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SUPPORT GUIDE

CDSA & CDSA 2.0

SUPPORT GUIDE

Digestion/Absorption

Pancreatic Elastase

What is Pancreatic Elastase?

PE is a digestive enzyme secreted exclusively by the human pancreas. This enzyme breaks down protein in food. Unlike other pancreatic enzymes, it is highly stable and is not degraded during passage through the gut.[1]

How can I tell if my patient has pancreatic insufficiency?

Pancreatic Elastase (PE) is a simple, noninvasive method of assessing exocrine pancreatic function, allowing the clinician to establish a prompt and reliable diagnosis with high degrees of sensitivity (63%-100%) and specificity (93%-98%) in suspected cases of pancreatic insufficiency. Sensitivity is lower in milder cases of pancreatic insufficiency (63%), but excellent (100%) in moderate and severe cases.[2-5]

Why is PE a superior noninvasive measurement of pancreatic insufficiency?

- PE has a strong correlation with the gold standard test for pancreatic insufficiency (secretin-pancreozymin test).[5] Thus there is a direct correlation between PE and pancreatic function.
- PE is produced exclusively in the pancreas and as such has absolute pancreatic specificity.[6] There is little or no interference by other enzymes in the gastrointesinal (GI) tract. What is measured is strictly the function of the pancreas.
- PE results are not affected by pancreatic enzyme replacement therapy.[6] Therefore, patients are not required to stop supplementation prior to providing a sample. Other non-invasive markers for exocrine pancreatic function require the cessation of enzyme supplements up to 72 hours before the test.
- PE is not degraded during intestinal transit, nor is it affected greatly by increases or decreases in intestinal transit times.[7] Thus, its fecal concentration accurately reflects pancreatic exocrine function. Only in cases of liquid diarrhea is the sensitivity affected.[8]
- PE levels are 5-fold to 6-fold higher in feces than in duodenal juice. This reflects the extraordinary stability of PE in the GI tract.[9]

How does the PE test from Genova Diagnostics differ from other from laboratories performing this test?

PE can be measured using monoclonal and, more recently, polyclonal antibodies. Genova Diagnostics assesses PE concentrations using an ELISA methodology based on monoclonal antibodies. These antibodies are highly specific for measuring exocrine pancreatic function and have been validated through research. In contrast, the polyclonal antibody assay for PE has been less validated, and measures an unknown antigen.[10]

What are the medical indications for this test?

- **Diabetes** —Reduced PE is found in over 50% of type 1 diabetics and 35% of type 2 diabetics.[3] Diabetes secondary to exocrine disease could be much more frequent than previously thought; studies have shown that low PE is closely related to glycemic control.[11]

Turn-around Time 14 days

• **Gallstone or post-cholecystectomy** —Exocrine pancreatic function is frequently impaired in gallstone sufferers and post-cholecystectomy patients. There is a high prevalence of pathological changes in exocrine pancreatic function in patients with gallstones.[12]

• **Osteoporosis** – Nearly one third of patients with osteoporosis have reduced concentrations of PE.[13] Vitamin D levels may also be significantly decreased in these patients.[14]

• **Age ≥35 years** —Pancreatic function decreases with age.[15] Nutrient deficiencies may develop as a result of inadequate nutrient metabolism, particularly if undiagnosed or untreated for a prolonged time.

• **Cystic fibrosis patients** —PE can be used to diagnose enzyme need and to monitor the effectiveness of pancreatic supplementation.[16]

PE is also useful in monitoring exocrine pancreatic function caused by:

• **Chronic pancreatitis**

• **Autoimmunopathies and connective tissue diseases**

• **Chronic inflammatory bowel disease**

• **Intestinal malabsorption with mucosal atrophy** – a transient reduction of PE can occur with villous atrophy. After mucosal regeneration, PE levels return to normal. PE can therefore be used as a monitoring tool in patients with celiac disease and other malabsorptive conditions.[17]

How do I interpret PE levels?

PE values > 200 mcg/g rule out significant pancreatic dysfunction [18]

PE values of 100-200 mcg/g suggest mild to moderate pancreatic insufficiency[19]

PE values <100 mcg/g reflect moderate to severe pancreatic insufficiency[7]

Healthy individuals produce on average 500 mcg/g of PE. Thus, levels below 500 mcg/g and above 200 mcg/g suggest a deviation from optimal pancreatic function. The clinician should therefore consider digestive enzyme supplementation if one or more of the following conditions is present:

- Loose, watery stools
- Undigested food in the stools
- Post-prandial abdominal pain
- Nausea or colicky abdominal pain
- Gastroesophageal reflux symptoms
- Bloating or food intolerance

References

1 Borowitz D et al. Use of fecal elastase-1 to classify pancreatic status in patients with cystic fibrosis. J Pediatr. 2004 Sep;145(3):322-6.

2 Gullo L, et al. Faecal elastase 1 in children with cystic fibrosis. Eur J Pediatrics 1997;156(10):770-2.

3 Hardt PD, et al. Pancreatic exocrine function in patients with type 1 and type 2 diabetes. Acta Diabetol 2000;37(3):105-10.

4 Stein J, Jet al. Immunoreactive elastase I: clinical evaluation of a new noninvasive test of pancreatic function. Clin Chem 1996 Feb;42(2):222-6

5 Loser C, Mollgaard A, Folsch UR. Faecal elastase 1: a novel, highly sensitive and specific tubeless pancreatic function test. Gut 1996;39(4):580-6.

6 Gullo L, et al. Fecal elastase 1 determination in chronic pancreatitis. Dig Dis Sci 1999;44(1):210-3.

7 Dominguez-Munoz JE, et al. Fecal elastase test: evaluation of a new noninvasive pancreatic function test. Am J Gastroenterology 1995;90(10):1834-7.

8 Brydon WG et al. Limitations of faecal elastase-1 and chymotrypsin as tests of exocrine pancreatic disease in adults. Ann Clin Biochem. 2004 Jan;41(Pt 1):78-81.

9 Sziegoleit A, Lindner D. Studies on the sterol-binding capacity of human pancreatic elastase-1. Gastroenterology 1991;100:768-74.

10 Hardt PD et al. The commercially available ELISA for pancreatic elastase 1 based on polyclonal antibodies does measure an as yet unknown antigen different from purified elastase 1. Binding studies and clinical use in patients with exocrine pancreatic insufficiency. Z Gastroenterol. 2003 Sep;41(9):903-6.

11 Rathmann W, et al. Pancreatic exocrine insufficiency in type 1 and type 2 diabetes. Exp Clin Endocrinol Diabetes 2000; 108(supp 1):16.

12 Hardt PD, et al. Pathological pancreatic exocrine function and duct morphology in patients with cholelithiasis. Dig Dis Sci. 2001 Mar;46(3):536-9.

13 Teichmann J, et al. Pancreatic elastase 1 in patients with osteoporosis [abstract] Kongress der Deutschen Gesellschaft fur Innere Medizin; 2001 Apr 22; Wiesbaden, Germany.

14 Moran CE, et al. Bone density in patients with pancreatic insufficiency and steatorrhea. Am J Gastroenterology 1997;92(5):867-71.

15 Laugier R, et al. Changes in pancreatic exocrine secretion with age: pancreatic exocrine secretion does decrease in the elderly. Digestion 1991;50(3-4):202-11.

16 Cade A, et al. Evaluation of fecal pancreatic function in children with cystic fibrosis. Pediatr Pulmonol 2000;29(3):172-6.

17 Walkowiak J, Herzig KH. Fecal elastase-1 is decreased in villous atrophy regardless of the underlying disease. Eur J Clin Invest. 2001 May;31(5):425-30.

18 Ka tschinski et al. Duodenal secretion and fecal excretion of pancreatic elastase-1 in healthy humans and patients with chronic pancreatitis. Pancreas. 1997 Aug;15(2):191-200.

Digestion/Absorption
Chymotrypsin

What is Chymotrypsin?

Chymotrypsin is a protein-digesting enzyme secreted by the pancreas. It is useful in monitoring patients who have moderate to severe pancreatic dysfunction.[1]

What are the clinical indications for assessing chymotrypsin?

Stool enzymes reflect the amount of proteolytic enzymes that the pancreas secretes into the intestine. As both pancreatic elastase (PE) and chymotrypsin are indicators of exocrine pancreatic function, the clinical indications for PE also apply to chymotrypsin. Therefore consider assessing patients with the following symptoms:

- Loose, watery stools
- Undigested food in the stools
- Post-prandial abdominal pain
- Nausea or colicky abdominal pain
- Gastroesophageal reflux symptoms
- Bloating or food intolerance

Other conditions that have been scientifically proven to be associated with reduced chymotrypsin include:

- Diabetes[2]
- Cystic Fibrosis[3]
- Chronic pancreatitis[4]
- Malabsorption[5]

What is the difference between chymotrypsin and pancreatic elastase (PE)?

While both chymotrypsin and PE are markers of exocrine pancreatic function, there are some distinct differences between the two tests. Chymotrypsin was the first non-invasive exocrine pancreatic test to be discovered and is a reflection of chymotrypsin activity in the pancreas. The more recently discovered PE reflects trypsin, chymotrypsin, amylase and lipase activity.[6] Both chymotrypsin and PE are highly accurate in distinguishing between pancreatic maldigestion and intestinal malabsorption (82% and 92%, respectively).[7] PE has been found to be more sensitive and specific than chymotrypsin.[8] In clinical studies, PE has been shown to correlate with the gold standard in pancreatic testing (secretin-pancreozymin test).[9] PE is not affect by bovine or porcine enzyme supplements[8], so patients do not have to discontinue therapy to assess baseline levels. Chymotrypsin is affected by exogenous supplementation which makes it an ideal tool to monitor dosing adequacy. When chymotrypsin values fall within the reference range in supplemented individuals, the clinician can be confident that an appropriate dosage of digestive enzymes is being administered. In summary, PE is a more accurate non-invasive marker to assess exocrine pancreatic function, and chymotrypsin is the preferred marker to monitor enzyme supplementation.[10]

Turn-around Time 14 days

How do I interpret the results?

Low levels of chymotrypsin (< 0.9 U/g) are indicative of exocrine pancreatic insufficiency. Therapy should include exogenous supplementation of pancreatic enzymes including lipase.[11]

Elevated levels of chymotrypsin (> 26.8 U/g) suggest a rapid transit time (diarrhea). A faster transit time reduces the intestinal degradation of chymotrypsin, which results in an increased recovery of this enzyme.[12] Chymotrypsin could also be elevated with excess pancreatic enzyme supplementation.

Are their any contra-indications with this test?

In general, chymotrypsin is nearly always present in the stool in measurable quantities in normal individuals. In those with exocrine pancreatic dysfunction, there is less activity. The exception is in patients with watery diarrhea. Levels may appear normal, however this is due to reduced proteolytic degradation from a rapid transit time, thus higher baseline levels.[12]

What further testing might be indicated?

Exocrine pancreatic dysfunction can mimic the symptoms of other diseases and imbalances such as celiac disease, giardiasis and small bowel bacterial overgrowth.[5] Consider the following additional tests to determine the underlying etiology of imbalance:

- **Celiac Panel**
- **Parasitology Profile**
- **Bacterial Overgrowth of the Small Intestine Breath Test**
- **Allergy Antibody Assessment**

Pancreatic dysfunction typically leads to malabsorption, the severity of which is relative to the degree of exocrine pancreatic impairment. Assessment of absorptive markers (triglycerides, long chain fatty acids, cholesterol, and phospholipids) will provide valuable insight into the degree of malabsorption present.

References

1 Bockus Gastroenterology. Haubrich WS,Schaffner F, Berk JE (eds). Philadelphia, WB Saunders Co, 5th Ed, 1995 p 2844.

2 Sidhu SS et al. Ketosis resistant diabetes of the young: a profile of its exocrine and endocrine pancreatic dysfunction. Indian J Physiol Pharmacol. 1994 Oct;38(4):289-93.

3 Scotta MS et al. Fecal chymotrypsin: a new diagnostic test for exocrine pancreatic insufficiency in children with cystic fibrosis. Clin Biochem. 1985 Aug;18(4):233-4.

4 Goldberg DM. Proteases in the evaluation of pancreatic function and pancreatic disease. Clin Chim Acta. 2000 Feb 15;291(2):201-21.

5 Keller J, Layer P. Pancreatic Enzyme Supplementation Therapy. Curr Treat Options Gastroenterol. 2003 Oct;6(5):369-374

6 Katschinski M et al. Duodenal secretion and fecal excretion of pancreatic elastase-1 in healthy humans and patients with chronic pancreatitis. Pancreas. 1997 Aug;15(2):191-200.

7 Carroccio A et al. Diagnostic accuracy of fecal elastase 1 assay in patients with pancreatic maldigestion or intestinal malabsorption: a collaborative study of the Italian Society of Pediatric Gastroenterology and Hepatology. Dig Dis Sci. 2001 Jun;46(6):1335-42.

8 Dominici R, Franzini C. Fecal elastase-1 as a test for pancreatic function: a review. Clin Chem Lab Med. 2002 Apr;40(4):325-32.

9 Loser C, Mollgaard A, Folsch UR. Faecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test. Gut. 1996 Oct;39(4):580-6.

10 Walkowiak J et al. Indirect pancreatic function tests in children. J Pediatr Gastroenterol Nutr. 2005 Feb;40(2):107-14.

11 Layer P, Groger G. Fate of pancreatic enzymes in the human intestinal lumen in health and pancreatic insufficiency. Digestion. 1993;54 Suppl 2:10-4.

12 Brydon WG, Kingstone K, Ghosh S. Limitations of faecal elastase-1 and chymotrypsin as tests of exocrine pancreatic disease in adults. Ann Clin Biochem. 2004 Jan;41(Pt 1):78-81.

Digestion/Absorption

Putrefactive Short Chain Fatty Acids

What are Putrefactive Short Chain Fatty Acids (SCFAs)?

Valerate, isovalerate and isobutyrate constitute the putrefactive short chain fatty acids (SCFAs). Elevated levels result from bacterial fermentation of undigested protein.

What is the clinical significance of elevated putrefactive SCFAs?

Elevated levels of these three SCFAs result from anaerobic bacterial fermentation of polypeptides and amino acids,[1] and suggest hypochlorhydria, exocrine pancreatic insufficiency,[2] and/or protein malabsorption.[3,4]

Are elevated levels of putrefactive SCFAs always due to maldigestion or malabsorption?

No. Other possible causes include:

- **Bacterial overgrowth in the small intestine** (BOSI)— proteins may be fermented before they are fully digested.[5,6] Look for elevated levels of Beneficial SCFAs and n-butyrate.
- **Gastrointestinal disease**— due to the fermentation of blood or mucosal cells delivered to the colon.[7]
- **Rapid transit time**— results in too little time for digestion and absorption of dietary peptides and amino acids.[8]

How can I determine the cause of elevated putrefactive SCFAs?

Signs, symptoms, and other laboratory abnormalities may help to suggest contributing factors:

- **Hypochlorhydria**—Possible positive *Helicobacter pylori* antigen, dyspepsia, or B12 insufficiency.
- **Pancreatic insufficiency**—Low level of pancreatic elastase or chymotrypsin, possible abdominal discomfort or bloating after eating.
- **Malabsorption**—Food in the stool, greasy stools, difficulty gaining weight, gluten intolerance, etc.
- **Bacterial Overgrowth of the Small Intestine** (BOSI)—Elevated Total SCFAs and n-butyrate, possible history of hypochlorhydria or bowel stasis, possible gas and post-prandial bloating. Consider a breath test for BOSI.
- **Gastrointestinal disease**—Positive fecal occult blood, possible mucus in the stool, signs and symptoms of various disorders.

