GI360™; stool

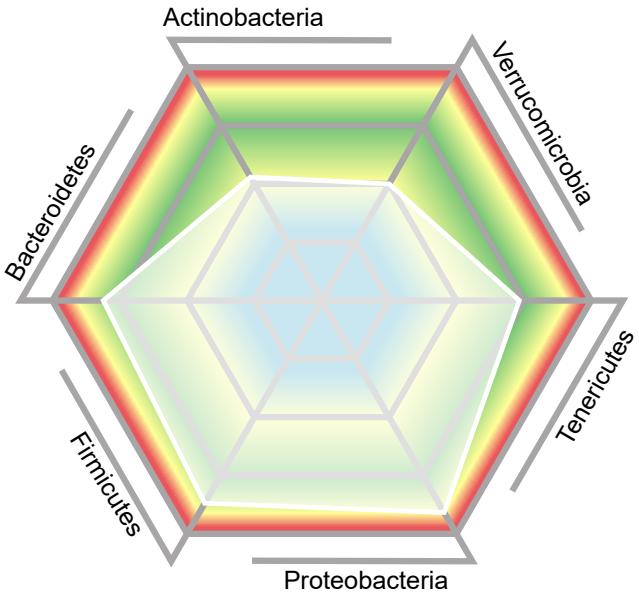
Order: 230407-0295
Client #: 31417
Rn Labs
18/93 Rivergate Pl
Murarrie, Qld, 4172 Australia

Patient: Mackenzie Jane Trivett
Id: P230970219
Age: 22 **DOB:** 11/06/2000
Sex: Female

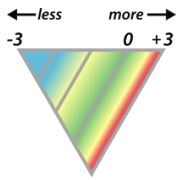
Sample Collection	Date/Time
Date Collected	04/02/2023
Date Received	04/07/2023
Date Reported	04/18/2023
Specimens Collected	3

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



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.

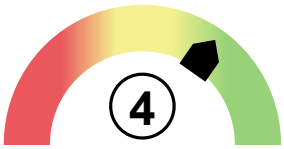
Dysbiosis and Diversity Index

These indexes are calculated from the results of the Microbiome Profile, with scores ranging from 1 to 5, and do not include consideration of dysbiotic and pathogenic bacteria, yeast, parasites and viruses that may be reported in subsequent sections of the GI360™ test.

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.

A diversity score of 3 indicates an expected amount of diversity, with 4 & 5 indicating an increased distribution of bacteria based on the number of different species and their abundance in the sample, calculated based on Shannon's diversity index. Scores of 1 or 2 indicate less diversity than the defined normobiotic reference population.

Dysbiosis Index



Diversity Score

Key Findings

Butyrate producing bacteria	●	*Blastocystis* spp., Observed
Gut barrier protective bacteria	●	Secretory IgA, Very Low
Gut intestinal health marker	●	β-glucuronidase, High
Pro-inflammatory bacteria	●	*Citrobacter freundii* complex, Cultured
Gut barrier protective bacteria vs. opportunistic bacteria	●	*Citrobacter freundii* complex,isolate 2, Cultured
		Klebsiella pneumoniae, Cultured

● = Expected ● = Imbalanced

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Microbiome Bacterial Abundance;Multiplex PCR

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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	0	0
Bifidobacterium family	-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	0	0
Catenibacterium mitsuokai	0	0

Notes:
The gray-shaded area of the bar graph represents reference values outside the reporting limits for this test.
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Methodology: Multiplex PCR

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Microbiome Bacterial Abundance;Multiplex PCR

