

ORDER: 999999-9999  
PATIENT: Sample Patient  
ID: 999999  
SEX: Male  
AGE: 2  
DOB: 08/22/2018

CLIENT #: 999999

## Comprehensive Stool Analysis + Parasitology

### BACTERIOLOGY CULTURE

#### Expected/Beneficial flora

NG *Bacteroides fragilis* group  
NG *Bifidobacterium* spp.  
4+ *Escherichia coli*  
1+ *Lactobacillus* spp.  
4+ *Enterococcus* spp.  
3+ *Clostridium* spp.

#### Commensal (Imbalanced) flora

#### Dysbiotic flora

4+ *Citrobacter farmeri*  
3+ *Citrobacter freundii* complex  
4+ *Klebsiella pneumoniae*

NG = No Growth



### BACTERIA INFORMATION

**Expected / Beneficial bacteria** make up a significant portion of the total microflora in a healthy & balanced GI tract. These beneficial bacteria have many health-protecting effects in the GI tract including manufacturing vitamins, fermenting fibers, digesting proteins and carbohydrates, and propagating anti-tumor and anti-inflammatory factors.

**Clostridia** are prevalent flora in a healthy intestine. *Clostridium* spp. should be considered in the context of balance with other expected/beneficial flora. Absence of clostridia or over abundance relative to other expected/beneficial flora indicates bacterial imbalance. If *C. difficile* associated disease is suspected, review the *Clostridium difficile* toxin A/B results from the GI Pathogens PCR section of this report.

**Commensal (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 known pathogenic bacteria and those that have the potential to cause disease in the GI tract. They can be present due to a number of factors including: consumption of contaminated water or food, 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. *Aeromonas*, *Plesiomonas*, *Salmonella*, *Shigella*, *Vibrio*, *Yersinia*, & *Edwardsiella tarda* have been specifically tested for and found absent unless reported.

### YEAST CULTURE

#### Normal flora

#### Dysbiotic flora

3+ *Candida parapsilosis*



### YEAST INFORMATION

Yeast may normally be present in small quantities in the skin, mouth, and GI tract as a component of the resident microbiota. Their presence is generally benign. Recent studies, however, show that high levels of yeast colonization is associated with several inflammatory diseases of the GI tract. Animal models suggest that yeast colonization delays healing of inflammatory lesions and that inflammation promotes colonization. These effects may create a cycle in which low-level inflammation promotes fungal colonization and this colonization promotes further inflammation. Consideration of clinical intervention for yeast should be made in the context of other findings and presentation of symptoms.

### SPECIMEN DATA

#### Comments:

Date Collected: 06/25/2021  
Date Received: 07/01/2021  
Date Reported: 07/14/2021

Specimens Collected: 3

Methodology: Culture and identification by MALDI-TOF and conventional biochemicals

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**GI Pathogen Profile, Multiplex PCR; stool**

Viruses	Result		Reference Interval
Adenovirus F40/41	No call-inhibited	<input type="checkbox"/>	Negative
Norovirus GI/GII	No call-inhibited	<input type="checkbox"/>	Negative
Rotavirus A	No call-inhibited	<input type="checkbox"/>	Negative
Pathogenic Bacteria	Result		Reference Interval
<i>Campylobacter</i> ( <i>C. jejuni</i> , <i>C. coli</i> and <i>C. lari</i> )	No call-inhibited	<input type="checkbox"/>	Negative
<i>Clostridioides difficile</i> (Toxin A/B)	No call-inhibited	<input type="checkbox"/>	Negative
<i>Escherichia coli</i> O157	No call-inhibited	<input type="checkbox"/>	Negative
Enterotoxigenic <i>Escherichia coli</i> (EPEC) lt/st	No call-inhibited	<input type="checkbox"/>	Negative
<i>Salmonella</i> spp.	No call-inhibited	<input type="checkbox"/>	Negative
Shiga-like toxin-producing <i>Escherichia coli</i> (STEC) stx1/stx2	No call-inhibited	<input type="checkbox"/>	Negative
<i>Shigella</i> ( <i>S. boydii</i> , <i>S. sonnei</i> , <i>S. flexneri</i> & <i>S. dysenteriae</i> )	No call-inhibited	<input type="checkbox"/>	Negative
<i>Vibrio cholerae</i>	No call-inhibited	<input type="checkbox"/>	Negative
Parasites	Result		Reference Interval
<i>Cryptosporidium</i> ( <i>C. parvum</i> and <i>C. hominis</i> )	No call-inhibited	<input type="checkbox"/>	Negative
<i>Entamoeba histolytica</i>	No call-inhibited	<input type="checkbox"/>	Negative
<i>Giardia duodenalis</i> (AKA <i>intestinalis</i> & <i>lamblia</i> )	No call-inhibited	<input type="checkbox"/>	Negative

