

Gut: Stool Testing - Part 7

Comprehensive Stool Analysis / Parasitology x1

DIGESTION / ABSORPTION			
	Within	Outside	Reference Range
Elastase	> 500	> 200	> 200 µg/mL
Fat Stain	None	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
Lactoferrin	60.7	< 7.3	< 7.3 µg/mL
Calprotectin*	406	<= 50	<= 50 µg/g
Lysozyme*	298	<= 600	<= 600 ng/mL
White Blood Cells	None	None - Rare	
Mucus	Neg	Neg	

Lactoferrin and Calprotectin are reliable markers for differentiating organic inflammation (IBD) from functional symptoms (IBS) and for management of IBD. Monitoring levels of fecal lactoferrin and calprotectin can play an essential role in determining the effectiveness of therapy, are good predictors of IBD remission, and can indicate a low risk of relapse. **Lysozyme*** is an enzyme secreted at the site of inflammation in the GI tract and elevated levels have been identified in IBD patients. **White Blood Cells (WBC)** and **Mucus** in the stool can occur with bacterial and parasitic infections, with mucosal irritation, and inflammatory bowel diseases such as Crohn's disease or ulcerative colitis.

IMMUNOLOGY			
	Within	Outside	Reference Range
Secretory IgA*	89.1	51 - 204	51 - 204 mg/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.

>200 µg/gm	50-200 µg/gm	<50 µg/gm
Active IBD, colitis, cancer	Chronic inflammation, NSAIDs, inactive IBD	Normal

Fecal calprotectin
disease association



Tracey O'Shea: Calprotectin is a small calcium-binding protein that's found in abundance, particularly in neutrophilic granulocytes. It's another marker of gut inflammation, and it can indicate the presence of neutrophils and inflammation within the [gastrointestinal] (GI) mucosa. Some research indicates calprotectin is more accurate for diagnosis of [inflammatory bowel disease] (IBD) than lactoferrin marker. Most labs now use the monoclonal fecal calprotectin antibody, which is superior to the older polyclonal technique.

New studies have also found that certain subsets of patients [with irritable bowel syndrome (IBS)] also demonstrate elevated calprotectin greater than 50. This finding is significant, as it suggests that inflammation plays an important role in the pathophysiology of IBS, especially in those individuals. Indications of elevated calprotectin depend on the level. As displayed [in] this table, you can see in the slide greater than 200 is thought to be indicative of active IBD, colitis, or even cancer; 50 to 200 usually is indicative of chronic inflammation, chronic high-dose [non-steroidal anti-inflammatory drug] (NSAID) use, or inactive IBD. And less than 50 for a fecal calprotectin is considered within [the] normal and optimal range. So the patient on this particular slide had been treated for [small intestinal bacterial overgrowth] (SIBO) and *Candida* several times. And it turned out that once they came, we did a full stool test, including the lactoferrin and calprotectin test. And at the end of the day, she was diagnosed with Crohn's [disease].

Intestinal Health			
Digestion			
	Result		Normal
Elastase-1	317		>200 ug/g
Steatocrit	14		<15 %
GI Markers			
	Result		Normal
b-Glucuronidase	2707	High	<2486 U/mL
Occult Blood - FIT	0		<10 ug/g
Immune Response			
	Result		Normal
Secretory IgA	198	Low	510 - 2010 ug/g
Anti-gliadin IgA	86		0 - 157 U/L
Inflammation			
	Result		Normal
Calprotectin	266	High	<173 ug/g



In my experience, I think the calprotectin numbers can be a little bit more variable than lactoferrin within these stool tests. We've had patients with calprotectin numbers [of] about 200 that are dealing with a gut infection or have an unknown gluten sensitivity or misdiagnosed celiac disease. So even though we gave those ranges about what those numbers indicate, I think you still have to use your judgment and your critical thinking skills to see what else is going on in the stool test, do your due diligence, [and] retest those markers, with Labcorp or Quest. But I have seen those numbers go up pretty high in people who do have, like we said, misdiagnosed celiac disease or a pretty significant gut infection. This particular patient in the slide is a 42-year-old male with rheumatoid arthritis, obesity, and chronic GI discomfort. He also had a parasite infection at the time that's not shown on this slide. All of these markers ended up improving when we treated the parasite with an antiparasitic protocol.