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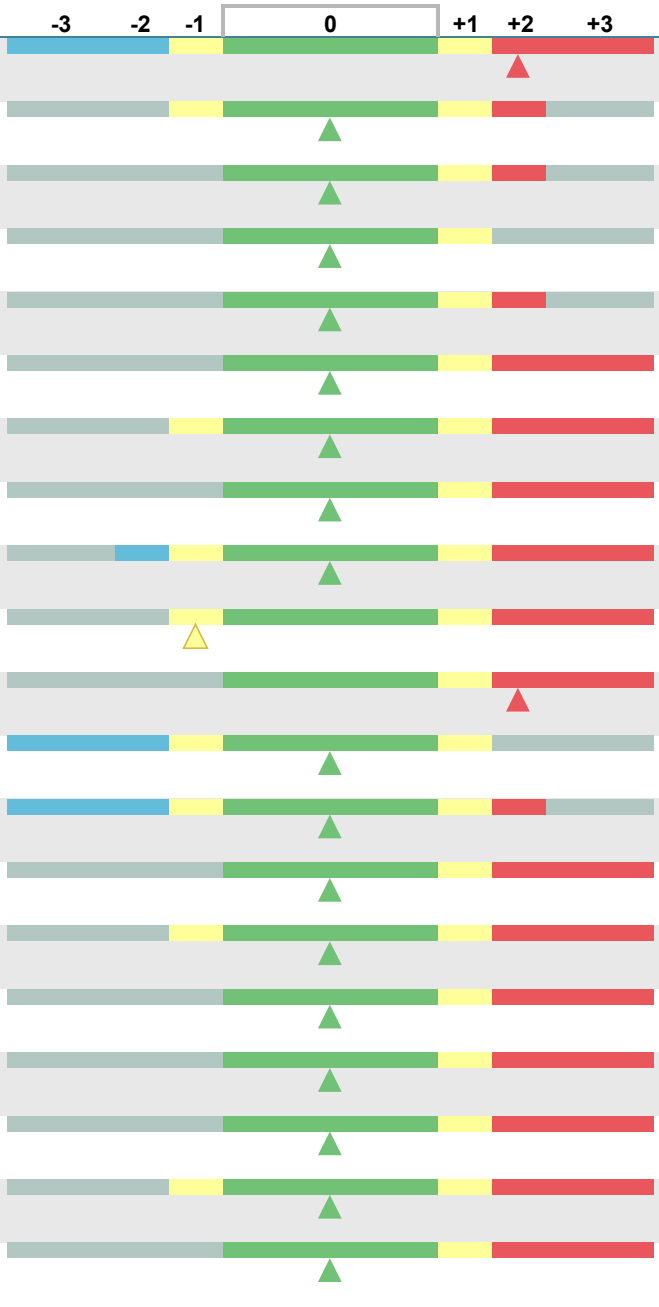
Firmicutes	Result	Reference Interval
Clostridia Class	+2	0
Clostridium methylpentosum	0	0
Clostridium L2-50	0	0
Coprobacillus cateniformis	0	0
Dialister invisus	0	0
Dialister invisus & Megasphaera micronuciformis	0	0
Dorea spp.	0	0
Eubacterium biforme	0	0
Eubacterium hallii	0	0
Eubacterium rectale	-1	0
Eubacterium siraeum	+2	0
Faecalibacterium prausnitzii	0	0
Lachnospiraceae	0	0
Lactobacillus ruminis & Pediococcus acidilactici	0	0
Lactobacillus family	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



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

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

Tenericutes	Result	Reference Interval
Mycoplasma hominis	0	0

Verrucomicrobia	Result	Reference Interval
Akkermansia muciniphila	-1	0

Microbiome Abundance Information:

- 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.

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Methodology: Multiplex PCR

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GI Pathogens;Multiplex PCR

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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)	Negative
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

Notes:
Methodology: Multiplex PCR

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Parasitology; Microscopy

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Protozoa	Result
Balantidium coli	Not Detected
Blastocystis spp.	Moderate
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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Parasitology; Microscopy

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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	Not Detected	Not Detected – Rare
RBC	Not Detected	Not Detected – Rare
WBC	Not Detected	Not Detected – Rare
Muscle fibers	Not Detected	Not Detected – Rare
Vegetable fibers	Rare	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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Parasitology; Microscopy

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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

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

Dysbiotic Bacteria	Result	NG 1+ 2+ 3+ 4+	Reference Interval
Citrobacter freundii complex	4+		No Growth
Citrobacter freundii complex,isolate 2	3+		No Growth
Klebsiella pneumoniae	3+		No Growth

