GI360 INTERPRETATION REPORT

THE 'BIRD'S EYE VIEW'

A 'GA Map' of the 6 key microbiome phyla.

(The white region indicates the patient's result and degree of balance across overall populations)

GI360™; stool

DOCTOR'S DATA INC

Order: 999999-9999
Client #: 999999
Doctor: Sample Doctor
Doctors Data Inc
123 Main St.
St. Charles, IL 60174 USA

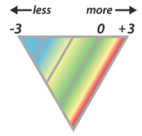
Patient: Sample Patient
Id: 999999
Age: 56
Sex: Female

Sample Collection	Date/Time
Date Collected	01/30/2020
Date Received	01/31/2020
Date Reported	02/01/2020
Specimens Collected	3

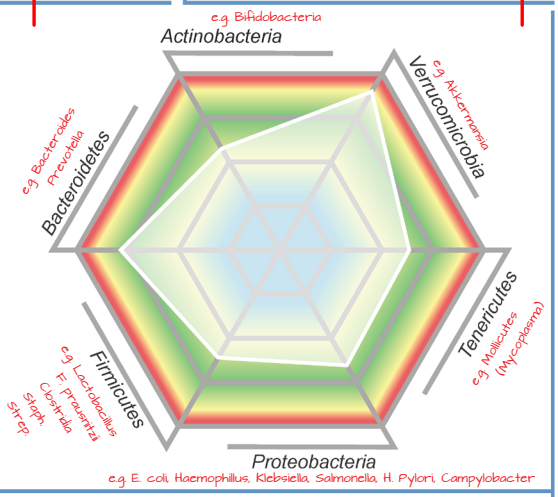
Microbiome Abundance and Diversity Summary

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 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

4

A standardised and evidence-based rating summarising degree of dysbiosis.

1-2 = normal/ healthy

3 = moderate dysbiosis

4-5 = high dysbiosis/disease risk

Key Findings

Eubacterium siraeum, Very Low	↓	Vegetable fibers, Abnormal	
Faecalibacterium prausnitzii, Very Low	↓	*Enterobacter cloacae* complex, Cultured	
Phascolarctobacterium spp., Very High	↑		
Actinobacteria, Low	↓		
Alistipes onderdonkii, Low	↓		
Bacteroides zoogleoformans, High	↑		
Bacilli Class, Low	↓		
Akkermansia muciniphila, High	↑		

A summary of all key findings in the report.

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After reviewing the overall balance of populations (on the first page) as the key clinical insight, these remaining 3 pages provide further details as to the levels of specific families and notable species (for potential further exploration and treatment opportunities).

GI 360 Microbiome Bacterial Abundance;Multiplex PCR

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Quantification via qPCR 'signal strength' is represented here as standard deviations (NOT CFU) from the healthy normobiotic population.

Certain markers provide quantification of:

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	-2	-1 to +1
Actinomycetales	0	0 to +1
Bifidobacterium spp.	-1	-1 to +1
Bacteroidetes	**Result**	**Reference Interval**
Alistipes spp.	0	-1 to +1
Alistipes onderdonkii	-2	-1 to +1
Bacteroides fragilis	0	0 to +1
Bacteroides spp. & *Prevotella* spp.	+1	-1 to +1
Bacteroides stercoris	0	0 to +1
Bacteroides zoogleoformans	+2	0 to +1
Parabacteroides johnsonii	0	0 to +1
Parabacteroides spp.	0	-1 to +1
Firmicutes	**Result**	**Reference Interval**
Firmicutes	0	-1 to +1
Bacilli Class	-2	-1 to +1
Catenibacterium mitsuokai	0	-1 to +1
Clostridia Class	-1	-1 to +1
Clostridium L2-50	0	0 to +1

Overall phyla

Genus level

Specific species

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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Note the lab methodology used (on each page)

