therefore, with moderately elevated levels of fecal lactoferrin, one should check for the presence of enteropathogens (eg. *Shigella*, *Campylobacter*, *Vibrio cholerae*, *Yersinia*).

Lysozyme

The level of lysozyme is elevated in this sample. Lysozyme is a biomarker of an inflammatory immune response in the gut. Moderate elevations in lysozyme are commonly associated with significant

overgrowth of enteropathogens such as yeast, dysbiotic or pathogenic bacteria. Markedly elevated levels of lysozyme may occur with inflammatory bowel disease (IBD), such as Crohn's disease and Ulcerative colitis as well as other non-IBD intestinal diseases with diarrhea. If lysozyme is markedly elevated check the levels of calprotectin and lactoferrin. If either or both are very elevated reassess the levels in about four weeks. Lysozyme is commonly elevated for actively breast feeding infants due to high maternal milk content.

Lysozyme is helpful in the determination of pathogen-induced inflammatory activity rather than IBD. Slightly-to moderately elevated levels of lysozyme may be remediated with elimination of an offending enteroinvasive microorganism and use of anti-inflammatory nutraceuticals.

Calprotectin (Very high)

The level of calprotectin is highly elevated in this specimen. Very high levels of calprotectin are associated with active IBD and gastrointestinal inflammation, colitis (not autoimmune), or sometimes cancer. Elevated fecal calprotectin levels indicate inflammation in the gastrointestinal mucosa. High levels of calprotectin have been highly correlated with inflammatory bowel disease (IBD). IBD includes autoimmune conditions such as Crohn's disease and ulcerative colitis (UC); these conditions may become life-threatening and require lifelong treatment.

Chronic inflammation of the gastrointestinal mucosa contributes to symptoms of IBD. Chronic stress is also known to contribute to symptom flare-ups and increased inflammation in IBD patients. Liver disease or the use of aspirin or nonsteroidal anti-inflammatory (NSAID) medications may variably elevate calprotectin levels.

Fecal Calprotectin should be reassessed after about 4 weeks for confirmation. A confirmatory finding warrants referral to a gastroenterologist for scoping.

Calprotectin (Moderately high)

The level of calprotectin is moderately elevated in this specimen. Moderate levels of calprotectin are an indicator of chronic inflammation. Inflammation at this level may be due to IBD in remission or inflammation caused by non-steroidal anti-inflammatories (NSAIDs).

Chronic inflammation of the gastrointestinal mucosa contributes to symptoms of IBD. Chronic stress is also known to contribute to symptom flare-ups and increased inflammation in IBD patients. Liver disease or the use of aspirin or nonsteroidal anti-inflammatory (NSAID) medications may variably elevate calprotectin levels.

Levels should be reassessed after about 4 weeks.

Calprotectin (Low)

The level of calprotectin is low in this specimen. Low levels of calprotectin may be normal or associated with a viral GI infections or non-inflammatory bowel conditions such as IBS.

Secretory IgA (sIgA) High

The concentration of sIgA is abnormally high in this fecal specimen. Secretory IgA represents the first line of defense of the gastrointestinal (GI) mucosa and is central to the normal function of the GI tract as an immune barrier. Elevated fecal sIgA is an appropriate response to antigens such as pathogenic bacteria, parasites, yeast, and viruses. Eradication of the pathogenic microorganisms will bring sIgA back down into the normal range. sIgA may remain elevated up to six weeks after a GI viral infection. Elevated fecal sIgA may also be associated with autoinflammatory conditions such as reactive arthritis and spondyloarthritis. Actively breast-feeding infants may exhibit high fecal sIgA due to high maternal milk content. Consumption of bovine colostrum does not artificially increase fecal sIgA because the assay is specific for human sIgA.

Secretory IgA (sIgA) Low

The concentration of sIgA is abnormally low in this fecal specimen. Secretory IgA represents the first line of defense of the gastrointestinal (GI) mucosa and is central to the normal function of the GI tract as an immune barrier. Immunological activity in the gastrointestinal tract can be accessed via fecal sIgA levels in a formed stool sample. However, sIgA may be artefactually low due to fluid dilution effects in a watery or loose/watery stool sample.

Chronic mental and physical stress as well as inadequate nutrition have been associated with low fecal sIgA concentrations. This includes dietary restrictions, excessive alcohol intake, body mass loss, negative moods, and anxiety. One study found decreased levels of sIgA in malnourished children, particularly protein malnourishment, which responded well to nutritional rehabilitation with a significant increase in sIgA. A possible explanation for this may be the synthesis and expression of sIgA requires adequate

intake of the amino acid L-glutamine. An increase of dietary L-glutamine may restore GI immune function by protection of cells that synthesize sIgA. *Saccharomyces boulardii* is a nonpathogenic yeast that has been used for the treatment of acute infectious enteritis and antibiotic-associated diarrhea. Restored levels of sIgA and subsequent enhanced host immune response have been found following *S. boulardii* administration (animal models). With low sIgA one might consider a salivary cortisol test.