**GI Pathogen information**

“No call-inhibited” results mean no determination can be made and are reported when the PCR reaction is inhibited by components in the stool sample. Approximately 1-2% of PCR samples will be inhibited and this is due to traces of PCR inhibitors that were carried through the extraction process. The PCR inhibition is a complex process that cannot be modeled by one specific compound. They may originate from diet, medication, supplements, and competing DNA. Excessive calcium, tannic acid, bile salts, hemoglobin, melanin, collagen, urea, degradation products, complex polysaccharides and polyphenolic substances have also been found to cause inhibition.

If a sample demonstrates inhibition of the PCR reaction, the sample is re-extracted with an additional wash step in an attempt to remove the inhibiting substances. The additional wash procedure can eliminate some of the inhibitors without compromising the sensitivity of the assay when compared to performing a 1:10 dilution (Internal DDI Validation). If a culture and parasite exam were performed, check for pathogens on the following pages. DDI combines bacterial culture and microscopic parasite detection with PCR. This approach results in a comprehensive, highly sensitive, and highly specific assessment of bacterial and parasitic infection(s).

Running another GI Pathogen PCR test is recommended if symptoms persist.

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 Methodology: Multiplex PCR

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## Parasitology; Microscopy

Protozoa	Result	
<i>Balantidium coli</i>	Not Detected	<input type="checkbox"/>
<i>Blastocystis spp.</i>	Not Detected	<input type="checkbox"/>
<i>Chilomastix mesnili</i>	Not Detected	<input type="checkbox"/>
<i>Dientamoeba fragilis</i>	Not Detected	<input type="checkbox"/>
<i>Endolimax nana</i>	Not Detected	<input type="checkbox"/>
<i>Entamoeba coli</i>	Not Detected	<input type="checkbox"/>
<i>Entamoeba hartmanni</i>	Not Detected	<input type="checkbox"/>
<i>Entamoeba histolytica/Entamoeba dispar</i>	Not Detected	<input type="checkbox"/>
<i>Entamoeba polecki</i>	Not Detected	<input type="checkbox"/>
<i>Enteromonas hominis</i>	Not Detected	<input type="checkbox"/>
<i>Giardia duodenalis</i>	Not Detected	<input type="checkbox"/>
<i>Iodamoeba bütschlii</i>	Not Detected	<input type="checkbox"/>
<i>Isospora belli</i>	Not Detected	<input type="checkbox"/>
<i>Pentatrichomonas hominis</i>	Not Detected	<input type="checkbox"/>
<i>Retortamonas intestinalis</i>	Not Detected	<input type="checkbox"/>
<b>Nematodes - Roundworms</b>		
<i>Ascaris lumbricoides</i>	Not Detected	<input type="checkbox"/>
<i>Capillaria hepatica</i>	Not Detected	<input type="checkbox"/>
<i>Capillaria philippinensis</i>	Not Detected	<input type="checkbox"/>
<i>Enterobius vermicularis</i>	Not Detected	<input type="checkbox"/>
<i>Strongyloides stercoralis</i>	Not Detected	<input type="checkbox"/>
<i>Trichuris trichiura</i>	Not Detected	<input type="checkbox"/>
Hookworm	Not Detected	<input type="checkbox"/>
<b>Cestodes - Tapeworms</b>		
<i>Diphyllobothrium latum</i>	Not Detected	<input type="checkbox"/>
<i>Dipylidium caninum</i>	Not Detected	<input type="checkbox"/>
<i>Hymenolepis diminuta</i>	Not Detected	<input type="checkbox"/>
<i>Hymenolepis nana</i>	Not Detected	<input type="checkbox"/>
<i>Taenia</i>	Not Detected	<input type="checkbox"/>