Turn-around Time 14 days

How do putrefactive SCFAs differ from the other SCFAs on the report?

Beneficial SCFAs include n-butyrate, propionate and acetate, and are produced by bacterial fermentation of carbohydrates, especially non-digestible fiber. These SCFAs are beneficial to the colon[9-11] and provide daily energy for the colonocytes.[12]

Valerate, isovalerate and isobutyrate are produced exclusively by fermentation of protein. These SCFAs are putrefactive, and suggest underlying protein maldigestion, malabsorption, or BOSI.

How should elevated levels of putrefactive short chain fatty acids be treated?

Treatment should be aimed at the underlying cause:

- Betaine HCl with pepsin, and/or digestive enzymes
- Eradication of *H. pylori* infection
- Treatment for BOSI

What further testing might be indicated?

- **Bacterial Overgrowth of the Small Intestine Breath test** – consider if Beneficial SCFAs and n-butyrate are also elevated
- ***Helicobacter pylori* stool antigen test**

References

1 Zarling EJ, Ruchim MA. Protein origin of the volatile fatty acids isobutyrate and isovalerate in human stool. J Lab Clin Med 1987 May;109(5):566-70.

2 Nakamura T, et al. Short-chain carboxylic acid in the feces in patients with pancreatic insufficiency. Acta Gastroenterol Belg 1993 Sep-Dec;56(5-6):326-31.

3 Vaisman N, Tabachnik E, Sklan D. Short-chain fatty acid absorption in patients with cystic fibrosis. J Pediatr Gastroenterol Nutr 1992 Aug;15(2):146-9.

4 Prizont R, Whitehead JS, Kim YS. Short chain fatty acids in rats with jejunal blind loops. I. Analysis of SCFA in small intestine, cecum, feces, and plasma. Gastroenterology 1975 Dec;69(6):1254-64.

5 Cater RE 2nd. The clinical importance of hypochlorhydria (a consequence of chronic Helicobacter infection): its possible etiological role in mineral and amino acid malabsorption, depression, and other syndromes. Med Hypotheses 1992 Dec;39(4):375-83.

6 Hoverstad T, et al. Short-chain fatty acids in the small-bowel bacterial overgrowth syndrome. Scand J Gastroenterol 1985 May;20(4):492-9.

7 Rasmussen HS, Holtug K, Mortensen PB. Degradation of amino acids to short-chain fatty acids in humans. An in vitro study. Scand J Gastroenterol 1988 Mar;23(2):178-82.

8 Oufir LE, et al. Relationships between transit time in man and in vitro fermentation of dietary fiber by fecal bacteria. Eur J Clin Nutr 2000 Aug;54(8):603-9.

9 Royall D. Clinical significance of colonic fermentation. Am J Gastroenterol 1990;85(10):1307-12.

10 Cummings JH. Short chain fatty acids in the human colon. Gut 1981;22:763-79.

11 Mortensen FV, et al. Short chain fatty acids dilate isolated human colonic resistance arteries. Gut 1990;31:1391-4.

12 Rombeau JL, Kripke SA. Metabolic and intestinal effects of short-chain fatty acids. JPEN J Parenter Enteral Nutr 1990;14(5):181S-4S.

Digestion/Absorption

Triglycerides, Long Chain Fatty Acids (LCFAs), Cholesterol, Phospholipids and Total Fecal Fat

What are fecal fats?

Fecal fats include triglycerides, long chain fatty acids(LCFAs), cholesterol and phospholipids. They are derived predominately from the dietary ingestion of fat, and provide important clues about digestion and absorption.

How are dietary fats metabolized?

Fat digestion and absorption involves four distinct phases and includes the pancreas, liver, intestinal mucosa and lymphatics. The average adult consumes about 125g of fat per day.[1] Initially, dietary fats need to be broken down to free fatty acids and monoglycerides. This requires pancreatic lipase and bile acids which transition the lipids into a water soluble form. In this capacity, they are able to enter the mucosal cell. Once inside the mucosal cells, free fatty acids and monoglycerides are repackaged into chylomicrons –a specific class of lipoproteins. Chylomicrons are then transported to the lymphatics and thereafter into general circulation for utilization in liver, muscle and adipose tissue.[2]

What is the significance of elevated fecal fats?

Fat malabsorption can occur from maldigestion and/or impaired uptake of fatty acids.[3] Physiological imbalances or disorders that impair lipase activity and bile acid production and release can lead to malabsorption. Therefore, consider the following conditions to help determine the cause of impaired absorption.[2]

- **Pancreatic insufficiency** (specifically lipase)
- **Cholestasis** (e.g., bilary obstruction or liver disease)
- **Interrupted enterohepatic circulation** (e.g., ileal disease or bile salt deconjugation from small bowel bacterial overgrowth)
- **Celiac disease**
- **Short bowel syndrome**
- **Whipple's disease** (rare)

In general, an elevation in any one of the fecal fat markers is suggestive of malabsorption. Refer below for the clinical significance of the individual absorptive markers:

Triglycerides

Triglycerides represent the major component of dietary fat (on average 120g of a 125g daily load).[2] Elevated levels are suggestive of incomplete fat hydrolysis which can be caused by exocrine pancreatic insufficiency[4] or bile acid insufficiency.[5] Elevated triglycerides with normal LCFAs have been noted in patients with steatorrhea due to pancreatic insufficiency.[4] Triglycerides may also be elevated with a rapid transit time, which impairs the breakdown and absorption of these lipids.[6]

Turn-around Time 14 days

Long chain fatty acids (LCFAs)

LCFAs are free fatty acids that are normally readily absorbed in a healthy mucosa. Elevated levels are suggestive of malabsorption, reduced pancreatic function or bile insufficiency.[1] Increased LCFAs have also been noted after acute intestinal infections.[7]

Cholesterol

Fecal cholesterol is derived from the diet, bile, and from mucosal epithelial breakdown. In a healthy gastrointestinal tract, about 40-60% of dietary cholesterol will be absorbed.[8] Elevated levels are a reflection of mucosal malabsorption.[2] Impaired absorption of fecal cholesterol occurs in celiac disease secondary to damaged mucosa from gluten ingestion.[9]

Phospholipids

Phospholipids are derived from three specific sources: bile (50%), diet (25%), and mucosal desquamation (25%). The major dietary- derived phospholipids include phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine and cardiolipin. In a healthy individual, nearly 85% of intestinal phospholipids are absorbed. As phospholipids are derived from more than one source, elevations could occur from the following: malabsorption, inadequate bile salt resorption, or increased mucosal cell turnover.[2] Referring to the other absorptive markers will help provide clues as to the cause of high fecal phospholipids.

Total Fecal Fats

Total fecal fats include the sum total of triglycerides, cholesterol, phospholipids, and long chain fatty acids. These fats are predominately derived from the diet, although an additional lipid content in the stool is derived from bile and from mucosal desquamation.[1] A random stool sample that measures the mg of total fat per gram of stool has been shown to correlate with the 72 hour fecal fat study.[10] An elevation of the total of fecal fats is representative of malabsorption.

What is the significance of a low recovery of fecal fats?

To date, there is minimal literature on the clinical significance of reduced fecal fats. As they are largely of dietary origin, an overall low recovery of these absorptive markers is suggestive of a diet low in fat. Another consideration would be increased intestinal absorption, particularly if dietary fat intake is adequate.

What further testing might be indicated?

Initially assess PE and/or chymotrypsin to rule out exocrine pancreatic insufficiency. Evaluate the levels of secondary bile acids for impaired bilary flow.

Consider also the following additional tests to help determine the underlying etiology:

- **Celiac Panel**
- **Bacterial Overgrowth of the Small Intestine Breath Test**
- **Intestinal Permeability Profile**

References

1 Jungas RJ. Chapter 4, The Digestion and Absorption of Lipids. In: Lectures on Gastrointestinal Physiology and the Hormonal Regulation of Energy Metabolism. Framington, Conneticut. University of Connecticut Health Center. 2002

2 http://gastroresource.com/GITextbook/en/chapter7/7-6-pr.htm cited on 4/06/2005.

3 Kalivianakis M et al. Detection of impaired intestinal absorption of long-chain fatty acids: validation studies of a novel test in a rat model of fat malabsorption. Am J Clin Nutr. 2000 Jul;72(1):174-80.

4 Voortman G et al. Quantitative determination of faecal fatty acids and triglycerides by Fourier transform infrared analysis with a sodium chloride transmission flow cell. Clin Chem Lab Med. 2002 Aug;40(8):795-8.

5 Kalivianakis M et al. Fat malabsorption in cystic fibrosis patients receiving enzyme replacement therapy is due to impaired intestinal uptake of long-chain fatty acids. Am J Clin Nutr. 1999 Jan;69(1):127-34.

6 Verkade HJ et al. Fat absorption in neonates: comparison of long-chain-fatty-acid and triglyceride compositions of formula, feces, and blood. Am J Clin Nutr. 1991 Mar;53(3):643-51.

7 Jonas A et al. Disturbed fat absorption following infectious gastroenteritis in children. J Pediatr. 1979 Sep;95(3):366-72.

8 Chen HC. Molecular mechanisms of sterol absorption. J Nutr. 2001 Oct;131(10):2603-5.

9 Vuoristo M, Miettinen TA. Increased biliary lipid secretion in celiac disease. Gastroenterology. 1985 Jan;88(1 Pt 1):134-42.

10 Van den Neucker AM, Forget PP, van Kreel B. Lipid, nitrogen, water and energy content of a single stool sample in healthy children and children with cystic fibrosis. Eur J Pediatr. 2003 Nov;162(11):764-6. Epub 2003 Aug 27.

Gut Immunology

Eosinophil protein X (EPX)

What is EPX?

Eosinophils are involved in a broad range of diseases, including those of inflammatory and neoplastic origin. There is increasing evidence that eosinophils are functionally involved in the pathophysiology of various inflammatory disorders of the gut.[1] In healthy individuals, eosinophils reside in the connective tissue layer of the gut, known as the lamina propria. It is not until damage occurs to the lamina propria that eosinophils migrate into the gut lumen. Eosinophils contain a number of highly cationic proteins such as eosinophil cationic protein, major basic protein, eosinophil peroxidase, and eosinophil protein X (EPX).[2] Upon eosinophil degranulation, these cationic proteins are released, all of which possess potent cytotoxic properties. Accumulation of EPX in the GI tract is therefore associated with inflammation and tissue damage.[3]

Why is EPX the "test-of-choice" to assess eosinophil activity in the gut?

There are a number of invasive procedures that assess eosinophilic activity in the intestinal mucosa, including histologic observation, immunochemistry markers, and gut lavage. However, all of these tests have limited utility for the office-based practitioner because they require colonoscopy or biopsy.

EPX offers the practitioner a sensitive, noninvasive alternative to these invasive procedures. Clinical research indicates a significant correlation between eosinophil mediators in stool, such as EPX, and whole gut lavage fluids, the "gold standard" assessment.[4] In addition, EPX is not prone to many of the clinical drawbacks of other noninvasive inflammatory markers. In fact, EPX is considered the superior cationic protein to assess, as it more accurately reflects the degree of mucosal damage.[5]

The clinical utility of EPX serves both as a diagnostic tool, and a monitoring tool for disease activity. Baseline levels can be used to determine intestinal inflammation associated with food allergy and to monitor dietary changes. Studies have demonstrated a significant reduction in EPX after three months on a successful elimination diet.[6]

Increased levels of EPX have also been found in Ulcerative Colitis and Crohn's Disease, with elevations correlating with disease activity.[5] As a non-invasive marker, EPX offers increased sensitivity for evaluating inflammatory disease activity and for predicting relapses in patients with Inflammatory Bowel Disease (IBD).[7]

How do I interpret the results?

Normal EPX levels (< 7.0 mcg/g) can indicate the clinical efficacy of an elimination diet or a clinical remission of IBD. It should be noted that corticosteroids can reduce circulating levels of EPX.[8]

Elevated levels of EPX (>7.0 mcg/g) are associated with the following conditions:

- Inflammatory Bowel Disease (IBD)[5]
- Intestinal parasites (helminthiasis)[9]
- Chronic diarrhea[10]
- Food allergy and/or atopic dermatitis[4]
- Gastroesophageal reflux[11]
- Chronic alcoholism[11]

Turn-around Time 14 days

- Protein-sensitive enteropathy[11]
- Allergic colitis[12]
- Bowel cancer[13]
- Eosinophilic gastroenteritis (rare)[12]

What further testing might be indicated?

The following tests should be considered in patients with elevated EPX:

- **Allergy Antibody Assessment**
- **Celiac Panel**
- **Intestinal Permeability Assessment**
- **Parasitology Profile** (including macroscopic evaluation for worms)
- **ImmunoGenomic™ Profile**

When making a differential diagnosis involving bowel cancer, also consider levels of calprotectin, as well as radiologic and/or endoscopic evaluation.

References

1 Furuta GT, Ackerman SJ, Wershil BK. The role of eosinophils in gastrointestinal diseases. Curr Opin Gastroenterol 1995;11:541-547.

2 Carlson M, Raab Y, Peterson C, et al. Increased intraluminal release of eosinophil granule proteins EPO, ECO, EPX, and cytokines in ulcerative colitis and proctitis in segmental perfusion. Am J Gastroenterol 1999; 94(7):1876-1883.

3 Rothenberg ME et al. Gastrointestinal Eosinophils. Immunol Rev. 2001 Feb;179:139-55.

4 Bischoff SC, Grabowsky MS, Manns MP. Quantification of inflammatory mediators in stool samples of patients with inflammatory bowel disorders and controls. Dig Dis Sci 1997;42(2):394-403.

5 Peterson CG et al. A new method for the quantification of neutrophil and eosinophil cationic proteins in feces: establishment of normal levels and clinical application in patients with inflammatory bowel disease.Am J Gastroenterol. 2002 Jul;97(7):1755-62.

6 Majamaa H et al. Eosinophil protein X and eosinophil cationic protein as indicators of intestinal inflammation in infants with atopic eczema and food allergy. Clin Exp Allergy. 1999 Nov;29(11):1502-6.

7 Saitoh O et al. Fecal eosinophil granule-derived proteins reflect disease activity in inflammatory bowel disease. Am J Gastroenterol. 1999 Dec;94(12):3513-20.

8 Levy AM, Kita K. Increased eosinophil granule proteins in gut lavage fluid from patients with inflammatory bowel disease. Mayo Clin Proc. 1997 Feb;72(2):117-23.

9 Liu LX, et al. Eosinophilic colitis associated with larvae of the pinworm enterobius vermicularis. Lancet 1995;346:410-412.

10 Clouse RE, et al. Pericrypt eosinophilic enterocolitis and chronic diarrhea. Gastroenterology 1992;103:168-176.

11 Rothenberg ME, et al. Gastrointestinal eosinophils. Immun Reviews 2001; 179:139-155.

12 Bischoff SC et al. Immunnohistological assessment of intestinal eosinophil activation in patients with eosinophilic gastroenteritis and inflammatory bowel disease. Am J Gastroenterol. 1999 Dec;94(12):3521-9.

13 Saitoh O, Kojima K, Sugi K, et al. Fecal eosinophil granule-derived proteins reflect disease activity in inflammatory bowel disease. Am J Gastroenterol 1999; 94(12):3513-3520.

Gut Immunology

Calprotectin

What is calprotectin?