So when you see an elevated calprotectin above 200, it's important to order the lactoferrin if you don't already have it as part of the lab. Follow up with the repeat calprotectin markers from another lab like Labcorp or Quest just to double-check the results and cover your bases.

Positive	Negative	Interpretative Report
Only pANCA	-	"Suggestive of ulcerative colitis."
Only one of ACCA, ALCA, AMCA or gASCA	pANCA	"Suggestive of Crohn's disease. Pattern is not conclusive for disease behavior risk stratification."
Only one of ACCA, ALCA, AMCA or gASCA + pANCA	-	"Suggestive of inflammatory bowel disease. Pattern is not conclusive for any specific disease form."
Any two of ACCA, ALCA, AMCA or gASCA + pANCA	pANCA	"Suggestive of Crohn's disease with high risk of aggressive disease behavior (development of strictures or fistulae)."
Any three or more of ACCA, ALCA, AMCA or gASCA + pANCA	pANCA	"Suggestive of Crohn's disease with the very high risk of aggressive disease behavior (development of strictures or fistulae)."
-	ALL markers	"Pattern is not suggestive of inflammatory bowel disease."

ASCA - anti-*Saccharomyces cerevisiae* antibodies
ALCA - antilaminaribioside carbohydrate antibodies (IgG)
ACCA - antichitobioside carbohydrate antibodies (IgA)

AMCA - antimannobioside carbohydrate antibodies (IgG)
gASCA - anti-*Saccharomyces cerevisiae* antibodies (IgG)
pANCA - atypical perinuclear antineutrophil cytoplasmic antibodies

When you see calprotectin above 200 or lactoferrin above 50, you can run this [IBD] expanded antibody panel with Labcorp. The test number is 162045, and it assesses new antibody markers that are quite sensitive and specific for IBD. So we've included an interpretation matrix on this slide here, and I'll let you read through it. But you can see that if you only have one that's positive, it's suggestive of ulcerative colitis. If you only have one of the ACCA, ALCA, AMCA, or gASCA, [that's] suggestive of Crohn's disease but not conclusive for disease behavior. So you can see that this goes through and ultimately, if three or more of the antibodies that we just talked about are positive, that's also suggestive of Crohn's disease with a very high risk of aggressive disease behavior. If all markers are negative, then the pattern would, of course, be suggested that [IBD] is not present.

We'll often use this as an interim step if the patient has positive levels that are suggestive of IBD and fecal calprotectin or lactoferrin that's elevated. Then, we'll run this blood test, and depending on the results of the blood test, we may refer out to a gastroenterologist so [the patient] can have a colonoscopy. Another option is that if the markers are borderline, stool markers are borderline for calprotectin or lactoferrin, or the lysozyme, then you can treat for any gut pathogens that are present that could be causing the elevations in the markers and then retest the stool a month later after treatment. If those markers are still elevated, then you could do this blood test, and depending on the results, then refer out. So there [are] lots of different ways to manage the different testing, and I think it makes sense to do these in a layered approach.



Comprehensive Stool Analysis / Parasitology x3

DIGESTION / ABSORPTION			
	Within	Outside	Reference Range
Elastase	197	> 200	> 200 µg/mL
Fat Stain	Few	None - Mod	None - Mod
Muscle fibers	None	None - Rare	None - Rare
Vegetable fibers	Rare	None - Few	None - Few
Carbohydrates	Int	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*	3800	<= 600	<= 600 ng/mL
Lactoferrin	1.3	< 7.3	< 7.3 µg/mL
White Blood Cells	None	None - Rare	None - Rare
Mucus	Neg	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*	436	51 - 204	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.