Short Chain Fatty Acids (SCFAs)

The total concentration and/or percentage distribution of the primary short chain fatty acids (SCFAs) are abnormal in this specimen. Beneficial bacteria that ferment non-digestible soluble fiber produce SCFAs that are pivotal in the regulation of intestinal health and function. Restoration of microbial abundance and diversity, and adequate daily consumption of soluble fiber and polyphenols can improve SCFA status.

The primary SCFAs butyrate, propionate and acetate are produced by predominant commensal bacteria via fermentation of soluble dietary fiber and intestinal mucus glycans. Key producers of SCFAs include *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Bacteroides fragilis*, *Bifidobacterium*, *Clostridium* and *Lactobacillus* spp. The SCFAs provide energy for intestinal cells, and regulate the actions of specialized mucosal cells that produce anti-inflammatory and antimicrobial factors, mucins that constitute the mucus barriers, and gut active peptides that facilitate appetite regulation and euglycemia. The SCFAs also contribute to a more acidic and anaerobic microenvironment that disfavors dysbiotic bacteria and yeast. Abnormal SCFAs may be associated with dysbiosis (including insufficiency dysbiosis), compromised intestinal barrier function (intestinal permeability) and inappropriate immune and inflammatory conditions.

"Seeding" with supplemental probiotics may contribute to improved production and status of SCFAs, but it is imperative to "feed" the beneficial microbes. Sources of soluble fiber that are available to the microbes include chick peas, beans, lentils, oat and rice bran, fructo- and galacto- oligosaccharides, and inulin.

pH high

The pH of this stool sample was higher than expected (>7.8, alkaline). Ideally, the colonic (stool) pH stool is slightly acidic. Predominant beneficial bacteria normally produce large amounts of short chain fatty acids (butyrate, acetate, propionate), which contribute to lower colonic pH. Check short chain fatty acid status (SCFA).

Many GI pathogens, including bacteria and yeast, thrive in an alkaline pH. When fecal pH is more towards the alkaline end of the reference range, it is not uncommon to find low levels of the predominant commensal/beneficial bacteria. There may also possibly be an increase in the imbalanced flora, dysbiotic bacteria and/or yeast present. Insufficient daily intake of fermentable soluble fiber is often associated with insufficiency dysbiosis, low absolute levels of butyrate and total SCFAs and high pH.

pH low

The pH of this stool sample is more acidic (<6.0) than expected. The pH of the stool, reflective of colonic pH, is normally slightly acidic. An acidic pH is commonly associated with rapid transit time, e.g. diarrhea or loose stools, more than three bowel movements per day. Check stool consistency. Further investigation of the cause of rapid transit such as food intolerance, and viral, bacterial, parasitic infection, may be warranted. An acidic pH is common in individuals with lactose malabsorption/intolerance. Unabsorbed lactose in the gut can be hydrolyzed by colonic bacteria forming volatile fatty acids which cause the stool to become acidic, often times accompanied by a sweet, sickly stool odor.

β-glucuronidase

β-glucuronidase (β-G) is an enzyme that breaks the tight bond between glucuronic acid and toxins in the intestines. The liver and intestine bind toxins, steroid hormones and some dietary components to glucuronic acid. That is a protective process that limits absorption and enterohepatic resorption of toxins, and enhances excretion. A high level of activity of β-G in the gut is not desirable. A low level of β-G activity is not known to be of any direct clinical consequence.

β -glucuronidase is produced by the intestinal epithelium and many species of intestinal bacteria. Observational studies have indicated a correlation between high β-G activity and certain cancers, but a definitive causal relationship has not been established. Higher levels of β-G have been associated with higher circulating estrogens and lower fecal excretion of estrogens in premenopausal women. A potential dietary carcinogen derived from grilled/smoked meat and fish induces high β-G activity and prolongs internal exposure to the toxin in an experimental animal model.

Diet and intestinal bacterial imbalance modulate β-G activity. High fat, high protein and low fiber diets are associated with higher β-G activity compared to vegetarian or high soluble fiber diets. Higher β-G may be associated with an imbalanced intestinal microbiota profile. Some major bacterial producers of fecal β-G include *Bifidobacterium*, *Lactobacillus*, *Escherichia coli*, *Clostridium*, *Bacteroides fragilis* and other *Bacteroides* spp., *Ruminococcus gnavus*, and species that belong to the genera Staphylococcus *and* Eubacterium.

