



LAB #: F131009-0114-1
 PATIENT: Anna M. Salanti
 ID: P130910009
 SEX: Female
 DOB: 01/26/1952 AGE: 61

CLIENT #: 27210
 DOCTOR: Brian Popiel, ND
 Lab Interpretation LLC
 18124 Wedge Pkwy 432
 Reno, NV 89511 USA

Comprehensive Stool Analysis

BACTERIOLOGY CULTURE

Expected/Beneficial flora

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

 3+ Clostridium spp.
 NG = No Growth

Commensal (Imbalanced) flora

2+ Enterobacter cloacae
 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

No yeast isolated

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: 10/06/2013
 Date Received: 10/09/2013
 Date Completed: 10/23/2013

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





LAB #: F131009-0114-1
 PATIENT: Anna M. Salanti
 ID: P130910009
 SEX: Female
 DOB: 01/26/1952 AGE: 61

CLIENT #: 27210
 DOCTOR: Brian Popiel, ND
 Lab Interpretation LLC
 18124 Wedge Pkwy 432
 Reno, NV 89511 USA

Comprehensive Stool Analysis

DIGESTION / ABSORPTION

	Within	Outside	Reference Range
Elastase	439		> 200 µg/mL
Fat Stain	None		None - Mod
Muscle fibers	Rare		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*		624	<= 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*	87.9		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.

Comments:

Date Collected: 10/06/2013
 Date Received: 10/09/2013
 Date Completed: 10/23/2013

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



LAB #: F131009-0114-1
 PATIENT: Anna M. Salanti
 ID: P130910009
 SEX: Female
 DOB: 01/26/1952 AGE: 61

CLIENT #: 27210
 DOCTOR: Brian Popiel, ND
 Lab Interpretation LLC
 18124 Wedge Pkwy 432
 Reno, NV 89511 USA

Comprehensive Stool Analysis

SHORT CHAIN FATTY ACIDS

	Within	Outside	Reference Range
% Acetate	67		36 - 74 %
% Propionate	11		9 - 32 %
% Butyrate	20		9 - 39 %
% Valerate	1.9		1 - 8 %
Butyrate	2.3		0.8 - 3.8 mg/mL
Total SCFA's	11		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	6.7		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	Loose/Watery	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.

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 (6.5 - 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.

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

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 I inflammatory bowel disease. *Gastroenterol* 1992;103:532-538.