



LAB #: F140721-0016-1
PATIENT: Anna M M. Salanti
ID: P130910009
SEX: Female
DOB: 01/26/1952 AGE: 62

CLIENT #: 27210
DOCTOR: Brian Popiel, ND
Lab Interpretation LLC
18124 Wedge Pkwy 432
Reno, NV 89511 U.S.A.

Comprehensive Stool Analysis

BACTERIOLOGY CULTURE

Expected/Beneficial flora

3+ Bacteroides fragilis group
3+ Bifidobacterium spp.
2+ Escherichia coli
3+ Lactobacillus spp.
NG Enterococcus spp.

1+ Clostridium spp.
NG = No Growth

Commensal (Imbalanced) flora

3+ Alpha hemolytic strep
2+ Gamma hemolytic strep
2+ Pseudomonas chlororaphis group
1+ Staphylococcus aureus

Dysbiotic flora

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

1+ Candida albicans

Dysbiotic flora

MICROSCOPIC YEAST

Result:

Expected:

None

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: 07/15/2014

Date Received: 07/21/2014

Date Completed: 07/28/2014

* *Aeromonas, Campylobacter, Plesiomonas, Salmonella, Shigella, Vibrio, Yersinia, & Edwardsiella tarda* have been specifically tested for and found absent unless reported.





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DIGESTION / ABSORPTION

	Within	Outside	Reference Range
Elastase	> 500		> 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*		779	<= 600 ng/mL
Lactoferrin	6.2		< 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*		286	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	70		40 - 75 %
% Propionate		8.4	9 - 29 %
% Butyrate	19		9 - 37 %
% Valerate	3.2		0.5 - 7 %
Butyrate	4.0		0.8 - 4.8 mg/mL
Total SCFA's		22	4 - 18 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		5.6	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.

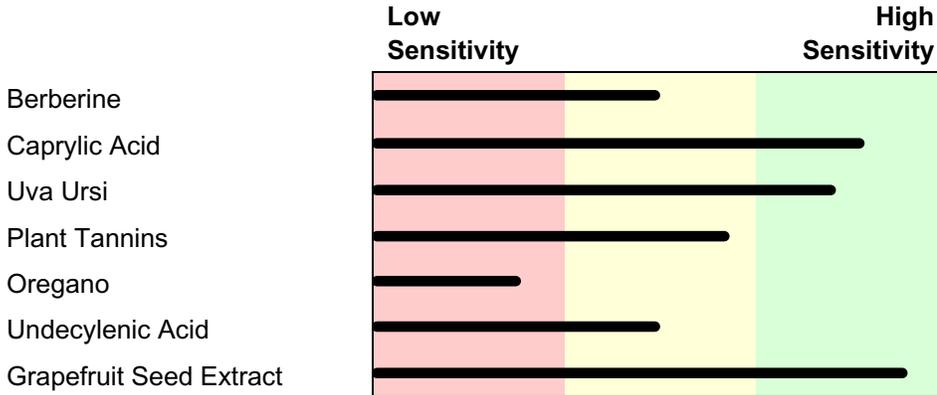


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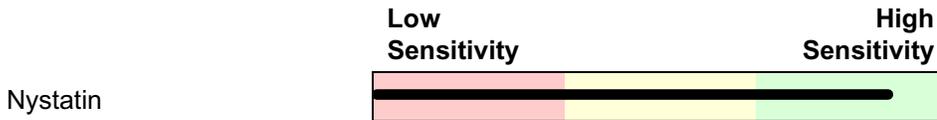
Yeast Susceptibilities: *Candida albicans*

NATURAL ANTIFUNGALS



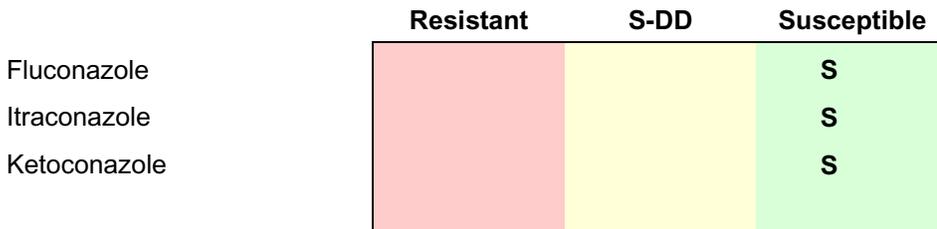
Natural antifungal agents may be useful for treatment of patients when organisms display in-vitro sensitivity to these agents. The test is performed by using standardized techniques and filter paper disks impregnated with the listed agent. Relative sensitivity is reported for each natural agent based 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.

