

Comprehensive Stool Analysis / Parasitology x3

MICROBIOLOGY

Bacteriology Culture

Beneficial flora		Imbalances		Dysbiotic flora	
Bifidobacterium	0+	Klebsiella pneumoniae ESBL	2+		
E. coli spp.	4+	Serratia ficaria	3+		
Lactobacillus spp.	4+				
Enterococcus spp.	4+				

Mycology (Yeast) Culture

Normal flora	Dysbiotic flora
No yeast isolated	

PARASITOLOGY / MICROSCOPY (TRICHROME STAIN & CONCENTRATION)

Sample 1	Sample 2	Sample 3
No Ova or Parasites	No Ova or Parasites	No Ova or Parasites

	Within	Outside	Ref. Range		Within	Outside	Ref. Range
Giardia Lamblia (EIA)	Neg		Neg	Cryptosporidium (EIA)	Neg		Neg

Beneficial flora: In a healthy balanced state of intestinal flora, the beneficial bacteria make up a significant proportion of the total microflora. The beneficial flora have many health-protecting effects in the gut including manufacturing vitamins, fermenting fibers, digesting proteins and the disaccharide lactose, and propagating anti-tumor and anti-inflammatory factors. Acidophilus, Bifidus, and Enterococcus produce lactic acid and short-chain fatty acids. The fermentation of fibers by beneficial bacteria and subsequent production of short chain fatty acids is crucial in lowering colonic pH and preventing the proliferation of microbial pathogens, including bacteria and yeast. Enterococcus has antibacterial activity against methicillin-resistant *S. aureus* (MRSA) and food-borne pathogens.

Parasitology: Intestinal parasites are abnormal inhabitants of the GI tract. Factors such as contaminated food and water supplies, day care centers, international travel, pets, carriers such as mosquitoes and fleas, and sexual transmission have contributed to an increased prevalence of intestinal parasites in the American population.

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CAMPYLOBACTER CULTURE

	Within	Outside	Ref. Range
Campylobacter jejuni	Neg		Neg

Campylobacter jejuni is a pathogenic bacteria and a common cause of diarrheal disease, often accompanied by abdominal cramping, fever, and vomiting. Campylobacter infection is often associated with raw or undercooked poultry, unpasteurized milk, or contaminated water.

DIGESTION / ABSORPTION

	Within	Outside	Ref. Range
Elastase	> 500		> 200 $\mu\text{g/mL}$
Fat stain	None		None - Mod
Muscle fibers	Rare		None - Rare
Vegetable fibers		Mod	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. **Meat/Vegetable fibers:** The presence of meat and/or vegetable fibers in the stool may be due to a number of factors including, improper mastication, excessive protein intake, a reduction of gastric HCL secretion, or insufficient output of pancreatic enzymes. **Carbohydrates:** The presence of reducing substances in stool specimens can indicate carbohydrate malabsorption.

INFLAMMATION

	Within	Outside	Ref. Range
Lysozyme*	128		≤ 600 ng/mL
Lactoferrin	< 0.5		< 7.3 $\mu\text{g/mL}$
WBC	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. **WBCs:** Elevated stool levels of white blood cells occur following an infiltration of leukocytes within the intestinal lumen during an inflammatory process. **Mucus** in the stool may result from prolonged mucosal irritation or in response to parasympathetic excitability such as spastic constipation or mucous colitis.

IMMUNOLOGY

	Within	Outside	Ref. Range
slgA*		17	51 - 204mg/dL

slgA: Secretory IgA is secreted by mucosal-associated lymphoid tissue and represents the first line of defense of the GI mucosa and is central to the normal function of the GI as an immune barrier. Elevated levels of slgA have been associated with an upregulated immune response.

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

SHORT CHAIN FATTY ACIDS

	Within	Outside	Ref. Range	
Acetate	50		36 - 74	%
Propionate	24		9 - 32	%
Butyrate	22		16 - 39	%
Valerate	5		1 - 8	%
Butyrate	1.2		0.8 - 3.8	mg/mL
Total SCFA's	5.5		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. Lactobacillus and Bifidus 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/g are important for assessing overall SCFA production, and are reflective of beneficial flora levels and/or adequate fiber intake.

INTESTINAL HEALTH MARKERS

	Within	Outside	Ref. Range	
RBC	None		None - Rare	
pH	7.1		6 - 7.8	
Occult Blood	Neg		Neg	
Yeast	None		None - Rare	

RBC: Red blood cells 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. **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. **pH:** Fecal pH is largely dependent on the fermentation of fiber by the beneficial flora of the gut. **Yeast:** A positive microscopic yeast level indicates the presence of fungi such as Candida albicans in the stool.

MACROSCOPIC APPEARANCE

	Appearance	Expected	
Color	Brown	Brown	
Consistency	Formed/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 beneficial bacteria are low in this specimen. Beneficial flora include Lactobacillus, Bifidus, Enterococcus sp., and beneficial E. 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 the disaccharide lactose, manufacture of vitamins and essential fatty acids, increasing the number of immune system cells, breaking down bacterial toxins and converting flavinoids into anti-tumor and anti-inflammatory factors [1]. Lactobacillus, Bifidus, and Enterococcus sp. secrete lactic acid as well as other acids including acetate, propionate, butyrate, and valerate. This 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. Lactobacillus acidophilus also secretes the antifungal and antimicrobial agents lactocidin, lactobacillin, acidolin, and hydrogen peroxide [2]. The beneficial flora of the GI have thus been found useful in the inhibition of microbial pathogens [3], prevention and treatment of antibiotic associated diarrhea [4], prevention of traveler's diarrhea [5], enhancement of immune function [6], and inhibition of the proliferation of Candida albicans [7,8].

Enterococcus sp. Are prominent non-anaerobic beneficial bacteria in the gastrointestinal tract. They are fermentative yet not gas producing bacteria that can survive in relatively harsh environments. Most importantly, Enterococcus sp. Provide antimicrobial activity against methicillin-resistant Staphylococcus aureus (MRSA), and impede the growth of food-borne pathogens. S. aureus strains, which are resistant to multiple antibiotics, have dramatically increased hospital associated infections. There is concern that the pharmaceutical industry cannot keep up the MRSA strains, therefore maintenance of healthy levels of Enterococcus sp. is important for antimicrobial activity against MRSA.

In a healthy balanced state of intestinal flora, the beneficial flora make up a significant proportion of the total non-anaerobic 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 [1]. Treatment may include the use of probiotic supplements containing various strains of Lactobacillus, Bifidobacter, and Enterococcus and/or consumption of cultured or fermented foods including yogurt, kefir, miso, tofu, tempen and tamari sauce. Polyphenols in green and ginseng tea have been found to increase the numbers of beneficial bacteria [9]. If dysbiosis is present, treatment may also include the removal of pathogenic bacteria, yeast, or parasites.

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Imbalanced flora

Imbalanced flora are those bacteria that are not pathogenic but are rather commensal. They reside in the host organism (GI tract) and neither injure nor benefit the host [1]. 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 (6.0 - 7.2). It is also not uncommon to find Haemolytic or NLF 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 dysbiosis category.

Vegetable Fibers

Excessive amounts of vegetable fibers were found in this stool specimen. This may be indicative of inadequate chewing, or eating "on the run". The presence of vegetable fibers must be

considered in conjunction with other parameters such as muscle fibers, Elastase-1, and triglycerides for a proper assessment of maldigestion. A rapid transit time may also result in elevated levels of vegetable fibers.

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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Patient:

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