Microbiome Bacterial Abundance;Multiplex PCR

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Firmicutes	Result	-3 -2 -1 0 +1 +2 +3	Reference Interval
Species — *Dialister invisus*	0		0 to +1
Species — *Dialister invisus & Megasphaera micronuciformis*	0		0 to +1
Genus — *Dorea* spp.	0		0 to +1
Eubacterium biforme	0		0 to +1
Species — *Eubacterium hallii*	0		-1 to +1
Species — *Eubacterium rectale*	0		0 to +1
Species — *Eubacterium siraeum*	-3		-1 to +1
Species — *Faecalibacterium prausnitzii*	-3		-1 to +1
Family — Lachnospiraceae	-1		-1 to +1
Species — *Lactobacillus ruminis & Pediococcus acidilactici*	0		0 to +1
Genus — *Lactobacillus* spp.	0		0 to +1
Genus — *Phascolarctobacterium* spp.	+3		0 to +1
Ruminococcus albus & R. bromii	0		0 to +1
Species — *Ruminococcus gnavus*	0		0 to +1
Streptococcus agalactiae & Eubacterium rectale	0		0 to +1
Subspecies — *Streptococcus salivarius* ssp. *thermophilus & S. sanguinis*	0		0 to +1
Subspecies — *Streptococcus salivarius* ssp. *thermophilus*	-1		-1 to +1
Genus — *Streptococcus* spp.	0		0 to +1
Genus — *Veillonella* spp.	-1		-1 to +1

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Methodology: Multiplex PCR

Continuation of PCR GA Map technology

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Microbiome Bacterial Abundance;Multiplex PCR

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	Result	-3 -2 -1 0 +1 +2 +3	Reference Interval
Proteobacteria	**Result**		**Reference Interval**
Phyla — *Proteobacteria*	0		0 to +1
Genus — *Escherichia* spp.	-1		-1 to +1
Tenericutes	**Result**		**Reference Interval**
Species — *Mycoplasma hominis*	-1		-1 to +1
Verrucomicrobia	**Result**		**Reference Interval**
Species — *Akkermansia muciniphila*	+2		0 to +1

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.

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Continuation of PCR GA Map technology

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
Clostridium 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

Note: The species will also be tested for via the MALDI-TOF and parasitology methods employed by the rest of the test. (providing considerable overlap of technologies for thorough and conclusive analysis).

Notes:
Methodology: Multiplex PCR

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Note: This 'FilmArray' type PCR is intended for prompt and accurate treatment of diarrhoeal illnesses, which may improve patient outcomes (however most are acute and self-limiting, and would need to be active at the time of stool collection to appear positive here)

*Tested for hundreds of potential parasites at multiple stages of their life-cycles i.e. O&P = ova & parasite stages.

*Consider the appropriate eradication strategy for any specific organisms detected in any of the samples tested.

GI 360 Parasitology; Microscopy

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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 - Round Worms	Result
Ascaris lumbricoides	Not Detected

Notes:
Methodology: Microscopy

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3 samples provides sufficient avoidance of false negatives in any one sample (for thorough parasite investigation)

NOTE: This is a summary list of key pathogens (analysis will investigate far more, and add to report if present)

Full ova & parasite analysis by trained parasitologist for 'Gold Standard' detection of parasites.

Parasitology; Microscopy

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

* These are key suspects but far more are tested for and will appear on report if detected.

Other Markers	Result	Reference Interval
Yeast	Few	Not Detected – Rare
RBC	Not Detected	Not Detected – Rare
WBC	Not Detected	Not Detected – Rare
Muscle fibers	Not Detected	Not Detected – Rare
Vegetable fibers	Moderate	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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The detection of dead yeasts (not culturable elsewhere on the test) is achieved here

(assess diet, immunity, stress and sIgA levels for correlation with this).

Animal Proteins

Fibre

Components of Eosinophils (elevations indicative of parasitic infection being fought by immune system or food allergic conditions such as eosinophilic gastritis or oesophagitis)

Possibly from honey, plant foods, propolis supplements etc. however may be mistaken for certain parasite egg stages (so simply confirms differentiation)

Nematodes detected via same rigorous methodology as previous page.

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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* Pathogenic species most likely to warrant treatment.

