BACTERIOLOGY CULTURE

Expected/Beneficial Bacteria

4+	*Bacteroides* family
3+	*Bifidobacterium* family
4+	*Escherichia coli*
4+	*Lactobacillus* family
NG	*Enterococcus* family
4+	*Clostridium* family

Commensal (Imbalanced) flora

1+	*Corynebacterium amycolatum*
2+	*Corynebacterium minutissimum*
2+	*Enterobacter cloacae* complex
1+	*Enterobacter cloacae* complex,isolate 2
2+	*Lelliottia amnigena*
4+	*Streptococcus anginosus*
4+	*Streptococcus mutans*

Dysbiotic flora

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

1+	*Candida albicans*

Dysbiotic flora

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.

Viruses	**Result**		**Reference Interval**
Adenovirus F40/41	Negative	🟩	Negative
Norovirus GI/GII	Negative	🟩	Negative
Rotavirus A	Negative	🟩	Negative

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

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

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	🟩

Nematodes - Roundworms		
Ascaris lumbricoides	Not Detected	🟩
Capillaria hepatica	Not Detected	🟩
Capillaria philippinensis	Not Detected	🟩
Enterobius vermicularis	Not Detected	🟩
Strongyloides stercoralis	Not Detected	🟩
Trichuris trichiura	Not Detected	🟩
Hookworm	Not Detected	🟩

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

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

Other Markers			**Reference Interval**
Yeast	Not Detected	🟩	None – Few
RBC	Not Detected	🟩	None – Few
WBC	Not Detected	🟩	None – Rare
Muscle fibers	Not Detected	🟩	None – Rare
Vegetable fibers	Rare	🟩	None – Few
Charcot-Leyden Crystals	Not Detected	🟩	None
Pollen	Not Detected	🟩	None
Mucus	Negative	🟩	Negative

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.

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".

Digestion / Absorption	Result	Unit		Reference Interval
Elastase	375	µg/g	🟩	> 200
Fat Stain	Not Detected		🟩	None – Moderate
Carbohydrates[†]	Negative		🟩	Negative

Inflammation	Result	Unit		Reference Interval
Lactoferrin	1.4	µg/mL	🟩	< 7.3
Calprotectin	20	µg/g	🟩	< 80
Lysozyme*	145	ng/mL	🟩	≤ 500

Immunology	Result	Unit		Reference Interval
Secretory IgA*	134	mg/dL	🟩	30 – 275

Short Chain Fatty Acids	Result	Unit		Reference Interval
% Acetate[‡]	68	%	🟩	50 – 72
% Propionate[‡]	16	%	🟩	11 – 25
% Butyrate[‡]	14	%	🟩	11 – 32
% Valerate[‡]	2.1	%	🟩	0.8 – 5.0
Butyrate[‡]	1.5	mg/mL	🟩	0.8 – 4.0
Total SCFA's[‡]	11	mg/mL	🟩	5.0 – 16.0

Intestinal Health Markers	Result	Unit		Reference Interval
pH	5.0		🟥	5.8 – 7.0
Occult Blood	Negative		🟩	Negative

Macroscopic Appearance	Result	Unit		Reference Interval
Color	Brown		🟩	Brown
Consistency	Soft		🟩	Soft

Chemistry Information

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

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.

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.

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.

The majority of reference intervals are established from adult populations. Results may differ in pediatric populations and care should be taken when interpreting these values.

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.

Imbalanced Flora

Imbalanced flora are those bacteria that reside in the host gastrointestinal tract and neither injure nor benefit the host. Certain dysbiotic bacteria may appear under the imbalanced category if found at low levels because they are not likely pathogenic at the levels detected. Imbalanced bacteria are commonly more abundant in association with insufficiency dysbiosis, and/or a fecal pH more towards the alkaline end of the reference range (5.8 - 7.0). Treatment with antimicrobial agents is unnecessary unless bacteria appear under the dysbiotic category.

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.

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

pH low

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