Yeast	Result	NG 1+ 2+ 3+ 4+	Reference Interval
No yeast isolated	NG		

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.
- **Dysbiotic bacteria** consist of those bacteria that have the potential to cause disease in the GI tract. They can be present due to a number of factors including: exposure to chemicals that are toxic to beneficial bacteria; the use of antibiotics, oral contraceptives or other medications; poor fiber intake and high stress 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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Stool Chemistries

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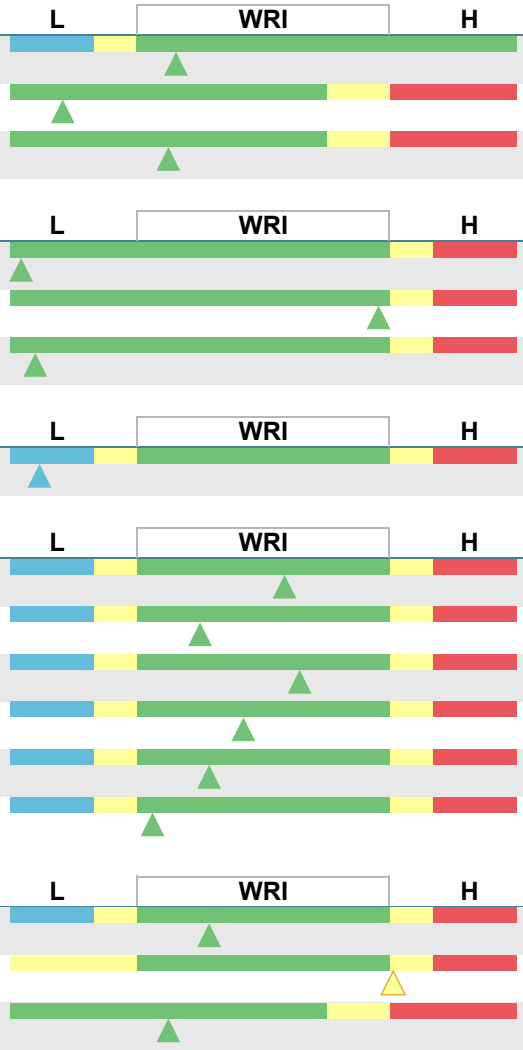
Digestion / Absorption	Result	Unit	Reference Interval
Elastase	231	µg/g	> 200
Fat Stain	None		None – Moderate
Carbohydrates†	Negative		Negative

Inflammation	Result	Unit	Reference Interval
Lactoferrin	0.7	µg/mL	< 7.3
Lysozyme*	485	ng/mL	≤ 500
Calprotectin	<10	µg/g	< 80

Immunology	Result	Unit	Reference Interval
Secretory IgA*	7.0	mg/dL	30 – 275

Short Chain Fatty Acids	Result	Unit	Reference Interval
% Acetate‡	62	%	50 – 72
% Propionate‡	14	%	11 – 25
% Butyrate‡	22	%	11 – 32
% Valerate‡	2.4	%	0.8 – 5.0
Butyrate‡	1.2	mg/mL	0.8 – 4.0
Total SCFA's‡	5.5	mg/mL	5.0 – 16.0

Intestinal Health Markers	Result	Unit	Reference Interval
pH	6.2		5.8 – 7.0
β-glucuronidase*	9680	U/h*g	4000 – 9400
Occult Blood	Negative		Negative



Chemistry Information:

- **Elastase** findings can be used for assessing pancreatic exocrine function and insufficiency.

Notes:
SIGA confirmed by repeat analysis.
RI= Reference Interval, L (blue)= Low (below RI), WRI (green)= within RI, Yellow= moderately outside RI, L or H, H (red)= High (above RI)
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†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: Turbidimetric immunoassay, Microscopy, Colormetric, Elisa, Gas Chromotography, ph Electrode, Enzymatic, Guaiac

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Stool Chemistries

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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.