NON-ABSORBED ANTIFUNGALS



Non-absorbed antifungals may be useful for treatment of patients when organisms display in-vitro sensitivity to these agents. The test is performed using standardized commercially prepared disks impregnated with Nystatin. Relative sensitivity is reported based upon the diameter of the zone of inhibition surrounding the disk.

AZOLE ANTIFUNGALS



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.

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

Comments:

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Yeast antifungal susceptibility testing is intended for research use only.
 Not for use in diagnostic procedures.

v10.11

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.

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 - 7.8). 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.

Mackowiak PA. The normal microbial flora. *N Engl J Med.* 1982;307(2):83-93.

Cultured Yeast

Yeast, such as *Candida* are normally present in the GI tract in very small amounts. Many species of yeast exist and are commensal; however, they are always poised to create opportunistic infections and have detrimental effects throughout the body. Factors that contribute to a proliferation of yeast include frequent use of wide-spread antibiotics/low levels of beneficial flora, oral contraceptives, pregnancy, cortisone and other immunosuppressant drugs, weak immune system/low levels of sIgA, high-sugar diet, and high stress levels.

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 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 for culturing. Therefore, both microscopic examination and culture are helpful in determining if abnormally high levels of yeast are present.

Lysozyme

The level of lysozyme, a biomarker of inflammation, is elevated in this specimen. Lysozyme is an enzyme that catalyzes the hydrolysis of specific glycosidic bonds in mucopolysaccharides that constitute the cell wall of gram-positive bacteria. Lysozyme is an antibacterial defense present in the G.I. tract and is secreted by granulocytes, macrophages, Paneth cells, and Brunner's Glands as well as normal colonic crypt cells [1]. The main source for fecal lysozyme is the intestinal granulocytes.

Moderate elevations in fecal lysozyme are commonly associated with significant overgrowth of enteropathogens such as yeast or dysbiotic bacteria. Markedly elevated levels of fecal lysozyme have been identified in colonic inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis as well as other non-IBD G.I. diseases with diarrhea, compared to healthy controls [2,3]. In Crohn's disease, excess lysozyme may be a result of active secretions of macrophages in the lamina propria, and monocytic cells in the granulomas (sites of G.I. inflammation) [4]. In ulcerative colitis, it has been postulated that elevations in fecal lysozyme may be secondary to intestinal loss of granulocytes and their secretory granules [5]. Additionally, Paneth cell metaplasia, a phenomenon that occurs with various inflammatory conditions of the large intestine, may be a minor contributor to fecal lysozyme elevations [5]. Paneth cells are part of the intestinal epithelial lining found in the deepest part of intestinal crypts which are the crypts of Lieberkühn. Paneth cells contain lysozyme in their secretory granules, and combined with their phagocytic capability, help to regulate intestinal microbial flora [5].

Lysozyme is helpful in the determination of colonic inflammatory activity rather than small bowel disease [2]. Slightly elevated levels of lysozyme may be treated with anti-inflammatory agents or by removing the antagonist, such as enteroinvasive microorganisms or allergens. Moderate to high levels of lysozyme (>2,000) may indicate an active inflammatory bowel condition which often requires further testing such as colonoscopy. To rule out IBD, check fecal lactoferrin levels (elevated with IBD).

1. Saito H, Ksajima T, Masuda A, et al. Lysozyme localization in human gastric and duodenal epithelium. *Cell Tissue Res* 1988; 251:3-7-313.
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3. Klass HJ, Neale G. Serum and faecal lysozyme in inflammatory bowel disease. *Gut* 1978;19:233-9.
4. Geboes K, Van den Oord JJ, Rutgeerts P, et al. Immunohistochemical identification of lysozyme in pseudopyloric gland metaplasia in Crohn's disease. *Hepatogastroenterology* 1986;90:1121-8.
5. Stamp GWH, Poulosom R, Chung LP, et al. Lysozyme gene expression in inflammatory bowel disease. *Gastroenterol* 1992;103:532-538.

Secretory IgA (sIgA)

The concentration of sIgA is abnormally high in this fecal 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 allowing them to be excreted directly in the feces.

Elevated fecal sIgA is an appropriate response to an antigenic presence. Microbial and microscopic studies of the stool are useful in identifying if bacteria, yeast, or parasites are present. Eradication of the pathogenic microorganisms will bring sIgA back down into the normal range. Elevated sIgA levels have been observed in the absence of bacteria, yeast or parasites, in individuals with atopic conditions such as food allergies, urticaria, and dermatitis.