Calprotectin belongs to a group of calcium-binding neutrophil-derived proteins. Calprotectin makes up about 5% of the total protein content of the neutrophil and about 60% of the cytosolic proteins.[1] It is very resistant to bacterial degradation in the gut and is stable in stool for up to one week at room temperature.[2]

Why is calprotectin the noninvasive "test of choice" for detecting and monitoring IBD?

Calprotectin is a sensitive, stable marker that is unaffected by medications, dietary supplements, or enzymatic degradation.[3,4] This neutrophil-derived protein:

- Reflects the flux of leukocytes into the intestinal lumen.[5]
- Is released upon activation and degranulation of neutrophils.[6]
- Correlates strongly with 111-indium-labeled leukocyte excretion (the "gold standard") as well as histologic and endoscopic grading of disease activity in ulcerative colitis.[2,5]
- Helps differentiate between Irritable Bowel Syndrome (IBS) and active Inflammatory Bowel Disease (IBD).[7]
- Predicts relapse in patients with IBD, and serves as an objective marker to assist in deciding when to treat.[8] Therapy can be instituted before inflammation reaches critical intensity.
- Assists in selecting patients for endoscopy and in monitoring response to treatment, especially in children who may require general anesthesia to undergo more invasive analyses.[9-11]

How should calprotectin be used?

Simple, reliable, and noninvasive, calprotectin can be utilized as an individual test for the following clinical uses:

- To assist in selecting patients with abdominal symptoms who may require further diagnostic procedures[12]
- To distinguish between IBD and IBS[7]
- To select children for endoscopy[10]
- To determine disease activity and risk of relapse in IBD[8]
- To monitor IBD treatment response and to determine when a full clinical remission has been achieved[13]
- To evaluate efficacy in trials of new treatments for IBD

Turn-around Time 14 days

How do I interpret test results?

Normal Calprotectin <50 mcg/g

Values below 50 mcg/g are not indicative of inflammation in the gastrointestinal tract[14]

Elevated Calprotectin 50-100 mcg/g

Values ranging from 50 to 100 mcg/g are associated with inflammation in the gastrointestinal tract. The inflammatory response could be due to IBD[5], infection[3], polyps[15], neoplasia[16], or the use of non-steroidal anti-inflammatory drugs (NSAIDs).[17] Calprotectin may also be elevated in children with chronic diarrhea secondary to cow's milk allergy or multiple food allergies.[18]

Elevated Calprotectin >100 mcg/g

Values above 100 mcg/g indicate significant inflammation in the gastrointestinal tract. Etiology could be associated with the following: IBD[5], infection[3], NSAID use[17], polyps[15], adenomas, or colorectal cancer[16]. Calprotectin may also be elevated in children with chronic diarrhea secondary to cow's milk allergy or multiple food allergies.[18] Further investigative procedures are necessary to determine the cause of inflammation.

Elevated Calprotectin >250 mcg/g (use in addition to >100 mcg/g Calprotectin)

For patients with IBD, levels between 250-500 mcg/g indicate low to moderate disease activity. Levels above 500 mcg/g suggest high disease activity. Patients with IBD in remission and levels above 250 mcg/g have a high risk of relapse within one year.[8]

What further testing might be indicated?

Whether inflammatory or neoplastic, the cause of elevated calprotectin MUST be ascertained by endoscopy or radiography. If these evaluations do not yield signs of overt disease, other tests may be considered to uncover causes of chronic bowel inflammation:

- **Intestinal Permeability Assessment**
- **Allergy Antibody Assessment**
- **Celiac Panel**
- **ImmunoGenomic™ Profile**
- **Comprehensive Parasitology Profile**

References

1 Fagerhol MK et al. Calprotectin (the L1 leukocyte protein). In: Smith VL, Dedman JR eds. Stimulus Responding Coupling: The Role of Intracellular Calcium-Binding Proteins. Boca Raton, Fla:CRC Press Inc; 1990:187-210

2 Roseth AG, et al. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. Digestion 1997;58:176-80.

3 Ton H et al. Improved assay for fecal calprotectin. Clin Chim Acta. 2000 Feb 25;292(1-2):41-54.

4 Johne B et al. A new fecal calprotectin test for colorectal neoplasia. Clinical results and comparison with previous method. Scand J Gastroenterol. 2001 Mar;36(3):291-6.

5 Roseth AG, Schmidt PN, Fagerhol MK. Correlation between faecal excretion of 111-indium labeled granulocytes and calprotectin, a granulocyte marker protein in patients with inflammatory bowel disease. Scand J Gastroenterol 1999;34:50-54.

6 Tibble JA, Bjarnason I. Non-invasive investigation of inflammatory bowel disease. World J Gastroenterol. 2001 Aug;7(4):460-5.

7 Tibble J, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000;47:506-513.

8 Tibble JA, et al. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. Gastroenterology 2000;119:15-22.

9 Bunn SK, et al. Fecal calprotectin: validation as a noninvasive measure of bowel inflammation in childhood inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2001;33:14-22.

10 Bunn Sk, et al. Fecal calprotectin as a measure of disease activity in childhood inflammatory bowel disease. J Pediatr Gastroenterol Nutr 2001;32:171-177.

11 Poullis A, et al. Emerging role of calprotectin in gastroenterology. J Gastroenterol Hepatol 2003;18:756-762.

12 Fagerhol MK. Calprotectin, a faecal marker of organic gastrointestinal abnormality. Lancet. 2000 Nov 25;356(9244):1783-4.

13 Bjarnason I, Sherwood R. Fecal calprotectin: a significant step in the noninvasive assessment of intestinal inflammation. J Pediatr Gastroenterol Nutr. 2001 Jul;33(1):11-3.

14 Roseth AG et al. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand J Gastroenterol. 1992 Sep;27(9):793-8.

15 Tibble J et al. Faecal calprotectin and faecal occult blood tests in the diagnosis of

colorectal carcinoma and adenoma. Gut. 2001 Sep;49(3):402-8.

16 Starkey BJ. Screening for colorectal cancer. Ann Clin Biochem. 2002 Jul;39(Pt 4):351-65.

17 Tibble JA et al. High prevalence of NSAID enteropathy as shown by a simple faecal test. Gut. 1999 Sep;45(3):362-6.

18 Corroccio A et al. Diagnostic accuracy of fecal calprotectin assay in distinguishing organic causes of chronic diarrhea from irritable bowel syndrome: a prospective study in adults and children. Clin Chem. 2003 Jun;49(6 Pt 1):861-7.

Gut Immunology
Lactoferrin

What is lactoferrin?

Lactoferrin is a 77-kDa iron-binding glycoprotein that serves as a marker for leukocyte activity.[1] In addition to being produced by neutrophils, it is also expressed by surface epithelial cells and secreted into the mucosa. It can therefore be found in most exocrine secretions, including breast milk, tears, nasal secretions, saliva, intestinal mucus and genital secretions.[2]

In the gastrointestinal tract, lactoferrin serves as a non-specific marker of inflammation. Lactoferrin is elevated with enteric infection,[3] active inflammatory bowel disease (IBD)[4], and colorectal cancer.[5]

Why do leukocytes release lactoferrin?

Lactoferrin is liberated from the granules in neutrophils in response to inflammation. It facilitates in the production of hydroxyl radicals and binds to iron, thereby impeding the growth of microbial organisms. In vitro, lactoferrin has demonstrated bacteriostatic and bacteriocidal activity against gram positive and gram negative bacteria, aerobes, yeast, and anaerobes.[1]

What is the clinical value of this test?

Lactoferrin is a very accurate marker to both identity and rule out inflammatory bacterial enteritis. A study by Silletti et al. showed the positive predictive value to be >98% and the negative predictive value >99%[6] Pathogenic bacteria require high amounts of iron, thus the recruitment of white blood cells and the subsequent release of lactoferrin is the body's innate way of fighting infection.

Lactoferrin has also been found to be a sensitive and specific marker for disease activity in IBD and can be used to monitor therapy in these patients.[7] In this patient cohort, lactoferrin acts as an anti-inflammatory agent by down-regulating inflammatory cytokine production. Lactoferrin also prevents the binding of lipopolysaccharide endotoxins to inflammatory cells.[2] Elevations in lactoferrin have also been found in colorectal cancer, with positivity comparable to fecal occult blood testing.[5] Animal studies suggest that lactoferrin plays a role in tumor inhibition by inducing apoptosis.[8,9]

What pathogens are associated with a positive lactoferrin?

Organisms that cause inflammatory bacterial enteritis are generally those that produce cytotoxins that disrupt or break down the epithelial lining of the gastrointestinal tract.[6]

Elevations in lactoferrin can occur from the following infections; Shigella, Salmonella, pathogenic E. coli[10] Campylobacter,[3] *Clostridium difficile*,[11] Cryptosporidium,[12] and the parasite *Entamoeba histolytica*.[13] To date, other microbial or viral infections that cause diarrhea have not been found to increase lactoferrin. This is because they usually do not elicit a severe enough inflammatory reaction to raise lactoferrin levels.

Turn-around Time 14 days

How do I interpret a positive result?

A positive lactoferrin test indicates significant inflammation of the intestinal mucosa. This is most commonly caused by enteric infection.[6] Another cause of increased lactoferrin is inflammatory bowel disease.[14] Both ulcerative colitis and Crohn's disease may raise the lactoferrin level, even in the absence of pathogenic bacteria, simply because of the degree of inflammation. Colorectal cancer can also be a cause of idiopathic elevations in lactoferrin.[5]

Will colostrum containing supplements affect the results?

The lactoferrin test is human-specific, so bovine and other derived lactoferrin containing products will not interfere with the results.[15]

What factors may affect the results?

False positives can occur in breast feeding infants.[16] This is because lactoferrin is naturally present in breast milk. False negative results may occur in immunocompromised individuals. This is because they are unable to mobilize white blood cells into the gut lumen in response to a pathogenic infection.[15]

What further testing might be indicated?

Initially assess the Microbiology and Parasitology sub-panels for infection. If pathogenic organisms were not recovered in the test, then consider evaluating EPX and calprotectin (Gut Immunology subpanel) to assess for mucosal damage and to quantify neutrophilic activity. If these two immune markers are also elevated, then radiographic/endoscopic assessment is warranted.

References

1 Martins CAP et al. Correlation of lactoferrin with neutrophilic inflammation in body fluids. Clin Diagn Lab Immunol. 1995 Nov;2(6):763-5.

2 Conneely OM. Antiinflammatory activities of lactoferrin. J Am Coll Nutr. 2001 Oct;20(5 Suppl):389S-395S; discussion 396S-397S.

3 Choi SW et al. To culture or not to culture: fecal lactoferrin screening for inflammatory bacterial diarrhea. J Clin Microbiol. 1996 Apr;34(4):928-32.

4 Fine KD et al. Utility of a rapid fecal latex agglutination test detecting the neutrophil protein, lactoferrin, for diagnosing inflammatory causes of chronic diarrhea. Am J Gastroenterol. 1998 Aug;93(8):1300-5.

5 Saitoh O et al. Comparison of tests for fecal lactoferrin and fecal occult blood for colorectal diseases: a prospective pilot study. Intern Med. 2000 Oct;39(10):778-82.

6 Silleti RP et al. Role of stool screening tests in diagnosis of inflammatory bacterial enteritis and in selection of specimens likely to yield invasive enteric pathogens. J Clin Microbiol. 1996 May;34(5):1161-5.

7 Buderus S et al. Fecal lactoferrin: a new parameter to monitor infliximab therapy. Dig Dis Sci. 2004 Jun;49(6):1036-9.

8 Fujita K et al. Lactoferrin modifies apoptosis-related gene expression in the colon of the azoxymethane-treated rat. Cancer Lett. 2004 Sep 15;213(1):21-9.

9 Fujita K et al. Lactoferrin enhances Fas expression and apoptosis in the colon mucosa of azoxymethane-treated rats. Carcinogenesis. 2004 Oct;25(10):1961-6. Epub 2004 Jun 10.

10 Greenberg DE et al. Markers of inflammation in bacterial diarrhea among travelers, with a focus on enteroaggregative Escherichia coli pathogenicity. J Infect Dis. 2002 Apr 1;185(7):944-9. Epub 2002 Mar 19.

11 Guerrant RL et al. Measurement of fecal lactoferrin as a marker of fecal leukocytes. J Clin Microbiol. 1992 May;30(5):1238-42.

12 Alcantara CS et al. Interleukin-8, tumor necrosis factor-alpha, and lactoferrin in immunocompetent hosts with experimental and Brazilian children with acquired cryptosporidiosis. Am J Trop Med Hyg. 2003 Mar;68(3):325-8.

13 Ruiz-Pelaez JG, Mattar S. Accuracy of fecal lactoferrin and other stool tests for diagnosis of invasive diarrhea at a Colombian pediatric hospital. Pediatr Infect Dis J. 1999 Apr;18(4):342-6.

14 Kane SV et al. Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. Am J Gastroenterol. 2003 Jun;98(6):1309-14.

15 IBD-CHEK™. An ELISA for the Detection of Lactoferrin, a Marker of Fecal Leukycytes and an Indicator of Intestinal Inflammation. Catalog No. T5008. TECHLAB, Blacksburg, VA 24060.

16 Huicho L et al. Fecal lactoferrin, fecal leukocytes and occult blood in the diagnostic approach to childhood invasive diarrhea. Pediatr Infect Dis J. 1997 Jul;16(7):644-7.

Metabolic Markers

Beneficial Short Chain Fatty Acids (SCFAs) and n-Butyrate

What are Beneficial SCFAs?

Beneficial SCFAs include acetate, proprionate and n-butyrate, and are produced through the fermentation of non-absorbed dietary fibers. In addition to providing energy for colonocytes and exerting a trophic effect on the intestinal lining, SCFAs have a number of other positive benefits in the gut.

What benefits do SCFAs provide?

SCFAs are produced by the anaerobic bacterial fermentation of primarily non-absorbed dietary fiber.[1] They serve several important functions:

- Provide energy for the colonocytes and exert a trophic effect on the intestinal lining[2]
- Act as anti-diarrheal agents by removing sodium and water from the colon[3]
- Improve colonic blood flow[4]
- Deter the colonization of pathogens in the bowel[2]
- Provide 5%-30% of systemic daily energy requirements[5]
- Reduce ammonia uptake from the intestine[2]

How does n-butyrate reduce the risk of colon cancer?

n-Butyrate is the preferred substrate for colonocytes, assisting in the maintenance of colonic integrity. Butyrate helps prevent colon cancer by stimulating healthy cellular growth and reducing DNA damage.[6] This SCFA has also been found to antagonize the hyperproliferation of colonic cells induced by the secondary bile acid deoxycholate.[7]

What is n-butyrate's role in Inflammatory Bowel Disease?

- Because of its trophic effect on the colonic epithelium, n-butyrate helps protect against ulcerative colitis (UC).[8]
- In Crohn's disease, n-butyrate decreases TNF-αlpha production and lipopolysaccharide-induced NFkappaB activity in intestinal cells (key factors in its pathogenesis).[9]

Do SCFAs play a role in Irritable Bowel Syndrome (IBS)?

Patients with Irritable Bowel Syndrome (especially diarrhea-predominant) tend to have lower total SCFAs.[10]

Are there problems associated with high levels of SCFAs?

Occasionally elevated levels of n-butyrate in patients with UC may indicate impaired transport of n-butyrate into the cell or defective metabolism within the cell,[10] or may result from bacterial fermentation of blood within the colon.[11]

Turn-around Time 14 days

Increased levels of SCFAs may also indicate:

- Malabsorption[12]
- Rapid transit time[13]
- Small bowel bacterial overgrowth[14]

How can low levels of total SCFAs or n-butyrate be increased?

