



LAB #: F000000-0000-0
 PATIENT: Sample Patient
 ID: P0000000000
 SEX: Male
 DOB: _____

AGE: 63

CLIENT #: 12345
 DOCTOR:
 Doctor's Data, Inc.
 3755 Illinois Ave.
 St. Charles, IL 60174 U.S.A.

Comprehensive Stool Analysis / Parasitology x2

BACTERIOLOGY CULTURE

Expected/Beneficial flora	Commensal (Imbalanced) flora	Dysbiotic flora
4+ Bacteroides fragilis group	4+ Alpha hemolytic strep	
4+ Bifidobacterium spp.	1+ Enterobacter sakazakii	
4+ Escherichia coli	4+ Gamma hemolytic strep	
NG Lactobacillus spp.	2+ Klebsiella pneumoniae ssp pneumoniae	
3+ Enterococcus spp.		
2+ Clostridium spp.		
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, 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
1+ Candida albicans	
1+ Candida parapsilosis	
1+ Zygosaccharomyces bailii	

MICROSCOPIC YEAST

Result:	Expected:
Mod	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: 03/02/2015
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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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PARASITOLOGY/MICROSCOPY *

Sample 1

None Ova or Parasites
 Few Yeast

Sample 2

None Ova or Parasites
 Mod Yeast

*A trichrome stain and concentrated iodine wet mount slide is read for each sample submitted.

PARASITOLOGY INFORMATION

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.

One negative parasitology x1 specimen does not rule out the possibility of parasitic disease, parasitology x3 is recommended. This exam is not designed to detect *Cryptosporidium* spp, *Cyclospora cayetanensis* or *Microsporidia* spp.

GIARDIA/CRYPTOSPORIDIUM IMMUNOASSAY

	Within	Outside	Reference Range
Giardia intestinalis	Neg	Neg	Neg
Cryptosporidium	Neg	Neg	Neg

Giardia intestinalis (lamblia) is a 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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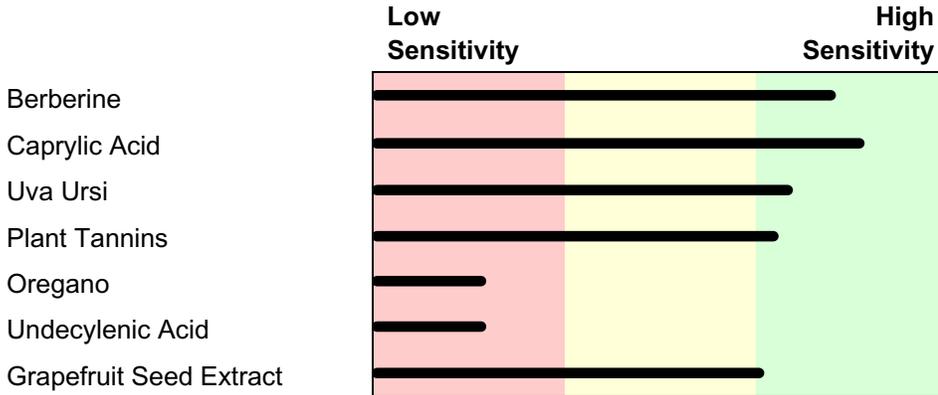


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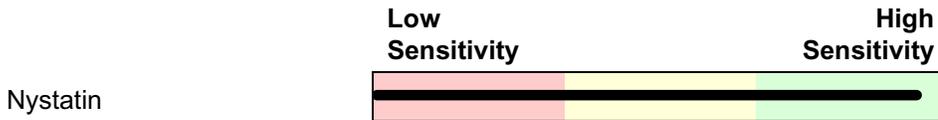
Yeast Susceptibilities: *Zygosaccharomyces bailii*

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.

Comments:

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

v10.11



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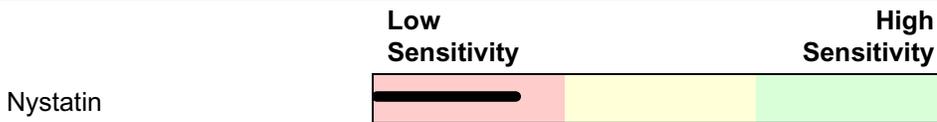
Yeast Susceptibilities: *Candida albicans*