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Bacterial Susceptibilities

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Citrobacter freundii complex

Natural Agents	Low Sensitivity	High Sensitivity
Berberine*		
Black Walnut*		
Caprylic Acid*		
Uva Ursi*		
Oregano*		
Grapefruit Seed Extract*		
Silver*		

Prescriptive Agents	Resistant	Intermediate	Susceptible
Amoxicillin-Clavulanic Acid	●		
Ampicillin	●		
Cefazolin	●		
Ceftazidime			●
Ciprofloxacin			●
Sulfamethoxazole / Trimethoprim			●

Susceptibility Information:

- **Natural antibacterial** agents may be useful for treatment of patients when organisms display in-vitro sensitivity to these agents. The test is performed by using standardized techniques and filter paper disks impregnated with the listed agent. Relative sensitivity is reported for each natural agent based upon the diameter of the zone of inhibition 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 sensitivity is defined for the natural agents tested.
- **Susceptible** results imply that an infection due to the bacteria may be appropriately treated when the recommended dosage of the tested antimicrobial agent is used. **Intermediate** results imply that response rates may be lower than for susceptible bacteria when the tested antimicrobial agent is used. **Resistant** results imply that the bacteria will not be inhibited by normal dosage levels of the tested antimicrobial agent.

Notes:
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Bacterial Susceptibilities

Order: 230407-0295
Client #: 31417
Rn Labs
18/93 Rivergate Pl
Murarrie, Qld, 4172 Australia

Patient: Mackenzie Jane Trivett
Id: P230970219
Age: 22 **DOB:** 11/06/2000
Sex: Female

Sample Collection	Date/Time
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Date Reported	04/18/2023
Specimens Collected	3

Citrobacter freundii complex,isolate 2

Natural Agents	Low Sensitivity	High Sensitivity
Berberine*		
Black Walnut*		
Caprylic Acid*		
Uva Ursi*		
Oregano*		
Grapefruit Seed Extract*		
Silver*		

Prescriptive Agents	Resistant	Intermediate	Susceptible
Amoxicillin-Clavulanic Acid	●		
Ampicillin	●		
Cefazolin	●		
Ceftazidime			●
Ciprofloxacin			●
Sulfamethoxazole / Trimethoprim			●

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Klebsiella pneumoniae

Natural Agents	Low Sensitivity	High Sensitivity
Berberine*	▲	
Black Walnut*	▲	
Caprylic Acid*	▲	
Uva Ursi*		▲
Oregano*	△	
Grapefruit Seed Extract*		▲
Silver*		▲

Prescriptive Agents	Resistant	Intermediate	Susceptible
Amoxicillin-Clavulanic Acid			●
Ampicillin	●		
Cefazolin			●
Ceftazidime			●
Ciprofloxacin			●
Sulfamethoxazole / Trimethoprim			●

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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.

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. 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.

Bacteroidetes (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 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.

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.

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.

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Microbiome Abundance Information continued...

***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.

***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.

***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.

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.

***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.

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.

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).

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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.

Parasitology

Parasites

Parasites were detected by microscopic examination in this stool specimen. Intestinal parasites are abnormal inhabitants of the GI tract that live off and have the potential to cause damage to their host. Factors such as contaminated food and water supplies, day care centers, increased international travel, pets, carriers such as mosquitoes and fleas, and sexual transmission have contributed to an increased prevalence of intestinal parasites.

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 and 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, decreased immune function, and fatigue.

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.

Microbiology

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.

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Microbiology continued...

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.

***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.

***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.

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Stool Chemistries

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.

β-glucuronidase

A high level of fecal β-glucuronidase (β-G) may promote enterohepatic reabsorption of toxins and steroid hormones that the body has conjugated to glucuronic acid for elimination via the biliary-fecal route of excretion. B-glucuronidase is an enzyme, produced by the intestinal epithelium and certain intestinal bacteria, which breaks down the tight bond between glucuronic acid and many different chemical entities. 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 cooked meat and fish induces high β-G and prolongs internal exposure to the toxin in an animal model.

Diet and intestinal bacterial imbalance modulate β-G. High fat, high protein and low fiber diets are associated with higher β-G 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* species, *Ruminococcus gnavus*, and species that belong to the genera *Staphylococcus* and *Eubacterium*.

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