- **Dietary fiber**[5]
- **Larch arabinogalactans** (*Larix* sp.)[15]
- **Normalization of pH**—In vitro SCFA production is higher when the colonic pH is between 5-8[16]
- **Normalization of transit time**—Chronically slow transit time can lead to bacterial overgrowth of the small intestine and high SCFAs; very rapid transit time can also lead to high SCFAs[17]
- **Probiotics and prebiotics** (e.g., fructooligosaccharides)—Lactobacillus produces lactic acid that acts as a substrate for other gut bacteria that ferment it to SCFAs[18]
- **Butyric acid** (oral or rectal)[19,20]

Are some dietary fibers more effective than others?

In general, the more slowly fermented (insoluble) forms of fiber tend to maintain low pH and raise SCFAs (especially butyrate) along the entire length of the bowel.[21] They are also more effective at raising n-butyrate levels by shifting much of the fermentation to the distal colon.[22]

In contrast, soluble non-starch polysaccharides, such as oat bran and guar gum, are rapidly fermented and have less effect on pH and SCFA production in the distal colon.[21]

What further testing might be indicated?

- **Bacterial Overgrowth of the Small Intestine Breath Test**—Consider when total SCFAs and n-butyrate levels are elevated along with the presence of gas, post-prandial bloating, or chronic bowel stasis (e.g., slow transit time).

References

1 Clausen MR, Bonnen H, Mortensen PB. Colonic fermentation of dietary fibre to short chain fatty acids in patients with adenomatous polyps and colonic cancer. Gut 1991;32;923-8.

2 Royall D. Clinical significance of colonic fermentation. Am J Gastroenterol 1990;85(10):1307-12.

3 Ramakrishna BS, Mathan VI. Colonic dysfunction in acute diarrhoea: the role of luminal short chain fatty acids. Gut. 1993 Sep;34(9):1215-8.

4 Mortensen FV, et al. Short chain fatty acids dilate isolated human colonic resistance arteries. Gut 1990;31:1391-4.

5 Rombeau JL, Kripke SA. Metabolic and intestinal effects of short-chain fatty acids. JPEN J Parenter Enteral Nutr 1990;14(5):181S-4S.

6 Roediger WE. The starved colon—diminished mucosal nutrition, diminished absorption, and colitis. Dis Colon Rectum 1990;33(10):858-62.

7 Scheppach W. Bartram, HP, Richter F. Role of short-chain fatty acids in the prevention of colorectal cancer. Eur J Cancer 1995 Jul-Aug;31A(7-8):1077-80.

8 Smith JG, Yokoyama WH, German JB. Butyric acid from the diet: actions at the level of gene expression. Crit Rev Food Sci Nutr 1998 May;38(4):259-97.

9 Segain JP, et al. Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. Gut 2000;47(3):397-403.

10 Treem WR, Aet al. Fecal short-chain fatty acids in patients with diarrhea-predominant irritable bowel syndrome: in vitro studies of carbohydrate fermentation. J Pediatr Gastroenterol Nutr 1996;23(3):280-6.

11 Holtug K, Rasmussen HS, Mortensen PB. Short chain fatty acids in inflammatory bowel diseases. The effect of bacterial fermentation of blood. Scand J Clin Lab Invest 1988;48(7):667-71.

12 Scheppach W, et al. The effect of starch malabsorption on fecal short-chain fatty acid excretion in man. Scand J Gastroenterol 1988 Aug;23(6):755-9.

13 Oufir LE, et al. Relationships between transit time in man and in vitro fermentation of dietary fiber by fecal bacteria. Eur J Clin Nutr 2000 Aug;54(8):603-9.

14 Hoverstad T, et al. Short-chain fatty acids in the small-bowel bacterial overgrowth syndrome. Scand J Gastroenterol 1985 May;20(4):492-9.

15 Kelly GS. Larch arabinogalactan: clinical relevance of a novel immune-enhancing polysaccharide. Altern Med Rev 1999 Apr;4(2):96-103.

16 Holtug K, et al. The colon in carbohydrate malabsorption: short-chain fatty acids, pH, and osmotic diarrhoea. Scand J Gastroenterol 1992;27(7):545-52.

17 Hoverstad T, Bjorneklett A. Short-chain fatty acids and bowel functions in man. Scand J Gastroenterol 1984;19:1059-1065.

18 Siigur U, et al. Effect of bacterial infection and administration of a probiotic on faecal short-chain fatty acids. Microb Ecol Health Dis 1996;9:271-7.

19 Vernia P, et al. Combined oral sodium butyrate and mesalazine treatment compared to oral mesalazine alone in ulcerative colitis: randomized, double-blind, placebo-controlled pilot study. Dig Dis Sci 2000 May;45(5):976-81.

20 Patz J, et al. Treatment of refractory distal ulcerative colitis with short chain fatty acid enemas. Am J Gastroenterol 1996 Apr;91(4):731-4.

21 Phillips J, et al. Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. Am J Clin Nutr 1995;62:121-30.

22 Morita T, et al. Psyllium shifts the fermentation site of high-amylose cornstarch toward the distal colon and increases fecal butyrate concentration in rats. J Nutr 1999;129(11):2081-7.

Metabolic Markers

Stool pH

What is Stool pH?

Fecal pH indicates the relative acidity or alkalinity of the colonic environment. The pH of the stool should not be confused with stomach pH, and therefore is not directly influenced by hydrochloric acid.

What are the key factors that determine stool pH?

Factors that have an impact on stool pH include fiber and food constituent intake,[1-3] fermentive processes, bacterial populations, antibiotics,[4] and stool transit time.[5]

What clinical conditions are associated with an imbalanced stool pH?

Acidity of the stool is often related to diarrhea[5] or carbohydrate malabsorption.[6] Additionally, use of osmotic laxative agents, herbal or chemical, can cause an acidic pH (<6.1).

Excessive protein consumption,[7] slow transit time/constipation,[5] and inadequate dietary fiber[8] may all contribute to an alkaline (> 7.9) stool pH.

How do I interpret the results?

If pH is too low/acidic (<6.1), address the cause of diarrheal syndromes: viral infection, possible malabsorption, or osmotic diarrhea[9] from food,[10] medications, or bacterial toxins.[11]

If stool pH is high/alkaline (>7.9), a key strategy is to correct constipation, address dietary protein excess, and improve transit time. It is also important to ensure adequate quantities of soluble and insoluble fiber intake.[12] Oat bran[2] and cellulose/hemicellulose from vegetables have been found to be highly effective in reducing stool pH.[13]

What further testing might be indicated?

If low/acidic (pH <6.1):

- **Lactose Intolerance Breath Test** - to rule out possible disaccharide intolerance.
- **Allergy Antibody Assessment** – food allergies can alter transit time, thereby reducing stool pH.
- **Intestinal Permeability Assessment** – helps rule out malabsorption.
- **Celiac Panel** – chronic diarrhea is common with gliadin intolerance.

If high/alkaline (pH > 7.9):

- Refer to the analytes n-butyrate, beta-glucuronidase, and secondary bile acids to assess overall long term risk for colorectal cancer. Unexplained elevations in EPX and Calprotectin warrant endoscopic/radiologic investigation.

Turn-around Time 14 days

References

1 Malhotra SL. Faecal urobilinogen levels and pH of stools in population groups with different incidence of cancer of the colon, and their possible role in its aetiology. J R Soc Med. 1982 Sep;75(9):709-14.

2 Kashtan H et al. Manipulation of fecal pH by dietary means. Prev Med. 1990 Nov;19(6):607-13.

3 Ahmed R et al. Fermentation of dietary starch in humans. Am J Gastroenterol. 2000 Apr;95(4):1017-20.

4 Hoverstad T et al. Influence of oral intake of seven different antibiotics on faecal short-chain fatty acid excretion in healthy subjects. Scand J Gastroenterol. 1986 Oct;21(8):997-1003.

5 Oufir LE et al. Relationships between transit time in man and in vitro fermentation of dietary fiber by fecal bacteria. Eur J Clin Nutr. 2000 Aug;54(8):603-9.

6 Eherer AJ, Fordtran JS. Fecal osmotic gap and pH in experimental diarrhea of various causes. Gastroenterology. 1992 Aug;103(2):545-51.

7 Newmark HL, Lupton JR. Determinants and consequences of colonic luminal pH: implications for colon cancer. Nutr Cancer 1990;14:161-73.

8 Muir JG et al. Combining wheat bran with resistant starch has more beneficial effects on fecal indexes than does wheat bran alone. Am J Clin Nutr 2004 Jun;79(6):1020-8.

9 Payne ML, Craig Wj, Williams AC. Sorbitol is a possible risk factor for diarrhea in young children. J Am Diet Assoc 1997 May;97(5):532-4.

10 Ament ME. Malabsorption of apple juice and pear nectar in infants and children: clinical implications. J Am Coll Nutr 1996 Oct;15(5 Suppl):26S-29S.

11 Borriello SP, Barclay FE. An in-vitro model of colonisation resistance to Clostridium difficile infection. J Med Microbiol. 1986 Jun;21(4):299-309.

12 Evans DF. Physicochemical environment of the colon. Eur J Cancer Prev. 1998 May;7 Suppl 2:S79-80.

13 Cummings JH, Bingham SA. Dietary fibre, fermentation and large bowel cancer. Cancer Surv. 1987;6(4):601-21.

Metabolic Markers

Beta-glucuronidase

What is Beta-glucuronidase?

Beta-glucuronidase is an inducible enzyme elaborated by anaerobic E. coli, Peptostreptococcus, Bacteroides, and Clostridia.[1,2] Increased activity of this enzyme has been implicated in increased enterohepatic recirculation of toxins, steroid hormones, drugs, and carcinogens.[3]

What is the clinical significance of elevated beta-glucuronidase?

Ordinarily, toxins, hormones and drugs are excreted from the body after being conjugated to a glucuronide molecule. By uncoupling glucuronides in the intestine, beta-glucuronidase can deconjugate potential toxins, increasing the formation of carcinogens in the bowel and promoting the enterohepatic recirculation of toxins, hormones,[4] and various drugs[5] in the body.

How strongly is beta-glucuronidase linked to colon cancer?

Research correlates elevated levels of beta-glucuronidase with increased colon cancer risk.[6] In fact, excessive beta-glucuronidase activity may be a primary factor in the etiology of colon cancer.[7]

Does excessive beta-glucuronidase increase the risk of other cancers?

Human studies that directly link fecal beta-glucuronidase and breast cancer are lacking. Animal studies, however, have shown reductions in breast cancer risk via administration of calcium D-glucarate, a compound known to inhibit the enzyme.[8]

Animal and in vitro studies suggest a similar relationship for liver,[9] lung,[10] and skin[11] cancers.

Are there any problems associated with LOW beta-glucuronidase levels?

A certain amount of beta-glucuronidase activity appears to be important for normal enterohepatic recirculation of endogenous compounds such as vitamin D,[12] thyroid hormone,[13] and estrogen.[14] Broad-spectrum antibiotics suppress intestinal microflora, which reduces beta-glucuronidase activity and intestinal reabsorption of estrogen.[15] This may cause reduced efficacy of oral contraceptives in a subset of women administered antibiotics. The bioavailability of the soy isoflavones, genestein and daidzein (cancer-preventive agents), depends upon initial hydrolysis by intestinal beta-glucuronidase and sulfatase enzymes. Low levels of beta-glucuronidase may therefore reduce the efficacy of these compounds.[16]

Turn-around Time 14 days

How can excessive beta-glucuronidase be reduced?

The following interventions may help to reduce beta-glucuronidase levels:

- Calcium-D-glucarate inhibits the enzyme.[17,18] Foods with the highest concentration of calcium-D-glucarate include oranges, apples, grapefruit, and cruciferous vegetables.[3]
- Silymarin (milk thistle).[19]
- Probiotics (including *Lactobacillus acidophilus*,[20] *Lactobacillus rhamnosus* GG,[2] and bifidobacteria[21]).
- Fructooligosaccharides (FOS). FOS may act as a substrate for bifidobacteria. Combining FOS with cellulose may be even more effective.[22]
- High-fiber diet (including both soluble and insoluble fiber)[23,24,25]
- Ascorbic acid.[26]
- Low- or non-meat diet[27] Lacto-vegetarian diets are associated with reduced levels of beta-glucuronidase.[28]
- Lowering colonic pH. In vitro studies had shown that raising fecal pH from 5 to 8 induces 11.5-fold increases in beta-glucuronidase levels.[29]

How do I increase beta-glucuronidase activity?

- **Probiotics** – to restore healthy colonic flora after broad-spectrum antibiotic therapy.
- **Cumin, cayenne** or **black pepper** – these spices have been found to increase levels of the enzyme.[30]
- **Assess stool pH** – raising fecal pH from 5 to 8 invitro induces 11.5-fold increases in beta-glucuronidase levels.[29]

What further testing might be indicated?

- **Women's Hormonal Health Assessment**, **Female Hormone Profile**, or **Menopause Profile**—to assess the possible impact of beta-glucuronidase on estrogen levels
- **Detoxification Profile**—to assess functioning of the glucuronidation pathway.

References

1 McConnell MA, Tannock GW. A note on lactobacilli and beta-glucuronidase activity in the intestinal contents of mice.J Appl Bacteriol. 1993 Jun;74(6):649-51.

2 Ling WH et al. Lactobacillus strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults. J Nutr. 1994 Jan;124(1):18-23.

3 Dwivedi C et al. Effect of calcium glucarate on beta-glucuronidase activity and glucarate content of certain vegetables and fruits. Biochem Med Metab Biol. 1990 Apr;43(2):83-92.

4 Adlercreutz H, et al. Intestinal metabolism of estrogens. J Clin Endocrin Metab 1976;43(3):497-505.

5 Kuhn JG. Pharmacology of irinotecan. Oncology 1998;12(8 Suppl 6):39-42.

6 Reddy BS, Wynder EL. Large-bowel carcinogenesis: Fecal constituents of populations with diverse incidence rates of colon cancer. J Natl Cancer Insti 1973;50(6):1437-42.

7 Dong-Hyun, Young-Ho Jin. Intestinal bacterial beta-glucuronidase activity of patients with colon cancer. Arch Pharm Res. 2001 Dec;24(6):564-7.

8 Walaszek Z, et al. Dietary glucarate as anti-promoter of 7,12-dimethylbenz[a]anthracene-induced mammary tumorigenesis. Carcinogenesis 1986 Sep;7(9):1463-6.

9 Oredipe OA, et al. Effects of calcium glucarate on the promotion of diethylnitrosamine-initiated altered hepatic foci in rats. Cancer Lett 1987 Dec;38(1-2):95-9.

10 Walaszek Z, et al. Dietary glucarate-mediated reduction of sensitivity of murine strains to chemical carcinogenesis. Cancer Lett 1986 Oct;33(1):25-32.

11 Dwivedi C, Downie AA, Webb TE. Modulation of chemically initiated and promoted skin tumorigenesis in CD-1 mice by dietary glucarate. J Environ Pathol Toxicol Oncol 1989 May-Jun;9(3):253-9.

12 Kumar R, et al. Enterohepatic physiology of 1,25-dihydroxyvitamin D3. J Clin Invest 1980 Feb;65(2):277-84.

13 Hazenberg MP, de Herder WW, Visser TJ. Hydrolysis of iodothyronine conjugates by intestinal bacteria. FEMS Microbiol Rev 1988 Feb;4(1):9-16.14 Gorbach SL. Estrogens, breast cancer, and intestinal flora. Rev Infect Dis 1984 Mar-Apr;6 Suppl 1:S85-90.