NATURAL ANTIFUNGALS



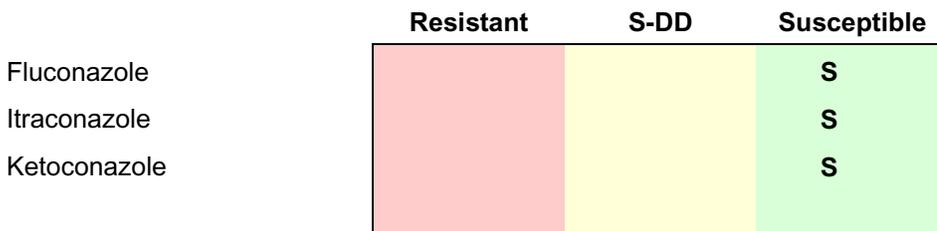
Natural antifungal agents may be useful for treatment of patients when organisms display in-vitro sensitivity to these agents. The test is performed by using standardized techniques and filter paper disks impregnated with the listed agent. Relative sensitivity is reported for each natural agent based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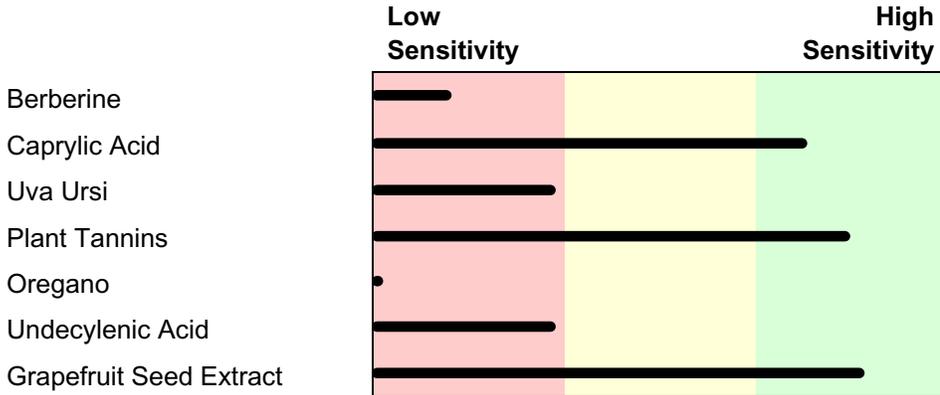


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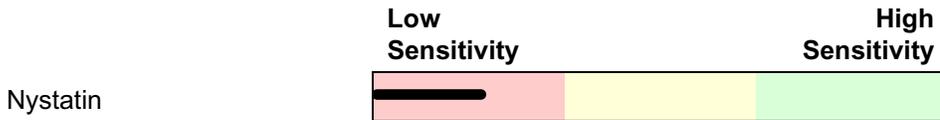
Yeast Susceptibilities: Candida parapsilosis

NATURAL ANTIFUNGALS



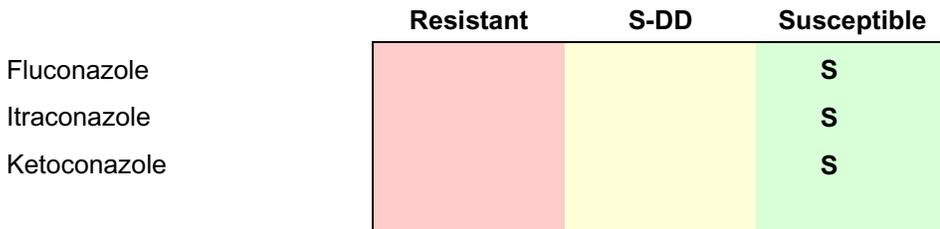
Natural antifungal agents may be useful for treatment of patients when organisms display in-vitro sensitivity to these agents. The test is performed by using standardized techniques and filter paper disks impregnated with the listed agent. Relative sensitivity is reported for each natural agent based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

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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.

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.

Washington W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, Woods, G. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th edition. Lippincott Williams and Wilkins; 2006. pg 931-939

Song Y, Liu C, Finegold SM. Real-Time PCR Quantitation of Clostridia in Feces of Autistic Children. Applied and Environmental Microbiology. Nov. 2004, 6459-6465.

Parracho H, Bingham MO, Gibson GR, McCartney AL. Differences Between the Gut Microflora of Children with Autistic Spectrum Disorders and That of Healthy Children. Journal of Medical Microbiology. 2005;54, 987-991.

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.

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.

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.
2. Van der Sluys Veer A, Brouwer J, Biemond I, et al. Fecal lysozyme in assessment of disease activity in inflammatory bowel disease. *Dig Dis & Sci.* 1998;43(3):590-5.
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, Poulsom 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:

1. Crago SS, Tomasi TB. Mucosal Antibodies, Food Allergy and Intolerance. Bailliere Tindall/W.B. Saunders 1987;167-89.
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.
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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.

Percival M. Intestinal Health. *Clin Nutr In.* 1997;5(5):1-6.

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Siitonen S, Vapaatalo H, Salminen S, et al. Effect of Lactobacilli GG Yoghurt in Prevention of Antibiotic Associated Diarrhea. *Ann Med.* 1990; 22:57-59.

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Valeur, N, et al. Colonization and Immunomodulation by Lactobacillus reuteri ATCC 55730 in the Human Gastrointestinal Tract. *Appl Environ. Microbiol.* 2004 Feb; 70(2):1176-81.

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