The next marker that we're going to talk about is lysozyme. You've heard us mention that a few times throughout this presentation. So [in] this test example here, it's important to note, it looks a little bit different because the calprotectin is missing off of this specific report before Doctor's Data added the calprotectin marker. So lysozyme is an enzyme that catalyzes the hydrolysis of specific glycosidic bonds in the mucopolysaccharides that constitute the cell wall of gram-positive bacteria. The antibacterial defense present in the GI tract is secreted by the granulocytes, macrophages, paneth cells, and Brunner's glands, as well as the normal colonic crypt cells. The main source for fecal lysozyme is the intestinal granulocytes, and it's considered a general marker of inflammation in the gut. Unlike calprotectin and lactoferrin, lysozyme is not specific for IBD, although it can be elevated. But by itself, [it] is not a marker that's specific for IBD.

Out of all the lab companies we've discussed, Doctor's Data is the only lab that still has this marker on its comprehensive stool panel. So when you see elevated lysozyme, and it's really elevated, and then you see lactoferrin and calprotectin are also significantly elevated, then that would point to IBD. But if you only see elevated lysozyme, and the lactoferrin and calprotectin are normal, then it is likely just a marker of gut infection or some other dysbiosis or imbalance. So the result on this test is somewhat in between because we have a low fecal elastase, indicating pancreatic insufficiency, we've got carbohydrate malabsorption, and we've got lysozyme of 3,800, which is quite high. And then you've got normal lactoferrin, [and] secretory [immunoglobulin A] (IgA) that's elevated. So there's definitely some inflammation here. And in this

situation, I [would] probably treat any gut infections that were present or SIBO if it were there, and then retest and see what happens with the lysozyme.

If this patient has symptoms [that] are indicative of [IBD], then I might go ahead and run the antibody panel that we just discussed on the last slide or do lactoferrin and calprotectin through Labcorp or Quest. And if those are positive, then we'll start referring to the colonoscopy, especially if the lysozyme and the other markers are high.

Fecal lysozyme disease association

600-2,000 ng/mL	>2,000 ng/mL
Yeast, dysbiotic bacteria, parasites	Active IBD

As I mentioned, moderate elevations of fecal lysozyme are commonly associated with significant overgrowth of gut pathogens or food antigens. So if you see levels between 600 and 2,000 nanograms per milliliter, you'll see that simultaneously with fungal overgrowth, dysbiotic bacteria, and parasites. But really high levels above 2,000 nanograms per milliliter are often indicators of chronic inflammatory bowel disease like Crohn's [disease] or ulcerative colitis. And also other non-inflammatory bowel disease, [GI] disorders with severe diarrhea.

Lysozyme is helpful in the determination of colonic inflammation activity rather than small bowel disease. So it's specific for colon inflammation as opposed to inflammation of the small intestine. When you see the slightly elevated levels [as] we talked about, you'd treat for any pathogens that are present, and then you would retest and see what's happening there. If it's still really high or slightly elevated, you would be looking for other causes of gut inflammation like IBD or something that you missed in the diagnostic workup. You also, of course, want to check calprotectin and lactoferrin, as we discussed, to determine the likelihood of IBD. So this can be a complement marker, and it can help guide you to further testing.



Comprehensive Stool Analysis / Parasitology x3

DIGESTION / ABSORPTION			
	Within	Outside	Reference Range
Elastase	438	> 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
Lactoferrin	59.3	< 7.3	µg/mL
Calprotectin*	145	<= 50	µg/g
Lysozyme*	5190	<= 600	ng/mL
White Blood Cells	None	None - Rare	
Mucus	Neg	Neg	

Lactoferrin and Calprotectin are reliable markers for differentiating organic inflammation (IBD) from functional symptoms (IBS) and for management of IBD. Monitoring levels of fecal lactoferrin and calprotectin can play an essential role in determining the effectiveness of therapy, are good predictors of IBD remission, and can indicate a low risk of relapse. **Lysozyme*** is an enzyme secreted at the site of inflammation in the GI tract and elevated levels have been identified in IBD patients. **White Blood Cells (WBC)** and **Mucus** in the stool can occur with bacterial and parasitic infections, with mucosal irritation, and inflammatory bowel diseases such as Crohn's disease or ulcerative colitis.