15 Weisberg E. Interactions between oral contraceptives and antifungals/antibacterials. Is contraceptive failure the result? Clin Pharmacokinet 1999 May;36(5):309-13.

16 Setchell KD, et al. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. Am J Clin Nutr 2002 Aug;76(2):447-53.

17 No authors listed. Calcium-D-glucarate. Altern Med Rev 2002;7(4):336-9.

18 Yoshimi N, et al. Inhibition of azoxymethane-induced rat colon carcinogenesis by potassium hydrogen D-glucarate. Int J Oncol 2000 Jan;16(1):43-8.

19 Kim DH, et al. Silymarin and its components are inhibitors of beta-glucuronidase. Biol Pharm Bull 1994 Mar;17(3):443-5.20 Goldin BR, Swenson L, Dwyer J, Sexton M, Gorbach SL. Effect of diet and Lactobacillus acidophilus supplements on human fecal bacterial enzymes. J Natl Cancer Inst 1980;64(2):255-61.

21 Kulkarni N, Reddy BS. Inhibitory effect of Bifidobacterium longum cultures on the azoxymethane-induced aberrant crypt foci formation and fecal bacterial beta-glucuronidase. Proc Soc Exp Biol Med 1994;207(3):278-83.

22 Gudiel-Urbano M, Goni I. Effect of short-chain fructooligosaccharides and cellulose on cecal enzyme activities in rats. Ann Nutr Metab 2002;46(6):254-8.

23 Gorbach SL, Goldin BR. The intestinal microflora and the colon cancer connection. Rev Infect Dis 1990;12(Suppl 2):S252-61.

24 Lampe JW,et al. Serum beta-glucuronidase activity is inversely associated with plant-food intakes in humans. J Nutr 2002;132(6):1341-4.

25 Roberts-Andersen J, Mehta T, Wilson RB. Reduction of DMH-induced colon tumors in rats fed psyllium husk or cellulose. Nutr Cancer 1987;10(3):129-36.

26 Young JC, Kenyon EM, Calabrese EJ. Inhibition of beta glucuronidase in human urine by ascorbic acid. Human & Experimental Toxicology 1990;9:165-70.

27 Reddy BS, Weisburger JH, Wynder EL. Fecal bacterial beta-glucuronidase: control by diet. Science 1974;183(123):416-7.

28 Goldin BR, et al. Estrogen excretion patterns and plasma levels in vegetarian and omnivorous women. N Engl J Med 1982 Dec 16;307(25):1542-7.

29 Kim DH, et al. pH inducible beta-glucuronidase and beta-glucuronidase of intestinal bacteria. Chem Pharm Bull 1992;40(6):1667-9.

30 Nalini N, et al. Influence of spices on the bacterial (enzyme) activity in experimental colon cancer. J Ethnopharmacol 1998 Aug;62(1):15-24.

Metabolic Markers

Secondary Bile Acids

What are Bile Acids?

Bile acids are end products of hepatic cholesterol metabolism that play an important role in fat emulsion and detoxification. High levels of secondary bile acids can result from an excess of dietary fat and animal protein, and may be associated with increased risk of gallstones and certain cancers.

How do bile acids reflect the relationship between diet and colorectal cancer risk?

The primary bile acids chenodeoxycholic acid (CDCA) and cholic acid (CA) are the end products of hepatic cholesterol metabolism. CDCA is derived from dietary cholesterol, whereas CA is produced from endogenous cholesterol production. Once they enter the colon, they are acted upon by anaerobic bacteria to produce secondary bile acids. CDCA is modified into lithocholic acid (LCA), and CA is modified into deoxycholic acid (DCA). The specific anaerobic bacteria involved in primary bile acid deconjugation include Clostridium, Enterococcus, Bacteroides and Lactobacillus.[1]

The correlation between diet and colorectal cancer appears to be related, at least in part, to bile acid metabolism. Consuming a diet rich in fat and animal protein and low in fiber results in a 2- to 5-fold increase in the excretion of secondary or unconjugated bile acids.[2-4]

Research suggests that the lithocholic acid:deoxycholic acid ratio (LCA:DCA) may be an important discriminate marker in colorectal cancer (CRC) susceptibility. A study by Owen et al. revealed that 75% of CRC patients exhibited a secondary bile acid ratio >1.0, whereas 86% of healthy controls had a ratio <1.0. [5]

What is the significance of an elevated LCA:DCA ratio?

According to the literature, a LCA:DCA ratio >1.0 is associated with gallstones, cholecystectomy, and increased risk of breast and colorectal cancers.[2,5-8]

Why is the LCA:DCA ratio considered important?

Evidence supports the role of both secondary bile acids as tumor promoters.[9] However, LCA is considered to be more toxic than DCA, possibly because LCA has a greater inhibitory effect on the enzyme glutathione-S-transferase than does DCA. The inhibition of glutathione-S-transferase results in the persistence of mutagens in colonocytes, which is linked to a greater frequency of neoplasia-associated mutations.[1,6,10]

Individuals with fibrocystic breast disease and adenocarcinoma of the breast have been found to have increased LCA levels and an elevated LCA:DCA ratio.[6] The mechanism by which secondary bile acids increase breast cancer risk may in part be due to the ability of the intestinal bacteria to synthesize estrogens from secondary steroids.[11]

What can be done to reduce the ratio?

Supplementation with fiber and probiotics can help reduce an elevated bile acid ratio. Fiber increases cholesterol absorption, which in turn reduces the concentration of secondary bile acids in the stool.4 Taking probiotics on a regular basis also has a beneficial effect on a high LCA:DCA ratio.[2-5,12-14] Prebiotics have been found to reduce the conversion of CDCA to LCA by over 90%.[16]

Turn-around Time 14 days

Which type of fiber is most effective in lowering the bile acid ratio?

Resistant starch, the insoluble fiber contained in wheat bran, legumes, and certain vegetables, decreases the level of secondary bile acids. Resistant starch enhances short-chain fatty acid production in the proximal colon, which lowers intestinal pH. A reduction in the colonic pH inhibits bacterial 7 alpha-hydroxylase activity, reducing the concentrations of LCA, DCA, and the LCA:DCA ratio.[4,13,16]

What other dietary interventions can modify levels of secondary bile acids?

Other dietary interventions that modify levels of secondary bile acids include increasing vegetable intake, reducing dietary fat, and supplementing with calcium.

Studies have shown that 30%-40% of secondary bile acids bind to lignin, a constituent of vegetable fiber. Plant sterols, in particular beta-sitosterol, can inhibit cholesterol absorption, which is thought to influence the absorption of cholesterol in the intestine.

Conversely, diets low in vegetables can augment cholesterol absorption, which in turn increases the synthesis of the primary bile acid CDCA.[17] An increase in CDCA raises levels of the secondary bile acid LCA, thereby elevating the LCA:DCA ratio.[5]

Reducing dietary fat can also help reduce CDCA synthesis in the liver. High-fat diets induce changes in the colonic flora, increasing levels of 7alpha-dehydroxylase. This enzyme is involved in the conversion of primary bile acids into the more toxic secondary bile acids.[2,5,7] A study by Lupton et al. found that calcium is able to modify the bile acids, via a mechanism that reduces CDCA in bile. Dietary calcium, along with the luminal concentrations of calcium-binding substances such as phosphate and fatty acids, determines the availability of ionized calcium. In its ionized form, calcium is able to form insoluble soaps with bile acids.[18]

What other analytes in the profile relate to colorectal cancer risk?

Other important analytes on the CDSA 2.0 to consider when assessing the bile acid ratio include: calprotectin, beta-glucuronidase, pH, n-butyrate, and occult blood.

When calprotectin results are above 100 mcg/g, one should further investigate the etiology, as elevated levels may be associated with neoplastic disease.

Beta-glucuronidase, pH, and n-butyrate are all modifiable markers that indicate an increased risk for colorectal cancer and breast cancer. A positive occult blood warrants further investigation, and should ideally commence with collecting three consecutive samples for repeat testing.

What is the significance of low secondary bile acids?

Since secondary bile acids are formed by anaerobic bacteria, broad spectrum antibiotic therapy can reduce these bile acids.[19] Reduced cholesterol intake[21] or impaired cholesterol absorption[21] can also result in lower than normal secondary bile acids. Having ruled out dietary insufficiencies of fat, it may be of value to assess hepatic function and serum cholesterol markers to determine the underlying cause of low bile acids.

What other tests might be indicated?

- **Allergy Antibody Assessment**— rule out oat and wheat antibodies, if using these fibers as therapeutic interventions.
- **Celiac Panel**—gluten intolerance can cause damage to gut mucosa that adversely affects lipid absorption.
- **Bacterial Overgrowth of the Small Intestine Breath Test** – Bacteria in the upper small intestine can deconjugate bile acids and affect gut barrier function.
- **Estrogen Metabolism Assessment** (2/16 α-hydroxyestrone ratio)—to assess additional breast cancer risk factors.
- **DetoxiGenomic™ Profile**—to test for glutathione-S-transferase polymorphisms.

References

1 Gaull GE, Wright CE. Taurine conjugation of bile acids protects human cells in culture. Adv Exp Med Biol. 1987;217:61-7.

2 Owen RW, et al. The importance of the ratio of lithocholic to deoxycholic acid in large bowel carcinogenesis. Nutr Cancer 1987;9(2-3):67-71.

3 Imray CH, et al. Faecal unconjugated bile acids in patients with colorectal cancer or polyps. Gut 1992 Sep;33(9):1239-45.

4 Nagengast FM, et al. The effect of a natural high-fiber diet on faecal and biliary bile acids, faecal pH and whole-gut transit time in man. A controlled study. Eur J Clin Nutr 1993 Sep;47(9):631-9.

5 Owen RW, et al. Fecal steroids and colorectal cancer. Nutr Cancer 1987;9(2-3):73-80.

6 Owen RW, Henly et al. Steroids and cancer: faecal bile acid screening for early detection of cancer risk. J Steroid Biochem 1986 Jan;24(1):391-4.

7 Mamianetti A, et al. Fecal bile acid excretion profile in gallstone patients. Medicina 1999;59:269-273.

8 Zuccato E, et al. Role of bile acids and metabolic activity of colonic bacteria in increased risk of colon cancer after cholecystectomy. Dig Dis Sci 1993 Mar;38(3):514-9.

9 Hill M.J. Bile flow and colon cancer. Mutat Res. 1990; 238: 313-320.

10 Schenider H. A factor in the increased risk of colon cancer due to ingestion of animal fat is inhibition of colon epithelial cell glutathione s-transferase, an enzyme that detoxifies mutagens. Med Hypotheses 1992;39:119-122.

11 Miller SR et al. Fecal steroid excretion and degradation and breast cancer stage.J Surg Res. 1983 Jun;34(6):555-9.

12 De Boever P, et al. Protective effect of the bile salt hydrolase-active Lactobacillus reuteri against bile salt cytotoxicity. Appl Microbiol Biotechnol 2000 Jun;53(6):709-14.

13 Hylla S, et al. Effects of resistant starch on the colon in healthy volunteers: possible implications for cancer prevention. Am J Clin Nutr 1998 Jan;67(1):136-42.

14 Orrhage K, et al. Binding of mutagenic heterocyclic amines by intestinal and lactic acid bacteria. Mutat Res 1994 Dec;311(2):239-48.

15 Owen RW. Faecal steroids and colorectal carcinogenesis. Scand J Gastroenterol Suppl. 1997;222:76-82.

16 Reddy B, Engle A, Katsifis S, Simi B, Bartram HP, Perrino P, Mahan C. Biochemical epidemiology of colon cancer: effect of types of dietary fiber on fecal mutagens, acid, and neutral sterols in healthy subjects. Cancer Res 1989 Aug 15;49(16):4629-35.

17 Breuer NF et al. Fecal bile acid excretion pattern in colonic cancer patients. Dig Dis Sci. 1985 Sep;30(9):852-9.

18 Lupton JR, et al. Calcium supplementation modifies the relative amounts of bile acids in bile and affects key aspects of human colon physiology. J Nutr 1996 May;126(5):1421-8.

19 Sedaghat A et al. Effects of neomycin on absorption, synthesis, and/or flux of cholesterol in man. J Clin Invest. 1975 Jan;55(1):12-21.

20 Reddy BS et al. Effect of low-fat, high-carbohydrate, high-fiber diet on fecal bile acids and neutral sterols. Prev Med. 1988 Jul;17(4):432-9.

21 Hakala K et al. Impaired absorption of cholesterol and bile acids in patients with an ileoanal anastomosis. Gut. 1997 Dec;41(6):771-7.

Metabolic Markers

Occult Blood

What is Occult Blood?

Fecal occult blood is hidden blood in the stool that is not detectable through macroscopic evaluation. Such blood may arise from anywhere along the gastrointestinal tract. Occult blood may be the first and, in many cases, the only warning sign of colorectal disease, including colorectal cancer.[1]

What conditions are associated with a positive Fecal Occult Blood Test (FOBT)?

The following conditions are associated with a positive result for fecal occult blood testing (FOBT):

- Colorectal Cancer[2]
- Peptic ulcers[3]
- Inflammatory Bowel Disease[4]
- Polyps[5]
- Diverticulosis[6]

Other conditions that may yield a positive result for FOBT include, but are not limited to: esophageal cancer, stomach cancer, duodenal cancer, small intestine cancer, gastritis, liver cancer, pancreatic cancer, cirrhosis, gallstones, and pancreatitis.[7]

What are the interfering factors that may affect results?

Non-intestinal sources of bleeding from conditions such as hemorrhoids, menstruation, hematuria and the use of rectal suppositories can create false positive results. It is therefore important to avoid testing this patient cohort when bleeding is active.

Certain medications can also interfere with the test results. Examples of drugs that affect the MonoHeam® test include; aspirin, indomethacin, phenylbutazone, corticosteroids, and reserpine. Discontinuing these medications (with physician's approval) for two days prior to testing will ensure that there is no iatrogenic interference.

Vitamin C at doses above 250 mg per day have been found to inactivate the MonoHeam® test, resulting in a false negative result.[8]

Is FOBT on the CDSA & CDSA 2.0 different from the conventional FOBT used to screen for colon cancer?

Essentially, there are two different methodologies that laboratories use for measuring FOBT. One uses polyclonal antibodies, and the other uses monoclonal antibodies. Genova Diagnostics uses monoclonal antibodies, specifically the MonoHeam® test. The advantage of this particular assay is that it is specific for human hemoglobin. This means that there is no cross-reactivity from non-human hemoglobin or vegetable peroxidases, so dietary restrictions are not necessary prior to testing.[8]

Turn-around Time 14 days

Are there any specific patient preparation instructions prior to testing?

In order to help uncover "silent" lesions that may only bleed intermittently, a high-roughage diet is recommended two days prior to testing. High roughage foods include bran cereal, peanuts, popcorn, raw fruits and vegetables.[8]

How do I follow up a positive FOBT result?

