

Comprehensive Stool Analysis / Parasitology x3

BACTERIOLOGY CULTURE

Expected/Beneficial flora	Commensal (Imbalanced) flora	Dysbiotic flora
3+ Bacteroides fragilis group 3+ Bifidobacterium spp. 3+ Escherichia coli 2+ Lactobacillus spp. NG Enterococcus spp. 4+ Clostridium spp. NG = No Growth	3+ Alpha hemolytic strep	

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, a Comprehensive Clostridium culture or toxigenic *C. difficile* DNA test is recommended.

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.

YEAST CULTURE

Normal flora	Dysbiotic flora
No yeast isolated	

MICROSCOPIC YEAST

Result:	Expected:
Rare	None - Rare

The microscopic finding of yeast in the stool is helpful in identifying whether there is proliferation of yeast. Rare yeast may be normal; however, yeast observed in higher amounts (few, moderate, or many) is abnormal.

YEAST INFORMATION

Yeast normally can be found in small quantities in the skin, mouth, intestine and mucocutaneous junctions. Overgrowth of yeast can infect virtually every organ system, leading to an extensive array of clinical manifestations. Fungal diarrhea is associated with broad-spectrum antibiotics or alterations of the patient's immune status. Symptoms may include abdominal pain, cramping and irritation. When investigating the presence of yeast, disparity may exist between culturing and microscopic examination. Yeast are not uniformly dispersed throughout the stool, this may lead to undetectable or low levels of yeast identified by microscopy, despite a cultured amount of yeast. Conversely, microscopic examination may reveal a significant amount of yeast present, but no yeast cultured. Yeast does not always survive transit through the intestines rendering it unviable.

Comments:

Date Collected: 06/23/2013
 Date Received: 06/26/2013
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** Aeromonas, Campylobacter, Plesiomonas, Salmonella, Shigella, Vibrio, Yersinia, & Edwardsiella tarda have been specifically tested for and found absent unless reported.*

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PARASITOLGY/MICROSCOPY *
<p>Sample 1 None Ova or Parasites</p>
<p>Sample 2 None Ova or Parasites</p>
<p>Sample 3 None Ova or Parasites Rare Yeast</p>
<p>*A trichrome stain and concentrated iodine wet mount slide is read for each sample submitted.</p>

PARASITOLGY INFORMATION
<p>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. 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. It is estimated that close to one billion people worldwide are infected. 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.</p> <p>There are two main classes of intestinal parasites that can cause human intestinal disease. 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 that are generally visible to the naked eye in their adult stages. Like protozoa, helminths can be either free-living or parasitic in nature. In their adult form, helminths cannot multiply in humans.</p> <p>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.</p> <p>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.</p>

GIARDIA/CRYPTOSPORIDIUM IMMUNOASSAY			
	Within	Outside	Reference Range
Giardia lamblia	Neg		Neg
Cryptosporidium	Neg		Neg

Giardia lamblia is flagellated protozoan that infects the small intestine and is passed in stool and spread by the fecal-oral route. Waterborne transmission is the major source of giardiasis.

Cryptosporidium is a coccidian protozoa that can be spread from direct person-to-person contact or waterborne transmission.

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DIGESTION / ABSORPTION

	Within	Outside	Reference Range
Elastase	472		> 200 µg/mL
Fat Stain	Few		None - Mod
Muscle fibers	None		None - Rare
Vegetable fibers	Rare		None - Few
Carbohydrates	Neg		Neg

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. **Fat Stain:** Microscopic determination of fecal fat using Sudan IV staining is a qualitative procedure utilized to assess fat absorption and to detect steatorrhea. **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". **Carbohydrates:** The presence of reducing substances in stool specimens can indicate carbohydrate malabsorption.

INFLAMMATION

	Within	Outside	Reference Range
Lysozyme*	87		<= 600 ng/mL
Lactoferrin	< 0.5		< 7.3 µg/mL
White Blood Cells	None		None - Rare
Mucus	Neg		Neg

Lysozyme* is an enzyme secreted at the site of inflammation in the GI tract and elevated levels have been identified in IBD patients. **Lactoferrin** is a quantitative GI specific marker of inflammation used to diagnose and differentiate IBD from IBS and to monitor patient inflammation levels during active and remission phases of IBD. **White Blood Cells (WBC):** in the stool are an indication of an inflammatory process resulting in the infiltration of leukocytes within the intestinal lumen. WBCs are often accompanied by mucus and blood in the stool. **Mucus** in the stool may result from prolonged mucosal irritation or in a response to parasympathetic excitability such as spastic constipation or mucous colitis.

IMMUNOLOGY

	Within	Outside	Reference Range
Secretory IgA*		8.8	51 - 204mg/dL

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.

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*For Research Use Only. Not for use in diagnostic procedures.

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SHORT CHAIN FATTY ACIDS

	Within	Outside	Reference Range
% Acetate	56		36 - 74 %
% Propionate	18		9 - 32 %
% Butyrate	23		9 - 39 %
% Valerate	3.5		1 - 8 %
Butyrate	3.1		0.8 - 3.8 mg/mL
Total SCFA's	14		4 - 14 mg/mL

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.

INTESTINAL HEALTH MARKERS

	Within	Outside	Reference Range
Red Blood Cells	None		None - Rare
pH	6.4		6 - 7.8
Occult Blood	Neg		Neg

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.
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.

MACROSCOPIC APPEARANCE

	Appearance	Expected
Color	Brown	Brown
Consistency	Soft	Formed/Soft

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 interpretive paragraphs are presented. If no significant abnormalities are found, interpretive paragraphs are not presented.