References:

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2. Roberts JA. Factors predisposing to urinary tract infections in children. *Ped Neph* 1996;10:517-522.
3. Carins J, Booth C. Salivary immunoglobulin-A as a marker of stress during strenuous physical training. *Aviat Space Environ Med* 2002;73(12):1203-7.
4. Teodosio MR, Oliveira ECM. Urinary secretory IgA after nutritional rehabilitation. *Braz J Med Biolog Res* 1999;32:421-426
5. Alverdy J. Effects of glutamine-supplemented diets on immunology of the gut. *J Parent Enteral Nutr* 1990;14(4):1095-1135.
6. Burke DJ, et al. Glutamine-supplemented total parenteral nutrition improves gut function. *Arch Surg* 1989;24:2396-2399.
7. Alverdy JA. The effect of total parenteral nutrition on gut lamina propria cells. *J Parent. Enteral Nutr* 1990;14(suppl).
8. Qamar A, Aboudola S, Warny M, et al. *Saccharomyces boulardii* stimulates intestinal immunoglobulin A immune response to clostridium difficile toxin A in mice. *Infect Immun* 2001;69(4):2762-5.
9. Buts JP, Bernasconi P, Vaerman JP, et al. Stimulation of secretory IgA and secretory component of immunoglobulins in small intestine of rats treated with *Saccharomyces boulardii*. *Dig Dis Sci* 1990;35(2):251-6.

Short Chain Fatty Acids (SCFAs)

The relative and/or total amounts of SCFAs are abnormal in this specimen. In infants, microbial colonization and subsequent SCFA production is a gradual process which is largely determined by environmental exposure, maternal gut microflora, breastfeeding, and possibly genetics [1]. The establishment of an adequate distribution of healthy flora in the gut is crucial in the health of both infants and adults because of the "competitive exclusion" process of dysbiotic flora [1]. Healthy microflora, such as *Lactobacillus* and *Bifidus* generate large amounts of SCFAs (acetic, propionic, butyric, and valeric) which decrease the pH of the intestine and therefore make the environment unsuitable for pathogens, including bacteria and yeast [1]. 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 prevention of intestinal dysbiosis [1].

The amount and types of SCFAs produced by colonic bacteria will depend on factors such as type of fiber consumed and overall intestinal health. For example, more SCFAs in general are produced by gums and pectins than oat fiber or corn bran; more propionate and butyrate are produced by bacterial activity on gums than pectins [2]. Antibiotic-induced diarrhea may be secondary to decreased colonic fermentation of carbohydrates and decreased overall production of SCFAs [3].

Studies show that SCFAs have numerous implications in gut physiology and health. SCFAs decrease inflammation, stimulate healing, and contribute to normal cell metabolism and differentiation [4]. Acetate, propionate and butyrate have been demonstrated to potentially improve the microcirculation in the intestinal mucosa thereby improving its growth and repair [5,6]. Rectal irrigation with SCFAs can result in improvement of ulcerative colitis [7]. Butyrate and propionate contribute to apoptosis of colorectal cells by increasing the production of reactive oxygen species in the gut [8]. Butyrate also has a positive effect on the differentiation of colonocytes, and this may account for the protective effect of dietary fiber against colorectal cancer [9]. Probiotics and increased dietary fiber can improve/normalize SCFA status.

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7. Breuer RI, Buto SK, Christ ML, et al. Rectal irrigation with short-chain fatty acids for distal ulcerative colitis. Preliminary report. *Dig Dis Sci* 1991;36(2):185-7.
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pH low

The pH of this stool sample (<6.0) is too acidic. Ideally, the pH of the stool is slightly acidic. This represents colonic pH, which is largely reflective of bacterial fermentation and putrefaction of intestinal contents. Healthy microflora such as *Lactobacillus* and *Bifidus* generate large amounts of short

chain fatty acids (acetic, propionic, butyric, and valeric), which lower colonic pH. Short chain fatty acids are byproducts of the bacterial fermentation process of dietary fiber by beneficial flora in the gut. An acidic pH, below 6.0, is usually reflective of a rapid transit time, e.g. diarrhea or loose stools. Further investigation as to the cause of diarrhea such as food allergy intolerance, viral, bacterial, parasitic infection, irritable bowel syndrome may be warranted. Additionally, research has indicated that an acidic pH (< 6.0) is common in individuals with lactose malabsorption [1]. Unabsorbed lactose in the gut can be hydrolysed by colonic bacteria forming volatile fatty acids which cause the stool to become acidic, often times accompanied by a sweet, sickly stool odor [1]. Hydrolysis of unabsorbed lactose and fermentation by colonic bacteria can also produce hydrogen (and carbon dioxide) which is then absorbed and excreted in the breath. This is the basis for the test for lactose malabsorption (lactose intolerance breath test).

Cooper BT. Lactase deficiency and lactose malabsorption. *Dig Dis* 1986;4:72-82.

Beneficial Flora

One or more of the expected or beneficial bacteria are low in this specimen. Normally abundant 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 lactobacillus and bifidobacterium species 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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