### SPECIMEN DATA

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**Methodology:** Microscopy

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## Parasitology; Microscopy

Trematodes - Flukes		
<i>Clonorchis sinensis</i>	Not Detected	<input checked="" type="checkbox"/>
<i>Fasciola hepatica/Fasciolopsis buski</i>	Not Detected	<input checked="" type="checkbox"/>
<i>Heterophyes heterophyes</i>	Not Detected	<input checked="" type="checkbox"/>
<i>Paragonimus westermani</i>	Not Detected	<input checked="" type="checkbox"/>

Other Markers			Reference Interval
Yeast	Rare	<input checked="" type="checkbox"/>	None – Rare
RBC	Not Detected	<input checked="" type="checkbox"/>	None – Rare
WBC	Not Detected	<input checked="" type="checkbox"/>	None – Rare
Muscle fibers	Not Detected	<input checked="" type="checkbox"/>	None – Rare
Vegetable fibers	Rare	<input checked="" type="checkbox"/>	None – Few
Charcot-Leyden Crystals	Not Detected	<input checked="" type="checkbox"/>	None
Pollen	Not Detected	<input checked="" type="checkbox"/>	None

Macroscopic Appearance	Result	
Mucus	Negative	<input checked="" type="checkbox"/>

This test is not designed to detect *Cyclospora cayatanensis* or *Microsporidia* 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.

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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**Methodology:** Microscopy, Macroscopic Observation

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*Parasitology; Microscopy*

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Methodology:

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## Stool Chemistries

Digestion / Absorption	Result	Unit		Reference Interval
Elastase	>500	µg/mL	<input checked="" type="checkbox"/>	> 200
Fat Stain	Few		<input checked="" type="checkbox"/>	None – Few
Carbohydrates <sup>†</sup>	Negative		<input checked="" type="checkbox"/>	Negative
Inflammation	Result	Unit		Reference Interval
Lactoferrin	<0.5	µg/mL	<input checked="" type="checkbox"/>	< 7.3
Calprotectin	<5	µg/g	<input checked="" type="checkbox"/>	≤ 50
Lysozyme*	119	ng/mL	<input checked="" type="checkbox"/>	≤ 500
Immunology	Result	Unit		Reference Interval
Secretory IgA*	19.2	mg/dL	<input type="checkbox"/>	30 – 275
Short Chain Fatty Acids	Result	Unit		Reference Interval
% Acetate <sup>‡</sup>	78	%	<input type="checkbox"/>	50 – 72
% Propionate <sup>‡</sup>	14	%	<input checked="" type="checkbox"/>	11 – 25
% Butyrate <sup>‡</sup>	8.1	%	<input type="checkbox"/>	11 – 32
% Valerate <sup>‡</sup>	0.2	%	<input type="checkbox"/>	0.8 – 5.0
Butyrate <sup>‡</sup>	0.41	mg/mL	<input type="checkbox"/>	0.8 – 4.0
Total SCFA's <sup>‡</sup>	5.1	mg/mL	<input checked="" type="checkbox"/>	5.0 – 16.0
Intestinal Health Markers	Result	Unit		Reference Interval
pH	6.9		<input checked="" type="checkbox"/>	5.8 – 7.0
Occult Blood	Negative		<input checked="" type="checkbox"/>	Negative
Macroscopic Appearance	Result	Unit		Reference Interval
Color	Brown		<input checked="" type="checkbox"/>	Brown
Consistency	Loose/Watery		<input type="checkbox"/>	Soft

### 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.

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**Methodology:** Elisa, Microscopy, Colormetric, Gas Chromotography, ph Electrode, Guaiac, Macroscopic Observation

RI= Reference Interval, Toggles: Green = within RI, Yellow = moderately outside RI, Red = outside 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.