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

Imbalance Bacteria	Result	NG 1+ 2+ 3+ 4+	Reference Interval
Beta hemolytic strep, group B	2+		No Growth
Citrobacter freundii complex	1+		No Growth
Comamonas jiangduensis	3+		No Growth
Gamma hemolytic strep	2+		No Growth

Dysbiotic Bacteria	Result	NG 1+ 2+ 3+ 4+	Reference Interval
Enterobacter cloacae complex	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.
- **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.
- **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

MALDI-TOF

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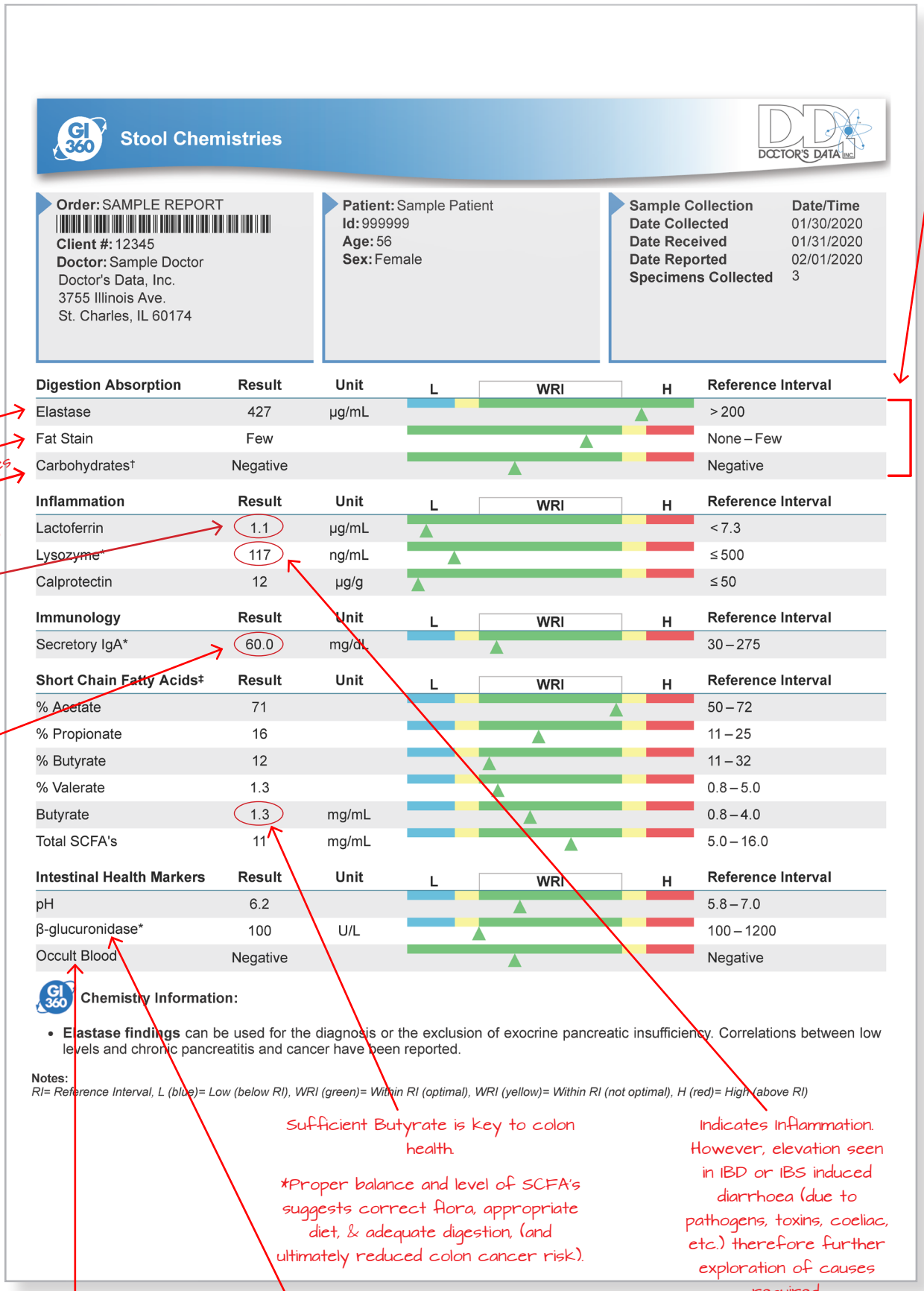
NOTE: Only key suspects listed, however 1000's tested for via advanced MALDI-TOF techniques (any non standard species detected will also be added to this list on certain reports)

Analysed by state of the art proteomic analysis (for accurate detection of specific pathogenic species beyond that of DNA methods e.g. including yeasts
Note: This method allows susceptibility testing for forming an eradication strategy.