Initially, it is important to ensure the above mentioned interfering factors have been ruled out. Bleeding from gastrointestinal lesions can be intermittent, so it is recommended that the MonoHeam® test be performed on three consecutive samples. If this is not possible, then collect from stool samples as closely spaced in time as possible.[8]

If the FOBT is positive, then other follow-up testing is indicated. The American College of Gastroenterology recommends flexible sigmoidoscopy with double contrast barium enema (barium X-ray) or colonoscopy for positive FOBT follow-up testing.[9]

References

1 Qin DX et al. New concept for cancer screening. Eur J Cancer Prev. 1996 Apr;5(2):121-4.

2 Greegor DH. Diagnosis of large-bowel cancer in the asymptomatic patient. JAMA. 1967 Sep 18;201(12):943-5.

3 Wroblewski M Ostberg H. Ulcer disease among geriatric inpatients with positive faecal occult blood test and/or iron deficiency anaemia. A prospective study. Scand J Gastroenterol. 1990 May;25(5):489-95.

4 Howarth GF et al. High prevalence of undetected ulcerative colitis: data from the Nottingham fecal occult blood screening trial. Am J Gastroenterol. 2002 Mar;97(3):690-4.

5 Abdul F et al. Diagnostic value of immunochemical fecal occult blood test for small colorectal neoplasms. Eur J Med Res. 1997 May 28;2(5):227-30.

6 Kronborg O. Diverticulitis: a new high-risk group for colorectal cancer? Scand J Gastroenterol. 2004 Aug;39(8):707-8.

7 Kawai, et al: Clinical study on MonoHaem, an immunological fecal occult blood test reagent. J Japan Soc Colo-Proctolo, 1987;40(3):308-313.

8 Package insert: Silenus. MonoHaemR. Monoclonal Antibody Screening Test for Faecal Occult Blood. Catalogue No: 991040995. Product code: MH101

9 Douglas F et al. ACG recommendations for colorectal cancer screening for average and higher risk patients in clinical practice. April 2000. http://www.acg.gi.org/patients/ccrk/CRC2000.pdf

Microbiology

Beneficial Bacteria

What are Beneficial Bacteria?

Beneficial bacteria, such as lactobacilli and bifidobacteria, play an important role in promoting a healthy gut microflora environment and ensuring proper digestion. These organisms can prevent the over-colonization of the gut with pathogenic organisms and may reduce the risk of certain gastrointestinal diseases.

What are the main functions of Beneficial Bacteria?

Beneficial bacteria have several important functions within the colon:[1]

- Control of potentially pathogenic organisms
- Nutrient production
- Removal of toxins from the gut
- Stimulation of the intestinal immune system

What factors affect the composition of bowel flora?

Several factors may affect the composition of the colonic flora, including diet, transit time, stool pH, age, microbial interactions, colonic availability of nutrients, bile acids, and sulfate, as well as the ability of the microbes to metabolize these substrates.[2]

How does transit time affect the beneficial flora?

Normal transit time can vary greatly from one individual to another, and this can affect the composition of the gut microflora. An increased transit time modifies bacterial pathways, which in turn increases short-chain fatty acid concentrations in the stool.[2]

In which clinical conditions may probiotics be indicated?

- **Colonization resistance**: Perhaps the greatest benefit attributed to probiotics is their contribution to the presence of large numbers of beneficial organisms that may limit the colonization of pathogenic organisms.[3]
- **Bacterial or viral diarrhea**: This condition is typically improved by probiotic therapy, whereas antibiotic-induced diarrhea may be less responsive.[4]
- **Lactose intolerance**: Lactose may be better digested when consumed in the form of a probiotic-containing food.[5]
- **Inflammatory bowel disease**: Probiotics possess immune modulating effects, and act as a first line of defense by preventing disruption of the mucosal barrier and gut associated lymphoid tissue dysfunction.[6]

How do I interpret the levels of Beneficial Bacteria?

Microbial analysis of stool samples provides clinical insight into the flora population of the distal colon. Provided the sample was appropriately obtained (numerous and contrasting small collections from one bowel motion), the quantitative growth on the agar plate should reflect the levels of beneficial bacteria in the distal colon.

Turn-around Time 14 days

The predominant beneficial flora in the large intestine are the bifidobacteria, which constitute as much as 25% of the overall colonic flora in healthy adults. Recovery of these organisms from the colon should therefore ideally be in the 3+ or 4+ ranges. Bifidobacteria are strict anaerobes. Lactobacilli, however, are facultative anaerobes, and as such are able to grow in the presence or absence of oxygen. In the colon, obligate anaerobes such as bifidobacteria predominate over facultative anaerobes such as lactobacilli by 1000:1. It is for this reason that lactobacilli growth as low as 1+ or 2+ is considered normal in healthy adults. Non-pathogenic E. coli populate the distal colon, although they are usually found in reduced quantities, comparable to levels of lactobacilli. A 1+ to 2+ concentration of non-pathogenic E. coli is therefore considered normal. [7-9]

Are probiotics safe to take long term?

Generally speaking, probiotics are extremely safe, even at high doses. No pathogenic or virulence properties have been identified for lactobacilli or bifidobacteria.[10] To date, there are no documented cases of septicemia associated with bifidobacteria. While lactobacillus has been associated with bacteremia, it has only been documented in severely immunocompromised patients, with prolonged hospitalization and after sugery.[11]

What doses are recommended?

Studies have shown that a dose of 10 billion colony forming units (CFU) twice a day is effective in colonizing the intestinal tract.[12]

When do I assess levels of beneficial bacterial relative to supplementation?

To date, it has been assumed, though not confirmed that probiotics adhere to the gastrointestinal mucosa.[13,14] In vitro studies have been able to detect the presence of adhesive substances from probiotics and demonstrate adherence to tissue cells.[15,16,17] A recent study in the animal model demonstrated colonization and translocation of lactobacillus strains into the mucosal lymphoid tissue.[18] However, one cannot conclude from this research that implantation in the human host occurs. It is clear that probiotics when administered exert a number of beneficial effects as mentioned above. These valuable effects might occur simply from transient passage through the gastrointestinal (GI) tract when supplementing, rather than actual colonization. Studies have shown that soon after exogenous supplementation ceases (as little as 7 days), probiotics are no longer recovered from the stool.[19] It is therefore doubtful in the absence of supplementation that beneficial bacteria will be recovered unless they are of indigenous origin.[20] Assessing levels of beneficial bacteria when taking probiotics will ensure that they are effectively delivered to the colon and thus able to exert their beneficial effects on the host.

How does FOS affect growth of the colonic flora?

Fructooligosaccharides (FOS) may act as a fermentative substrate. Because they particularly favor the bifidobacteria population[21] in the gut, regular ingestion can help these organisms to become predominant. A dose of 4 g/day appears sufficient to have this effect in vivo.[22] Even at a dose 5 times higher (i.e., 20 g/day), there is a negligible amount of intact FOS found in the stool, indicating that FOS have been nearly completely fermented in the colon.[23] FOS have been observed to reduce ammonia and isovalerate in stool.[24] FOS also appear to improve calcium absorption from the colon.3 Klebsiella and some strains of E. coli are also able to ferment FOS.[25]

What substances contain FOS?

FOS are contained in many vegetables (and some fruits) that have a significant "starchy" character. Examples include onions, asparagus, chicory, bananas, and artichokes.[20]

References

1 Bengmark S, Jeppsson B. Gastrointestinal surface protection and mucosa reconditioning. JPEN J Parenter Enteral Nutr. 1995 Sep-Oct;19(5):410-5.

2 El Oufir L, et al. Relations between transit time, fermentation products, and hydrogen consuming flora in healthy humans. Gut 1996 Jun;38(6):870-7.

3 Chow J. Probiotics and prebiotics: A brief overview. J Ren Nutr 2002 Apr;12(2):76-86.

4 Hove H, et al. Lactic acid bacteria and the human gastrointestinal tract. Eur J Clin Nutr 1999 May;53(5):339-50.

5 Goossens D et al. Probiotics in gastroenterology: indications and future perspectives. Scand J Gastroenterol Suppl. 2003;(239):15-23.

6 Sartor RB. Probiotic therapy of intestinal inflammation and infections. Curr Opin Gastroenterol. 2005 Jan;21(1):44-50.

7 Modler HW, McKellar RC, Yaguchi M. Bifidobacteria and Bifidogenic factors. J Inst Can Sci Technol Ailment 1990;23(1):29-41.

8 Von Wright A, Salminen S. Probioitics: established effects and open questions. Eur J Gastroenterol Hepatol. 1999 Nov;11(11):1195-8.

9 Tannock GW. The normal microflora: new concepts in health promotion. Microbiol Sci. 1988 Jan;5(1):4-8.

10 Aguirre M, Collins MD. Lactic acid bacteria and human clinical infection. J Appl Bacteriol. 1993 Aug;75(2):95-107.

11 Salminen MK et al. Lactobacillus bacteremia, clinical significance, and patient outcome, with special focus on probiotic L. rhamnosus GG. Clin Infect Dis. 2004 Jan 1;38(1):62-9. Epub 2003 Dec 04.

12 Gupta P, et al. Is lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. J Pediatr Gastroenterol Nutr. 2000 Oct;31(4):453-7.

13 Bezkorovainy A.Probiotics: determinants of survival and growth in the gut. Am J Clin Nutr. 2001 Feb;73(2 Suppl):399S-405S.

14 Reid G. The importance of guidelines in the development and application of probiotics. Curr Pharm Des. 2005;11(1):11-6.

15. Gopal PK et al. In vitro adherence properties of Lactobacillus rhamnosus DR20 and Bifidobacterium lactis DR10 strains and their antagonistic activity against an enterotoxigenic Escherichia coli. Int J Food Microbiol. 2001 Aug 5;67(3):207-16.

16 He F et al. Differences in composition and mucosal adhesion of bifidobacteria isolated from healthy adults and healthy seniors. Curr Microbiol. 2001 Nov;43(5):351-4.

17 Chauviere G et al. Adhesion of human Lactobacillus acidophilus strain LB to human enterocyte-like Caco-2 cells. J Gen Microbiol. 1992 Aug;138 Pt 8:1689-96.

18 Ibnou-Zekri N et al. Divergent patterns of colonization and immune response elicited from two intestinal Lactobacillus strains that display similar properties in vitro. Infect Immun. 2003 Jan;71(1):428-36.

19 Goldin BR et al. Survival of Lactobacillus species (strain GG) in human gastrointestinal tract. Dig Dis Sci. 1992 Jan;37(1):121-8.

20 Reuter G. The Lactobacillus and Bifidobacterium microflora of the human intestine: composition and succession. Curr Issues Intest Microbiol. 2001 Sep;2(2):43-53.

21 Salemans JM, et al. Effect of ageing on postprandial conjugated and unconjugated serum bile acid levels in healthy subjects. Eur J Clin Invest 1993 Mar;23(3):192-8.

22 Gibson GR. Dietary modulation of the human gut microflora using prebiotics. Br J Nutr 1998 Oct;80(4):S209-12.

23 Molis C, et al. Digestion, excretion, and energy value of fructooligosaccharides in healthy humans. Am J Clin Nutr 1996 Sep;64(3):324-8.

24 Swanson KS, et al. Fructooligosaccharides and Lactobacillus acidophilus modify bowel function and protein catabolites excreted by healthy humans. J Nutr 2002 Oct;132(10):3042-50.

25 Van Laere KM, et al. Fermentation of plant cell wall derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria. J Agric Food Chem 2000 May;48(5):1644-52.

Microbiology

Additional Bacteria/Mycology

What are Additional Bacteria and Mycology?

"Additional Bacteria" refers to all aerobic bacterial organisms, whereas "Mycology" refers to fungal organisms." These organisms are classified as Non-Pathogens, Potential Pathogens, or Pathogens. Pathogens are generally treated with antimicrobial agents. Potential pathogens should be confirmed with repeat testing before treatment.

Can you explain the grading system of organisms in the Microbiology sub panel of the CDSA and CDSA 2.0?

Organisms that constitute "Additional Bacteria" and "Mycology" are divided into three categories relative to their pathogenicity:

- **Non-Pathogen** (NP): Organisms in this category are normal flora or commensal flora. Because they have not been recognized as etiological agents of disease, their presence is not considered clinically significant.
- **Potential Pathogens** (PP): Organisms in this category are considered opportunistic pathogens at high concentration, and have referenced scientific literature to support this classification. Only when clinical symptoms persist in the absence of another clearly defined infection, should one consider these organisms etiological agents of disease.
- **Pathogen** (P): Organisms in this category are well-recognized pathogens in the clinical literature. They have a clearly recognized mechanism of pathogenicity and are considered significant regardless of the quantitative growth in culture.

What is the difference between Pathogens and Potential Pathogens?

Organisms defined as Pathogens satisfy Koch's postulates:

- The organism must be present in every case of the disease
- It must be possible to isolate the organism from the disease host and grow it in pure culture
- The pure culture of the organism, when inoculated into a new susceptible host, must produce the same symptoms of disease
- It must be possible to recover the organism from the experimental host

Potential Pathogens do not meet the criteria of Koch's postulates. However, in certain susceptible hosts, they have the potential to be etiological agents of disease. For example, Klebsiella and Proteus are not routinely reported in conventional laboratories, but both have demonstrated antigenic cross-reactivity to HLA antigens.[1] Klebsiella has been associated with ankylosing spondylitis[2] when cross-reactivity occurs with the HLA-B27 antigen.[3] Similarly, *Proteus mirabilis* has been associated with reactive arthritis and is known to cross-react with the HLA-DR4 antigen.[4]

Organisms that are considered pathogens should be eradicated with antimicrobial agents in most cases. Organisms classified as potential pathogens should ideally be confirmed with a positive repeat culture before the practitioner considers therapeutic intervention.

Turn-around Time 14 days

Why is repeat testing recommended when Potential Pathogens are isolated?

These organisms are often normal inhabitants of the gastrointestinal (GI) tract, and may be "transient visitors" in the colon. They are not consistently associated with disease; rather, they are considered opportunistic organisms. It is only when they are in heavy or pure culture (i.e., the sole organism growing) that they may be clinically significant. Repeat testing provides confirmation of the likelihood of pathogenicity and protects the patient against unnecessary antimicrobial therapy.

When should repeat testing be performed after isolating a Potential Pathogen?

Ideally, once the results from the initial profile have been received, a repeat sub-panel should be sent to the laboratory. If the organism is recovered a second time, and patient symptoms persist, then therapeutic intervention should be considered. If the organism is not isolated upon reculture, then it is unlikely to be an opportunistic pathogen.

Should all Potential Pathogens be treated with antimicrobial agents?

No. As mentioned above, Potential Pathogens are not always associated with disease. Ideally, it is advised to repeat a sub-section of the **CDSA** or **CDSA 2.0** (**Bacteriology Culture** or **Microbiology Analysis**) to help determine if the organism may be an opportunistic pathogen. If there are no other pathogenic bacteria, and the patient has GI-related symptoms, then the Potential Pathogen may well be the etiological agent of disease.

Which pathogenic organisms should not be treated with antimicrobial agents?

Antimicrobial therapy is not recommended for uncomplicated Salmonella gastroenteritis. Susceptibility testing for this organism is predominately for surveillance purposes. Antimicrobial therapy for 0157 STEC is not advised, as treatment may enhance toxin release and predispose the host to Hemolytic Uremic Syndrome. Treatment for *Staphylococcus aureus* infection is also not necessary, as complete recovery usually occurs after cessation of symptoms.

Generally speaking, if an antimicrobial agent does not reduce the frequency and duration of the diarrhea, or does not shorten the post-infective shedding—or if the organism is self-limiting by nature—there is little therapeutic value in administering antimicrobial therapy.[5-8]

What is the difference between the Bacteriology Culture and the Microbiology Analysis?