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## Stool Chemistries

**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 functional 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.

**Color:** Stool is normally brown because of pigments formed by bacteria acting on bile introduced into the digestive system from the liver. While certain conditions can cause changes in stool color, many changes are harmless and are caused by pigments in foods or dietary supplements.

**Consistency:** Stool normally contains about 75% water and ideally should be formed and soft. Stool consistency can vary based upon transit time and water absorption.

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*Citrobacter farmeri*

NATURAL ANTIBACTERIALS

	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			✓

**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.

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**Date Collected:** 06/25/2021      **Specimens Collected:** 3  
**Date Received:** 07/01/2021  
**Date Reported:** 07/14/2021  
**Methodology:** Disk Diffusion

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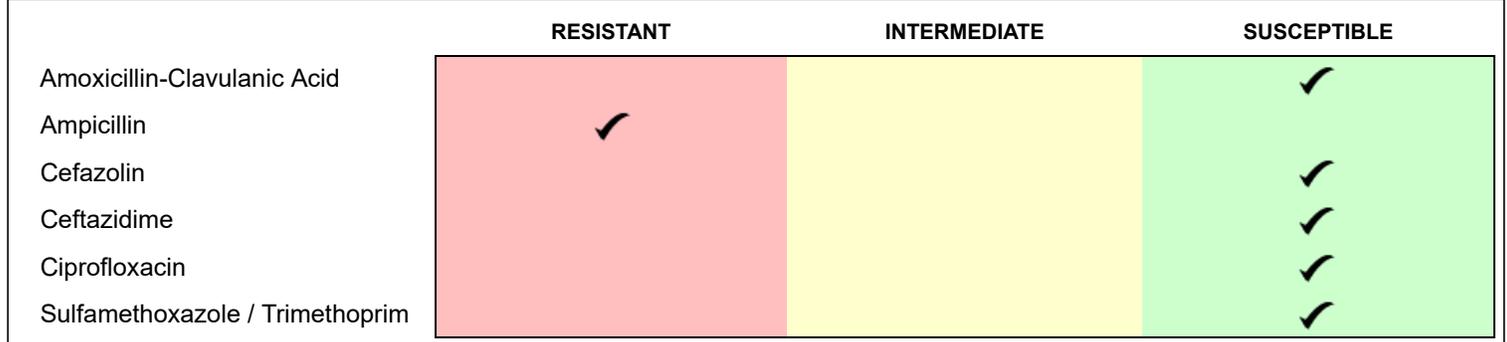
CLIENT #: 999999

*Klebsiella pneumoniae*

NATURAL ANTIBACTERIALS



PRESCRIPTIVE AGENTS



**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.

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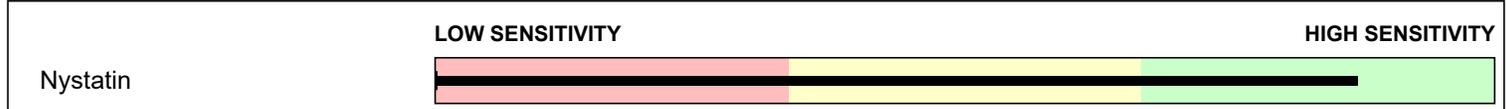
CLIENT #: 999999