*Note: Cross-reference each of these markers with the degree of intakes of each in the diet prior to stool collection.



Stool Chemistries

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Digestion Absorption	Result	Unit	L	WRI	H	Reference Interval
Elastase	427	µg/mL				> 200
Fat Stain	Few					None – Few
Carbohydrates†	Negative					Negative

Inflammation	Result	Unit	L	WRI	H	Reference Interval
Lactoferrin	1.1	µg/mL				< 7.3
Lysozyme*	117	ng/mL				≤ 500
Calprotectin	12	µg/g				≤ 50

Immunology	Result	Unit	L	WRI	H	Reference Interval
Secretory IgA*	60.0	mg/dL				30 – 275

Short Chain Fatty Acids‡	Result	Unit	L	WRI	H	Reference Interval
% Acetate	71					50 – 72
% Propionate	16					11 – 25
% Butyrate	12					11 – 32
% Valerate	1.3					0.8 – 5.0
Butyrate	1.3	mg/mL				0.8 – 4.0
Total SCFA's	11	mg/mL				5.0 – 16.0

Intestinal Health Markers	Result	Unit	L	WRI	H	Reference Interval
pH	6.2					5.8 – 7.0
β-glucuronidase*	100	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)

Indicates competency of digestion of:

Proteins

Fats

Carbohydrates

Elevated in IBD but NOT IBS, may serve as a differential indicator of active IBD.

Excessive IgA production can 'deplete' (and result in low sIgA levels)

*Consider what potential food allergen or intestinal pathogen (especially yeasts) that could be increasing/depleting secretion.

*Persistent low sIgA levels leave GI tract vulnerable to infection, invasion and systematic inflammation and the development of IgG/E food sensitivities..

(Consider immune system suppression from stress, steroid medications, etc.)

***The persistent presence of blood in consecutive stool tests indicates referral for endoscopic investigation (as does persistently elevated lactoferrin & lysozyme levels).

Sufficient Butyrate is key to colon health.

*Proper balance and level of SCFA's suggests correct flora, appropriate diet, & adequate digestion, (and ultimately reduced colon cancer risk).

Indicates Inflammation. However, elevation seen in IBD or IBS induced diarrhoea (due to pathogens, toxins, coeliac, etc.) therefore further exploration of causes required.

Excessive B-Glucuronidase activity may be associated with bacterial overgrowth (e.g. E. coli and clostridia) and undermines phase II Glucuronidation (chemical & hormone detoxification). Consider Cal-D-Glucarate to counteract.

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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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*This level of specific and targeted eradication strategy is only possible through the culture growth methods of stool analysis provided by the test.

Bacterial Susceptibilities

DOCTOR'S DATA INC

Order: 999999-9999
Client #: 999999
Doctor: Sample Doctor
Doctors Data Inc
123 Main St.
St. Charles, IL 60174 USA

Patient: Sample Patient
Id: 999999
Age: 56
Sex: Female

Sample Collection	Date/Time
Date Collected	01/30/2020
Date Received	01/31/2020
Date Reported	02/01/2020

Specific identified pathogen

Enterobacter cloacae complex

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

Best 'natural' eradication agents for the detected pathogen, indicated by high activity.

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

Any antibiotics in this column are potential eradication agents for the detected pathogen.

*NOTE: Agents with the least disruption potential to rest of flora should be considered to be the most desirable to avoid further flora imbalances/ infections.

Susceptibility Information:

- **Natural antibacterial** 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 susceptibility 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 susceptibility 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:
*Natural antibacterial agent susceptibility testing 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 approve 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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