IMMUNOLOGY			
	Within	Outside	Reference Range
Secretory IgA*	553	51 - 204	mg/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.

This patient is a 22-year-old female with right lower quadrant pain and chronic loose stool. You can see that she had significantly elevated lactoferrin, calprotectin, and lysozyme. Her lysozyme is technically in the active IBD category that we talked about, above 2,000. And the calprotectin and lactoferrin are in this inactive IBD category. Remember, those are just averages. There's not a hard and fast rule. So we still have to do some diving. We sent this patient to the GI [doctor] for a colonoscopy; there wasn't any evidence of IBD in the study. Her symptoms are somewhat controlled by a GAPS (gut and psychology syndrome) diet or a specific carbohydrate diet, but still symptomatic and still had structural issues. At the end of the day, we also found [*Cryptosporidium*], fungal overgrowth, and dysbiosis. After treating with a couple of different protocols, the numbers [dropped] significantly. So her numbers were really driven by the infections and the dysbiosis.



Comprehensive Stool Analysis / Parasitology x3

DIGESTION / ABSORPTION			
	Within	Outside	Reference Range
Elastase	> 500	> 200	> 200 µg/mL
Fat Stain	Few	None - Mod	None - Mod
Muscle fibers	None	None - Rare	None - Rare
Vegetable fibers	Few	None - Few	None - Few
Carbohydrates	Neg	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*	717	<= 600	<= 600 ng/mL
Lactoferrin	7.6	< 7.3	< 7.3 µg/mL
White Blood Cells	None	None - Rare	None - Rare
Mucus	Neg	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*	2.7	51 - 204	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.

Here's another example of a more moderate elevation. This is a 44-year-old male with IBS and SIBO and also fungal overgrowth, which is possibly contributing to the really low secretory IgA that you see here. Earlier, we talked about how *Candida* can secrete substances that break down secretory IgA, and that's likely the connection. The lysozyme is only mildly elevated at 717, so that's definitely in the inactive IBD range. And the lactoferrin was just barely out of the reference range. Again, [that's] not in the active IBD range by any stretch. The calprotectin wasn't on the test when we did this. But in this case, I would not refer out for a colonoscopy because of these numbers. At least certainly not immediately, and we would just treat the underlying gut conditions, which we did, and then retest. And if the markers are normal, which they were, then you move on to the next thing, which is what happened in this case.



Comprehensive Stool Analysis / Parasitology x3

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		Int	Neg

Elastase findings can be used for the diagnosis of 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
Lactoferrin	< 0.5		< 7.3 µg/mL
Calprotectin*	< 10		10 - 50 µg/g
Lysozyme*	324		<= 600 ng/mL
White Blood Cells	None		None - Rare
Mucus		Pos	Neg

Lactoferrin and **Calprotectin** are reliable markers for differentiating organic inflammation (IBD) from function symptoms (IBS) and for management of IBD. Monitoring levels of fecal lactoferrin and calprotectin can play an essential role in determining the effectiveness of therapy, are good predictors of IBD remission, and can indicate a low risk of relapse. **Lysozyme*** is an enzyme secreted at the site of inflammation in the GI tract and elevated levels have been identified in IBD patients. **White Blood Cells** (WBC) and **Mucus** in the stool can occur with bacterial and parasitic infections, with mucosal irritation, and inflammatory bowel diseases such as Crohn's disease or ulcerative colitis.

IMMUNOLOGY			
	Within	Outside	Reference Range
Secretory IgA*		50.0	51 - 204 mg/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.

There are two other inflammatory markers we haven't discussed yet: white blood cells and mucus. These can occur with bacterial and parasite infections or gut inflammation. This is a 34-year-old female result on this slide with [the] chief complaint [of] difficulty of losing weight, inflamed lymph nodes, poor dental health, and fatigue. She also had chronic constipation with dry hard stool, and she had SIBO, which was impairing her carbohydrate absorption, as you can see by the positive result for carbohydrate malabsorption. So all of these markers normalized after she was successfully treated for SIBO.