The **Bacteriology Culture** identifies all aerobic bacterial organisms, whereas the **Microbiology Analysis** identifies both bacterial and fungal organisms in the colon. Therefore, to repeat a culture of yeast and bacteria, it would be necessary to perform the **Microbiology Profile**. If the initial **CDSA** or **CDSA 2.0** panel did not identify any organisms under the "Mycology" section, then a Bacteriology culture would suffice.

What if my patient's culture results only revealed a fungal organism and all the other bacteria isolated were non-pathogens?

In this situation, the **Candida Intensive Culture**. This profile includes microscopic visualization of the organism, culture, and measurement of IgG antibodies against C. albicans. Detection of the organism with three different diagnostic methods provides greater confirmation that it is an etiological agent of disease.

Why does Genova Diagnostics identify and report yeast if they are not conventionally recognized as Pathogens?

Currently Genova Diagnostics is one of the few laboratories that identify and report yeast in stool. This is because a growing body of evidence supports the ability of Candida to colonize, bind to the mucosa, and translocate across the mucosal barrier. Colonization is often initiated with broad-spectrum antibiotics, which are commonly prescribed in modern medical practice. Yeast are then capable of becoming opportunistic pathogens after disruption of the mucosal barrier and/or impaired immunity.[9-11]

What further testing may be indicated?

If the initial culture reveals organisms of clinical significance, or potential clinical significance, additional testing may be helpful. The **Intestinal Permeability Profile** can assess whether the patient has a permeable intestinal barrier, also known as "leaky gut." Abnormalities of the immune or mechanical barriers can lead to enhanced uptake of inflammatory luminal macromolecules and pathogenic bacteria. Bacterial antigens are capable of inducing antibodies, which cross-react with host antibodies, forming systemic immune complexes.[12, 13]

When the microbiology results do not recover an etiological agent of infection, parasites should be ruled out with the **Parasitology Profile**. Protozoa such as *Blastocystis hominis* was originally classified as a yeast, and can mimic the symptoms of yeast overgrowth. This can make it challenging at best to distinguish between the two organisms without testing.[14]

The microbiology sub-panel in the **CDSA** and **CDSA 2.0** is reflective of colonic flora only. If a small bowel overgrowth is suspected, consider the **Bacterial Overgrowth of the Small Intestine Breath test**. Generally speaking, when there is a small bowel bacterial overgrowth, post prandial bloating occurs within an hour or two after eating, reflective of early fermentation by bacteria.

Where can I find additional information on microbial pathogens?

Refer to our website: **www.GDX.net** for detailed information on dysbiotic organisms which includes: a description of the microbe, sources of isolation, pathogenicity, symptoms and treatment guidelines.

References

1 Wilson C et al. Cytotoxicity responses to peptide antigens in rheumatoid arthritis and ankylosing spondylitis. J Rheumatol. 2003 May;30(5):972-8.

2 Sahly H, et al. Serum antibodies to Klebsiella capsular polysaccharides in ankylosing spondylitis. Arthritis Rheum 1994 May;37(5):754-9.

3 Ebringer A. Ankylosing spondylitis and Klebsiella—the debate continues. J Rheumatol. 1991 Mar;18(3):312-3.

4 Ebringer A, Khalafpour S, Wilson C. Rheumatoid arthritis and Proteus: a possible aetiological association. Rheumatol Int 1989;9(3-5):223-8.

5 Bopp CA, et al. Eschericia, Shigella, and Salmonella. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology. 7th ed. Washington DC:ASM Press;1999:470.

6 Bopp CA, et al. Eschericia, Shigella, and Salmonella. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology. 7th ed. Washington DC:ASM Press;1999:465.

7 Varnum AH, Evans MG. Foodborne pathogens: an illustrated text. St Louis: Mosby Year Book;1991.

8 Chapter 18.Gastrointestinal infections. In: Lee G, Bishop OP. Microbiology and infection control for health professionals. Sydney (AU): Prentice Hall, 1997:406.

9 Cole GT, Halawa AA, Anaissie EJ. The role of the gastrointestinal tract in hematogenous candidiasis: from the laboratory to the bedside. Clin Infect Dis 1996 May;22 Suppl 2:S73-88.

10 Levinger L, et al. Matrices of paired substitutions show the effects of tRNA D/T loop sequence on Drosophila RNase P and 3'-tRNase processing. J Biol Chem 1998 Jan 9;273(2):1015-25.

11 Matthews HL, Witek-Janusek L. Host defense against oral, esophageal, and gastrointestinal candidiasis. In: Calderone R, ed. Candida and Candidiasis. Washington, DC:ASM Press;2002:180-181.

12 Peters TJ, Bjarnason I. Uses and abuses of intestinal permeability measurements. Can J Gastroenterol 1988;2(3):127-132.

13 Madara JL, et al. Structure and function of the intestinal epithelial barrier in health and disease. In: Gastrointestinal Pathololgy:306-24.

14 Nigro L et al. A placebo-controlled treatment trial of Blastocystis hominis infection with metronidazole. J Travel Med. 2003 Mar-Apr;10(2):128-30.

Microbiology

Helicobacter pylori Specific Antigen (HpSA)

What is Helicobacter pylori*?*

H. pylori is the bacterium which causes peptic ulcer disease and has been associated with increased risk of gastric cancer.[1] *H. pylori* stool antigen (HpSA) testing reveals *H. pylori* antigens shed directly into the stool.[2]

Why should I test for Helicobacter pylori *Stool Antigen (HpSA)?*

Helicobacter pylori remains a highly prevalent infection in the United States and throughout the world. Prevalence is highest areas of low socioeconomic status, with infection rates reaching over 80% in middle-aged adults in many developing countries. This compares to infection rates of 20%-50% in most industrialized countries.[3]

What health risks are associated with H. pylori *infection?*

H. pylori is the major cause of peptic ulcer disease. For those infected, the lifetime risk of developing *H. pylori*-associated peptic ulcer is estimated at 10%-20%.[4]

H. pylori infection can lead to gastric adenocarcinoma (with clinical sequelae of gastric atrophy, intestinal metaplasia and, ultimately, gastric carcinoma). Gastric cancer is the second most frequent cause of cancer-related death. *H. pylori* has been classified as a Type I (definite) carcinogen since 1994. In one Japanese study that tracked patients over 7.8 years, gastric cancer developed in 2.9% of 1246 patients with *H. pylori* infection, whereas no gastric cancer was observed in 280 non-infected controls.[5]

Infection with *H. pylori* can lead to mucosa-associated lymphoid tissue (MALT) lymphoma. As many as 72%-98% of patients with gastric MALT are infected with *H. pylori*. Eradication of *H. pylori* induces regression of gastric MALT lymphoma in 70%-80% of cases. Most patients whose lymphomas respond to *H. pylori* eradication therapy remain in remission for several years. However, evidence concerning long-term outcomes is still limited.[6]

The relationship between *H. pylori* infection and non-ulcer dyspepsia and gastroesophageal reflux disease (GERD) is controversial. However, *H. pylori* eradication therapy appears to result in greater long-term symptom improvement and cost-effectiveness compared with antisecretory therapy. Because of the potential to reduce long-term risk of ulcer disease and possibly gastric cancer, it is widely recommended that patients with GERD and non-ulcer dyspepsia be considered for eradication therapies.[7]

Who should be tested?

- **Patients with duodenal ulcer disease or with a history of duodenal ulcers**— Over 90% of patients with duodenal ulcers are infected with *H. pylori.* Eradication therapy virtually cures the disease, with approximately 5% of patients relapsing after treatment at one year. Without eradication therapy, the relapse rate is 20%-70%, depending on the type of acid suppression therapy used for maintenance.[4]
- **Patients with gastric ulcer disease or with a history of peptic ulcers**— Although *H. pylori* is associated with gastric ulcers as well as duodenal ulcer disease, infection should not always be assumed in these patients. Testing should be done to avoid unnecessary therapy.[8]

Turn-around Time 14 days

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Innovative Testing for Optimal Health

• **Patients using or considering NSAID therapy**— Studies have shown that nonsteroidal anti-inflammatory drug (NSAID) use and *H. pylori* infection are independent risk factors for peptic ulcer disease; when both factors are present, risk increases synergistically.[9] Over 16,000 people die every year in the United States due to NSAID therapy. Most of these patients are elderly individuals who are more likely to be infected with *H. pylori.*

The benefit of diagnosing and eradicating *H. pylori* infection prior to commencing NSAID therapy was recently confirmed in a randomized trial.[10]

• **Patients with GERD**— Although this clinical approach is controversial, patients with GERD symptoms should be tested for *H. pylori* infection and treated, if necessary. One primary justification for this approach is that it may help patients avoid health risks associated with long-term proton pump inhibitor therapies, which can aggravate *H. pylori*-mediated gastritis and thus increase the risk of gastric cancer.[11]

• **Patients with non-ulcer dyspepsia**— A number of studies have shown symptomatic improvement (>70%) in this group of patients after eradication therapy for *H. pylori.* However, there is some evidence that adopting a "test-and-treat" strategy, particularly in the elderly, could be misleading and possibly dangerous if a full diagnostic approach with endoscopy is not adopted.[12]

• **Pediatric patients with allergies**— Recent studies have demonstrated a significant association between *H. pylori,* allergic reactions, food allergy and other allergic diseases.[13] When *H. pylori* colonizes the gastric mucosa, it alters gastric barrier function. This in turn increases the passage of intact molecules across the epithelial barrier with resultant allergies and atopic manifestations.[14]

• **Patients previously treated for *H. pylori***— Because of the high prevalence of metronidazole and clarithromycin resistance, patients who have completed eradication treatments in the past without a confirmatory test for eradication should be tested. Antibiotic-resistance poses a major threat to continued efficacy of the therapeutic regimens.[4]

How is H. pylori *treated once it is diagnosed?*

According to a recent article in BMC Gastroenterology, the greatest success rates for eradication of *H. pylori* were achieved with the regimens outlined in table 1. New research favors a 14 day duration of therapy for successful eradication. The consideration of shortening therapy to 7-10 days is pending results from US-based studies.[15]

Table 1 Garbanz 2005 Treatment choices for the eradication of *Helicobacter pylori*

Treatment Regime for *H. pylori* Eradication	Duration
PPI* + clarithromycin + amoxicillin	2 weeks
PPI + clarithromycin + metronidazole	2 weeks
Ranitidine bismuth citrate + clarithromycin + amoxicillin or metronidazole, or tetracycline	2 weeks
Ranitidine bismuth citrate + clarithromycin + amoxicillin or metronidazole, or tetracycline + PPI or omeprazole	1-2 weeks

**PPI = proton pump inhibitor*

Are their alternative therapies for the eradication of H. pylori*?*

Mastic gum, a resinous exudate from the stem and leaves of *Pistacia lentiscus* has been reported to be effective in the treatment of benign gastric and duodenal ulcers.[16] In vitro studies have demonstrated antibacterial activity against *H. pylori*, thus explaining the anti-peptic-ulcer properties of mastic gum.[17]

Zinc carnosine has demonstrated an inhibitory effect on the growth of *H. pylori*.[18] It has been found to inhibit urease activity and superoxide production, stabilize gastric membranes and promote wound healing.[19] Research shows that zinc carnosine increases the effectiveness of triple treatment therapy when administered concomitantly.[20]

How can I be sure H. pylori *has been eradicated after treatment?*

Invasive testing for cure is not reasonable in the primary care setting. Serologic testing cannot definitively evaluate post-treatment infection status, due to its lack of specificity following treatment.

H. pylori stool antigen (HpSA) testing provides a simple alternative to urea breath testing. Sensitivity and specificity are both 96%. HpSA testing is appropriate for diagnosis and follow-up of infection, and can be used 2 weeks after discontinuing treatment to verify eradication.[21] Because HpSA performs well in children of all ages, it may be the noninvasive test of choice for this group.[4]

What further testing might be indicated?

ImmunoGenomics™ – Research has demonstrated that individuals with single nucleotide polymorphisms in the cytokines interleukin-1beta, tumor necrosis factor-alpha, and interleukin-10 have increased risk for gastric cancers.[22]

Where can I find additional information on Helicobacter pylori*?*

Refer to our website: **www.GDX.net** for detailed information on *H. pylori* which includes: a description of the microbe, sources of isolation, pathogenicity, symptoms and treatment guidelines.

References

1 Uemura N, et al. Helicobacter pylori infection and the development of gastric cancer. New Engl J Med 2001;345:784-89.

2 Vaira D et al. Noninvasive antigen-based assay for assessing Helicobacter pylori eradication: a European multicenter study. The European Helicobacter pylori HpSA Study Group. Am J Gastroenterol. 2000 Apr;95(4):925-9.

3 Feldman RA. Epidemiologic observations and open questions about disease and infection caused by Helicobacter pylori. In: Achtman M, Suerbaum S, eds. Helicobacter pylori: molecular and cellular biology. Wymondham, United Kingdom: Horizon Scientific Press, 2001:29-51.

4 Duggan A. Helicobacter pylori: when is treatment now indicated? Int Med J 2002;32:465-469.

5 Uemura N, et al. Helicobacter pylori infection and the development of gastric cancer. New Engl J Med 2001;345:784-89.

6 Suerbaum S, Michetti P. Helicobacter pylori infection. New Engl J Med 2002;347(15):1175-86.

7 Katelaris PH, et al. A randomized comparison of quadruple and triple therapies for Helicobacter pylori eradication: the QUADRATE study. Gastroenterology 2002; 123:1763-89.

8 Ong SP, Duggan A. Eradication of Helicobacter pylori in clinical situations. Clin Exp Med. 2004 Sep;4(1):30-8. Review.

9 Huang JQ, Sridhar S, Hunt RM. Role of Helicobacter pylori and nonsteroidal anti-inflammatory drugs in peptic ulcer disease: a meta-analysis. Lancet 2002;359:14-22.

10 Chan FK, et al. Eradication of Helicobacter pylori and risk of peptic ulcers in patients starting long-term treatment with non-steroidal anti-inflammatory drugs: a randomized trial. Lancet 2002;359:9-13.

11 Meining A, Kiel G, Stolte M. Changes in Helicobacter pylori-induced gastritis in the antrum and corpus during and after 12 months of treatment with ranitidine and lansoprazole in patients with duodenal ulcer disease. Aliment Pharmacol Ther 1998;12:735-40.

12 Pilotto A, et al. Efficacy of 7 day lansoprazole-based triple therapy for H. pylori infection in elderly patients. J Gastroenterol Hepatol 1999;14:464-71.

13 Corrado G et al. Helicobacter pylori seropositivity in children with atopic dermatitis as sole manifestation of food allergy. Pediatr Allergy Immunol. 2000 May;11(2):101-5.

14 Matysiak-Budnik T, Heyman M. Food allergy and Helicobacter pylori.J Pediatr Gastroenterol Nutr. 2002 Jan;34(1):5-12.

15 Canbaz S et al. Survey of general practitioners' knowledge about Helicobacter pylori infection. BMC Gastroenterol. 2005 Jan 26;5(1):4.

16 Marone P et al. Bactericidal activity of Pistacia lentiscus mastic gum against Helicobacter pylori. J Chemother. 2001 Dec;13(6):611-4.

17 Huwez FU et al. Mastic gum kills Helicobacter pylori.N Engl J Med. 1998 Dec 24;339(26):1946.

18 Matsukura T, Tanaka H. Applicability of zinc complex of L-carnosine for medical use. Biochemistry (Mosc). 2000 Jul;65(7):817-23.

19 Amakawa T. Clinical effect of Z-103 tablets against gastric ulcers: phase III clinical study. Jpn Pharmacol Ther 1992;20:199-223.