Beneficial Flora

One or more of the expected (beneficial) bacteria are low in this specimen. Beneficial flora include lactobacilli, bifidobacteria, clostridia, *Bacteroides fragilis* group, enterococci, and some strains of *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 flavinoids 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 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 flora make up a significant proportion of the total microflora. Healthy levels of each of the beneficial bacteria are indicated by either a 3+ or 4+ (0 to 4 scale). However, some individuals have low levels of beneficial bacteria and an overgrowth of nonbeneficial (imbalances) or even pathogenic microorganisms (dysbiosis). Often attributed to the use of antibiotics, individuals with low beneficial bacteria may present with chronic symptoms such as irregular transit time, irritable bowel syndrome, bloating, gas, chronic fatigue, headaches, autoimmune diseases (e.g., rheumatoid arthritis), and sensitivities to a variety of foods. Treatment may include the use of probiotic supplements containing various strains of lactobacilli, bifidobacteria and enterococci and consumption of cultured or fermented foods including yogurt, kefir, miso, tempeh and tamari sauce. Polyphenols in green and ginseng tea have been found to increase the numbers of beneficial bacteria. If dysbiosis is present, treatment may also include the removal of pathogenic bacteria, yeast, or parasites.

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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. Clostridium 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.

If these disease associations are a concern further testing may be necessary.

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Imbalanced flora

Imbalanced flora are those bacteria that reside in the host gastrointestinal tract and neither

injure nor benefit the host. Certain dysbiotic bacteria may appear under the imbalances category if found at low levels because they are not likely pathogenic at the levels detected. When imbalanced flora appear, it is not uncommon to find inadequate levels of one or more of the beneficial bacteria and/or a fecal pH which is more towards the alkaline end of the reference range (6.5 - 7.2). It is also not uncommon to find hemolytic or mucoid *E. coli* with a concomitant deficiency of beneficial *E. coli* and alkaline pH, secondary to a mutation of beneficial *E. coli* in alkaline conditions (DDI observations). Treatment with antimicrobial agents is unnecessary unless bacteria appear under the dysbiotic category.

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Microscopic yeast

Microscopic examination has revealed yeast in this stool sample. The microscopic finding of yeast in the stool is helpful in identifying whether the proliferation of fungi, such as *Candida albicans*, is present. Yeast is normally found in very small amounts in a healthy intestinal tract. While small quantities of yeast (reported as none or rare) may be normal, yeast observed in higher amounts (few, moderate to many) is considered abnormal.

An overgrowth of intestinal yeast is prohibited by beneficial flora, intestinal immune defense (secretory IgA), and intestinal pH. Beneficial bacteria, such as *Lactobacillus* colonize in the intestines and create an environment unsuitable for yeast by producing acids, such as lactic acid, which lowers intestinal pH. Also, *Lactobacillus* is capable of releasing antagonistic substances such as hydrogen peroxide, lactocidin, lactobacillin, and acidolin.

Many factors can lead to an overgrowth of yeast including frequent use of antibiotics (leading to insufficient beneficial bacteria), synthetic corticosteroids, oral contraceptives, and diets high in sugar. Although there is a wide range of symptoms which can result from intestinal yeast overgrowth, some of the most common include brain fog, fatigue, recurring vaginal or bladder infections, sensitivity to smells (perfumes, chemicals, environment), mood swings/depression, sugar and carbohydrate cravings, gas/bloating, and constipation or loose stools.

A positive yeast culture (mycology) and sensitivity to prescriptive and natural agents is helpful in determining which anti-fungal agents to use as part of a therapeutic treatment plan for chronic colonic yeast. However, yeast are colonizers and do not appear to be dispersed uniformly throughout the stool. Yeast may therefore be observed microscopically, but not grow out on culture even when collected from the same bowel movement.

Secretory IgA (sIgA)

The concentration of sIgA is abnormally low in this specimen. Immunological activity in the gastrointestinal tract can be assessed using secretory immunoglobulin A (sIgA). Secretory IgA is the predominant antibody, or immune protein the body manufactures and releases in external secretions such as saliva, tears, and milk [1]. It is also transported through the epithelial cells that line the intestines out into the lumen. Secretory IgA 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 [1]. As the principal immunoglobulin isotype present in mucosal secretions, sIgA plays an important role in controlling intestinal milieu which is constantly presented with potentially harmful antigens such as pathogenic bacteria, parasites, yeast, viruses, abnormal cell antigens, and allergenic proteins [1]. Secretory IgA antibodies exert their function by binding to antigenic epitopes on the invading microorganism, limiting their mobility and adhesion to the epithelium of the mucus membrane [2]. This prevents the antigens from reaching systemic circulation and allowing them to be excreted directly in the feces.

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 [3]. One study found depressed levels of sIgA in malnourished children, particularly protein malnourishment, that responded well to nutritional rehabilitation with a significant increase in sIgA [4]. This may be because the synthesis and expression of sIgA requires adequate intake of the amino acid L-glutamine [3]. Animal studies have demonstrated that a glutamine-restricted diet can result in a 50% decrease in sIgA levels [5]. An increase of dietary L-glutamine can restore GI immune function by protection of cells that synthesize sIgA [6]. *Saccharomyces boulardii* is a nonpathogenic yeast that has been used for the treatment of acute infectious enteritis and antibiotic-associated diarrhea [7]. Significantly elevated levels of sIgA and subsequent enhanced host immune response have been found following *S. boulardii* administration in mice and rats [8,9].

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