Low β-G activity is an indicator of abnormal metabolic activity among the intestinal microbiota that may be influenced by dietary extremes, diminished abundance and diversity of the intestinal microbiota, or heavy probiotic and/or prebiotic supplementation. A low fat, low meat and high fiber diet, such as consumed by strict vegetarians, may be benignly associated with lower β-G activity compared to a typical "Western diet." High-end consumption of soluble fiber (e.g. inulin) and supplementation with *Lactobacillus acidophilus* may be inconsequentially associated with lower fecal β-G.

Occult Blood

Occult blood was detected in this specimen. In many cases, a positive occult blood results from gastrointestinal bleeding from the upper small intestine or higher. Gastric ulceration could, for example, result in a finding of occult blood rather than blood in the feces. However, a positive finding of occult blood may also be associated with colon cancer, ulcerative colitis (check fecal calprotectin and lactoferrin levels), ulceration of the esophagus, stomach or duodenum, diverticulitis, and gastric carcinoma. Positive occult blood findings require confirmation and further investigation may be indicated.

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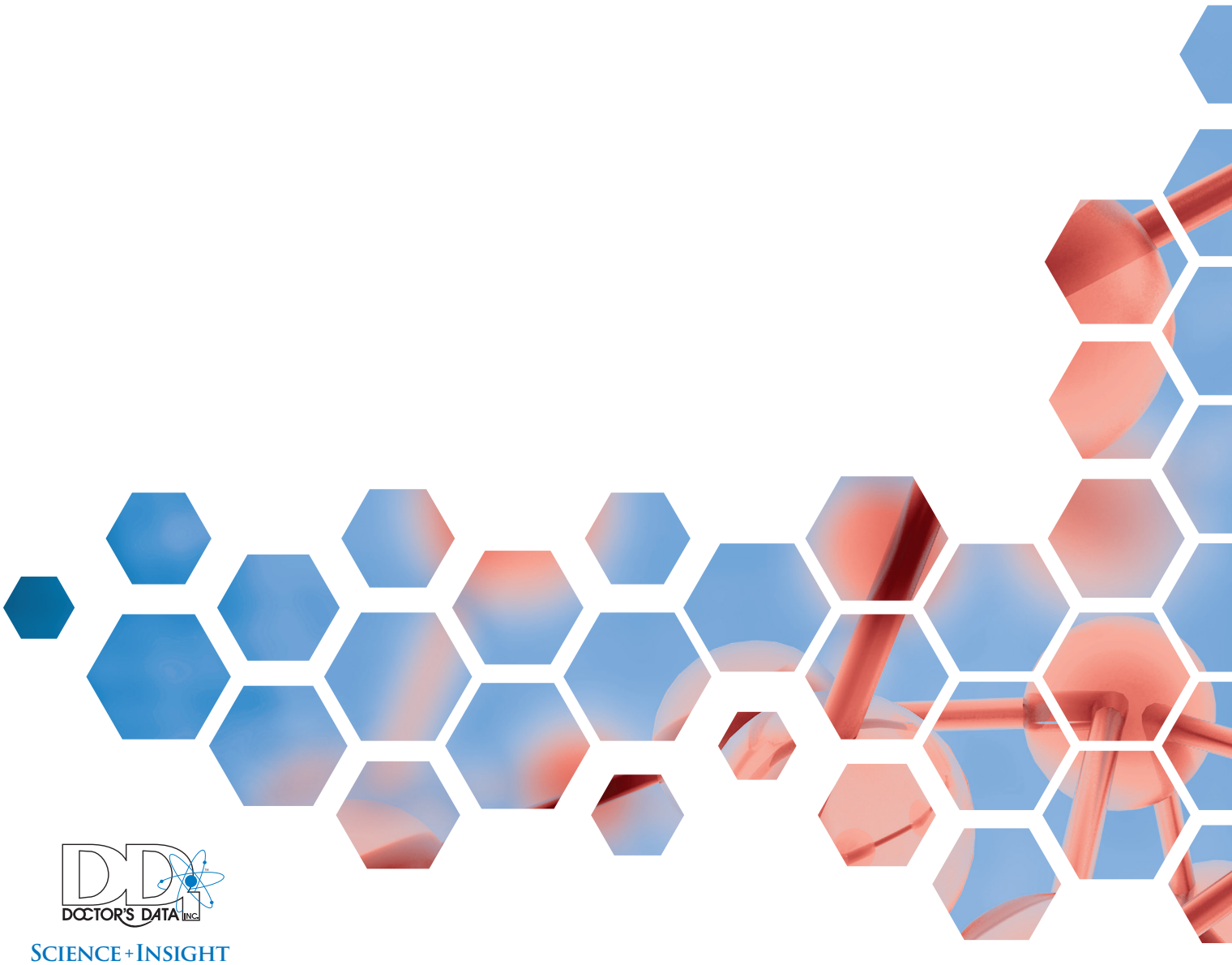

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