*Candida parapsilosis*

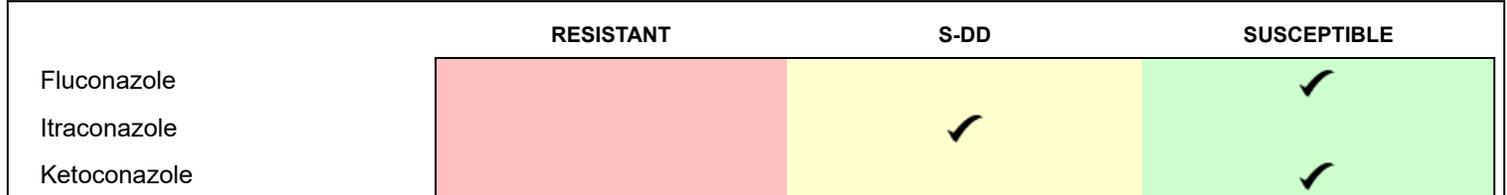
NATURAL ANTIFUNGALS



NON-ABSORBED ANTIFUNGALS



AZOLE ANTIFUNGALS



Standardized test interpretive categories established for *Candida* spp. are used for all yeast isolates.

**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.

**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.

**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.

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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.

## Microbiology

### Beneficial Flora

One or more of the expected or beneficial bacteria are low in this specimen. Normally abundant bacteria include *Lactobacillus* spp, *Bifidobacteria* spp, *Clostridium* spp, *Bacteroides fragilis* group, *Enterococcus* spp, and *Escherichia coli*. The beneficial flora have many health-protecting effects in the gut, and as a consequence, are crucial to the health of the whole organism. Some of the roles of the beneficial flora include digestion of proteins and carbohydrates, manufacture of vitamins and essential fatty acids, increase in the number of immune system cells, break down of bacterial toxins and the conversion of flavonoids into anti-tumor and anti-inflammatory factors. *Lactobacilli*, *bifidobacteria*, *clostridia*, and *enterococci* secrete lactic acid as well as other acids including acetate, propionate, butyrate, and valerate. This secretion causes a subsequent decrease in intestinal pH, which is crucial in preventing an enteric proliferation of microbial pathogens, including bacteria and yeast. Many GI pathogens thrive in alkaline environments. *Lactobacilli* also secrete the antifungal and antimicrobial agents lactocidin, lactobacillin, acidolin, and hydrogen peroxide. The beneficial flora of the GI tract have thus been found useful in the inhibition of microbial pathogens, prevention and treatment of antibiotic associated diarrhea, prevention of traveler's diarrhea, enhancement of immune function, and inhibition of the proliferation of yeast.

In a healthy balanced state of intestinal flora, the beneficial bacteria make up a significant proportion of the total microflora. Healthy levels of each of the beneficial bacteria are indicated by either a 2+, 3+ or 4+ (0 to 4 scale). However, in some individuals there is an imbalance or deficiency of beneficial flora and an overgrowth of non-beneficial (imbalance) or even pathogenic microorganisms (dysbiosis). 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, 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.

Antibacterial and antifungal susceptibility testing to a variety of prescriptive and natural agents may be provided for the pathogenic organisms that are cultured from this patient's specimen. This testing is intended to provide the practitioner with useful information to help plan an appropriate treatment regimen. A comprehensive program may be helpful in individuals in whom a dysbiotic condition has caused extensive GI damage.

Note: Not all genera or species can be tested for susceptibilities in the laboratory due to their specific growth requirements. In addition, the Centers for Disease Control and Prevention recommend not testing certain organisms such as those associated with food poisoning. If a practitioner has specific questions, please contact customer service.

### Clostridium spp

*Clostridia* are expected inhabitants of the human intestine. Although most *clostridia* in the intestine are not virulent, certain species have been associated with disease. *Clostridium perfringens* is a major cause of food poisoning and is also one cause of antibiotic-associated diarrhea. *Clostridioides difficile* is a causative agent in antibiotic-associated diarrhea and pseudomembranous colitis. Other species reported to be prevalent in high amounts in patients with Autistic Spectrum Disorder include *Clostridium histolyticum* group, *Clostridium cluster I*, *Clostridium bolteae*, and *Clostridium tetani*.

### 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.

### **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. murlinae*, *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 a 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.

#### **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.

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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.

## 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.

### 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.