20 Kashimura H et al. Polaprezinc, a mucosal protective agent, in combination with lansoprazole, amoxycillin and clarithromycin increases the cure rate of Helicobacter pylori infection. Aliment Pharmacol Ther. 1999 Apr;13(4):483-7.

21 Package insert: Premier Platinum HpSATM (Patent No 5716,971). Enzyme Immunoassay for the Detection of Helicobacter pylori Antigens in Stoll Specimens for Diagnosis and Monitoring.

22 Blanchard TG et al. Helicobacter infection: pathogenesis. Curr Opin Gastroenterol. 2004 Jan;20(1):10-5.

Microbiology

Shiga Toxin *E. coli* (STEC)

What is Shiga Toxin E. coli*?*

Shiga toxin-producing *Escherichia coli* (STEC) is a group of bacterial strains that have been identified as a worldwide cause of severe gastrointestinal disease. Infection with STEC is a major public health threat because of its ability to cause potentially life-threatening illnesses.[5]

How common is Shiga toxin E. coli*?*

In the U.S. alone, an estimated 20,000 STEC infections occur annually, causing approximately 250 deaths from Hemolytic Uremic Syndrome.[1,2]

What are the major strains of E. coli*?*

The subgroup enterohemorrhagic *E. coli* includes over 100 different serotypes, with 0157:H7 being the primary serotype occurring in over 80% of all cases.[3] However, infections caused by non-0157 stains are also being increasingly identified in many countries.[3]

Are all E. coli *considered pathogenic?*

No, there are many *E. coli* strains—some beneficial, some part of the normal flora, and some pathogenic. Only the strains with virulence factors that cause infection are of clinical concern.[4]

How is STEC spread?

STEC is believed to reside primarily in the intestinal tract of animals, most commonly dairy and beef cattle. Foods associated with infection include ground beef, sausage, salami, roast beef, raw milk, mayonnaise, apple cider, and raw vegetables. Because the organism has a low infective dose, food outbreaks may affect a large number of people.[3,5] Less frequently, infection may occur via direct contact or contaminated drinking water.[3]

How does STEC affect the host?

STEC may produce Shiga-like Toxin I and/or Shiga-like Toxin II, which are similar to the toxins expressed by Shigella dysenteriae.[2,6,7] These toxins destroy the colonic epithelium and may cause small blood vessel damage in various tissues including the kidney. Red blood cells passing through the damaged vessels can fragment as a result, causing anemia and thrombocytopenia.[4]

Is E. coli *the only organism that can produce the toxins?*

Because the toxin produced by the organism *Shigella dysenteriae*[1]is almost identical to Shiga-like Toxin I, infection with this organism may result in a positive test. To determine the etiology of a positive STEC result , the Microbiology department at Genova Diagnostics routinely examines each specimen for the presence of *Shigella* spp. and enterohemorrhagic *E. coli*.[6-8]

Turn-around Time 14 days

What are the main symptoms of infection?

Symptoms of STEC vary, and may include mild non-bloody diarrhea, severe bloody diarrhea, abdominal cramping, and fever.[3] Approximately 6% of patients with infected STEC go on to develop Hemolytic Uremic Syndrome (HUS). While HUS can occur in any age group, it is most common in children under the age of five.[1,3-5]

Who should be tested?

Patients who present with diarrhea with or without the presence of blood in the stool should be routinely assessed.[1] A clinical picture of alternating diarrhea and constipation does not warrant testing for STEC. Positive results are immediately reported to the authorizing physician (and when from the U.S., the patient's residing State) for epidemiological purposes and outbreak prevention.

How should STEC be treated?

Prevention is considered the best form of treatment, as antibiotics and antimotility agents are not safe or effective for STEC (i.e., they do not reduce the duration of diarrhea and may increase the risk of HUS).[2,9] Only when cystitis or pyelonephritis occur is antibiotic therapy warranted.[10] Probiotics may help prevent infection, but cannot nullify the effects of STEC once it has attached and released its toxin.[10]

Where can I find additional information about Shiga Toxin **E. coli***?*

Refer to our website: **www.GDX.net** for detailed information on STEC which includes: a description of the microbe, sources of isolation, pathogenicity, symptoms and treatment guidelines.

References

1 Mahon BE et al. Hemolytic Uremic Syndrome Surveillance to Monitor Trends in Infection with Escherichia coli O157:H7 and Other Shiga Toxin-Producing E. coli. Emerging Infectious Diseases National Center for Infectious Diseases Centers for Disease Control and Prevention Atlanta, GA. Updated: 02/28/2005.

2 Pai CH, Ahmed N, Lior H, Johnson WM, Sims HV, Woods DE. Epidemiology of sporadic diarrhea due to verocytotoxin-producing Escherichia coli: a two-year prospective study. J Infect Dis 1988;157(5):1054-7.

3 Bopp CA, et al. Escherichia, Shigella, and Salmonella. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of Clinical Microbiology. 7th ed. Washington, DC:ASM Press;1999:460-465.

4 Lee G, Bishop P. Microbiology and infection control for health professionals. Sydney (AU): Prentice Hall;1997.

5 Karch H, et al. Epidemiology and diagnosis of Shiga toxin-producing Escherichia coli infections. Diagn Microbiol Infect Dis 1999 Jul;34(3):229-43.

6 Stockbine NA, et al. Two toxin-converting phages from Escherichia coli O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. Infect Immun 1986;53(1):135-40.

7 O'Brien AO, et al. Escherichia coli O157:H7 strains associated with haemorrhagic colitis in the United States produce a Shigella dysenteriae 1 (SHIGA) like cytotoxin. Lancet 1983;1(8326 Pt 1):702.

8 O'Brien AD, Holmes RK. Shiga and Shiga-like toxins. Microbiol Rev 1987;51(2):206-20.

9 Paton JC, Paton AW. Pathogenesis and diagnosis of Shiga-toxin-producing Escherichia coli infections. Clin Microbiol Rev 1998;11(3):450-79.

10 Reid G, Burton J. Use of Lactobacillus to prevent infection by pathogenic bacteria. Microbes Infect 2002;4(3):319-24.

Microbiology
Campylobacter

What is Campylobacter jejuni*?*

Campylobacter jejuni is a gram-negative, microaerophilic, thermophilic rod first identified as an important human pathogen in the late 1970s. *C. jejuni* and *C. coli* have both been recognized as primary agents of gastrointestinal infection.[5,6]

How common is Campylobacter jejuni *infection?*

Enteric illness is one of the most common diseases throughout the world. Clinical and epidemiological studies have identified *C. jejuni* as the most frequent cause of bacterial-induced diarrhea. The isolation rate exceeds that of both Salmonella and Shigella combined. In the U.S., the infection rate has been reported as high as 1,000 per 100,000 population. The prevalence is even greater in developing countries.[1-6]

How is C. jejuni *spread?*

Ingestion of 100-1000 organisms is all that is required for the host to become infected. Possible sources of infection include:

- Fecal-oral transmission
- Ingestion of contaminated animal-based foods (e.g., poultry, red meat, milk)
- Consumption of untreated surface water

Epidemiological studies reveal a marked increase in the incidence of infection over the summer months and into the early fall.[2,3,5-7]

How does the infection affect the host?

C. jejuni affects the host by infiltrating and ultimately damaging the intestinal mucosa. From there it may spread to underlying tissue, causing inflammation and ulceration extending along the jejunum, ileum, and colon. While several toxins have been identified, their exact role in the pathogenesis of infection has not been clearly elucidated.[2,7-9]

What are the signs and symptoms of infection?

Patients may be asymptomatic to severely ill. Overt symptoms may include fever, abdominal cramping, and diarrhea (with or without the presence of blood or fecal leukocytes). Muscular pain, headaches, or nausea may also be present.

What are important susceptibility factors?

The highest incidence of *C. jejuni* infection occurs in infants and young children under age 5. Day care centers frequently harbor the organism. Adults aged 20-40 are the next most commonly affected segment of the population.[4,7]

Turn-around Time 14 days

What is the length of time between exposure and onset of symptoms?

Symptoms can occur anywhere between 1-5 days after exposure and generally last 7-10 days in duration[3,4,7]

Are there any other diseases or complications associated with infection?

Although uncommon, infection has been known to occur concurrently with the following conditions:

- Reactive arthritis
- Hemolytic uremic syndrome
- Recurrent colitis
- Acute cholecystitis
- Meningitis
- Guillain-Barre syndrome[3,4,8]
- Invasion and bacteremia (especially in neonates, debilitated adults, and persons with HIV)[5,6]
- Spontaneous abortion, stillbirth, prematurity, and neonatal sepsis[11]

Who should be tested?

- All patients with diarrheal illness[7]
- Infants and children with gastrointestinal symptoms
- Recent travelers to Third World countries

Positive results are immediately reported to the authorizing physician (and when from the U.S., the patient's residing State) for epidemiological purposes and outbreak prevention.

When should the infection be treated?

C. jejuni is generally a self-limiting infection with symptoms seldom lasting beyond 7 to 10 days. However, it is estimated that 5% -10% of those infected may develop persistent diarrhea, and in these cases antibiotic therapy is warranted.[7,10]

What is the treatment of choice for persistent infection?

Erythromycin is the preferred drug (sensitive in >95% of cases) and ciprofloxacin is usually the next most suitable alternative, although increasingly resistant strains are emerging.[7,8] The use of antibiotics decreases the shedding of the offending pathogen and lthe secondary spread, but does not necessarily reduce the duration of symptoms.[1,2]

Where can I find additional information on Campylobacter?

Refer to our website: **www.GDX.net** for detailed information on dysbiotic organisms which includes: a description of the microbe, sources of isolation, pathogenicity, symptoms and treatment guidelines.

References

1 Mahon BE et al. Hemolytic Uremic Syndrome Surveillance to Monitor Trends in Infection with Escherichia coli O157:H7 and Other Shiga Toxin-Producing E. coli. Emerging Infectious Diseases National Center for Infectious Diseases Centers for Disease Control and Prevention Atlanta, GA. Updated: 02/28/2005.

2 Pai CH, Ahmed N, Lior H, Johnson WM, Sims HV, Woods DE. Epidemiology of sporadic diarrhea due to verocytotoxin-producing Escherichia coli: a two-year prospective study. J Infect Dis 1988;157(5):1054-7.

3 Bopp CA, Brenner FW, Wells JG, Strockbine NA. Escherichia, Shigella, and Salmonella. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of Clinical Microbiology. 7th ed. Washington, DC:ASM Press;1999:460-465.

4 Lee G, Bishop P. Microbiology and infection control for health professionals. Sydney (AU): Prentice Hall;1997.

5 Karch H, Bielaszewska M, Bitzan M, Schmidt H. Epidemiology and diagnosis of Shiga toxin-producing Escherichia coli infections. Diagn Microbiol Infect Dis 1999 Jul;34(3):229-43.

6 Stockbine NA, Marques LR, Newland JW, Smith HW, Holmes RK, O'Brien AD. Two toxin-converting phages from Escherichia coli O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. Infect Immun 1986;53(1):135-40.

7 O'Brien AO, Lively TA, Chen ME, Rothman SW, Formal SB.Escherichia coli O157:H7 strains associated with haemorrhagic colitis in the United States produce a Shigella dysenteriae 1 (SHIGA) like cytotoxin. Lancet 1983;1(8326 Pt 1):702.

8 O'Brien AD, Holmes RK. Shiga and Shiga-like toxins. Microbiol Rev 1987;51(2):206-20.

9 Paton JC, Paton AW. Pathogenesis and diagnosis of Shiga-toxin-producing Escherichia coli infections. Clin Microbiol Rev 1998;11(3):450-79.

10 Reid G, Burton J. Use of Lactobacillus to prevent infection by pathogenic bacteria. Microbes Infect 2002;4(3):319-24.

Microbiology

Clostridium difficile

What is Clostridium difficile*?*

C. difficile is an anaerobic, spore-forming, gram-positive bacterium which can be part of the normal intestinal flora. After a disturbance of the gut flora (usually caused by antibiotics) colonization with *C. difficile* can occur. Toxigenic *C. difficile* produces two toxins, A and B, which are the main virulence factors.

Could C. difficile *be a more common cause of diarrhea than previously thought?*

Riley et al.[1] suggest that *C. difficile* may be underestimated as a cause of community-acquired diarrhea due to lack of awareness and inadequate investigation by physicians. A carrier state has been identified in approximately 3% of healthy adults. In the United States alone, *C. difficile* causes approximately 3 million cases of diarrhea and colitis per year. In many hospitals, *C. difficile* is the most common enteropathogen isolated from stool cultures.[2] One study found that up to 11% of patients admitted to the hospital were positive for *C. difficile*. However, only one third of these patients developed diarrhea; the remainder were asymptomatic carriers.[3]

Does C. difficile *infection always cause severe diarrhea?*

Human infection with *C. difficile* can take many forms. Some infected individuals may be "carriers" and appear clinically healthy. Some may have recurrent mild to moderate diarrhea and other symptoms resembling Irritable Bowel Syndrome (IBS). Others may have recurrent severe cramps and diarrhea, with or without flatulence.

C. difficile may also present as a condition indistinguishable from colitis, with cramps, diarrhea, urgency, mucus, and blood.[4] Severe, fulminant pseudomembranous colitis accounts for less than 3% of cases.[5]

Unless it is clearly diagnosed by testing for both toxins A and B, infection with *C. difficile* may be misdiagnosed as IBS or Inflammatory Bowel Disease.

Who should be tested?

All patients with recurrent diarrhea who have been treated with antibiotics within the last 8 weeks should be tested for *C. difficile*.

Given the high prevalence and variable clinical presentations associated with *C. difficile* infections, screening should be done on any patient with chronic, unexplained gastrointestinal complaints. These symptoms include abdominal pain, diarrhea, bloating, gas, and flatus.

How do I interpret this test?

If the test is negative, it indicates the absence of both toxins A and B, or an extremely low toxin level that is below the assay's detection limit (>= 0.2 ng/ml).

If the test is positive, it indicates the presence of *C. difficile* toxin A and/or toxin B.

Sensitivity of this test is 82-93% and specificity is 94-96%.6 Positive results are immediately reported to the authorizing physician (and when from the U.S., the patient's residing State) for epidemiological purposes and outbreak prevention.

Turn-around Time 14 days

Why does Genova Diagnostics perform testing for both toxins A and B?

Many laboratories use an Enzyme Immuno Assay (EIA) method that only captures the toxin A antibody. However, some isolates from *C. difficile* clinical cases have been shown to elicit only toxin B. In England, these strains have accounted for 3% of the samples referred to the reference laboratory. Strains producing only toxin B have also been described in Canada.[7-9]

What are therapeutic options for patients testing positive?

- **Antibiotic withdrawal** - In many cases, withdrawing antibiotics allows the normal gut flora to re-grow and enables the patient to spontaneously recover from mild infections. This is particularly true when beneficial bacteria antagonistic to *C. difficile* (such as Bacteroides) have not been damaged. However, withdrawal of antibiotics is often not sufficient to rid the patient of the infection, which may persist for life.[10]
- **Metronidazole** (Flagyl®) – This is the first-line medication against *C. difficile*, although at higher doses, nausea may be a problem. However, studies have shown that metronidazole alone may not be sufficient to eradicate *C. difficile*. Bowel flora must also be intact.[11]
- **Vancomycin** – This medication is recommended as the second line of therapy, or when metronidazole cannot be tolerated or is contraindicated (such as in pregnancy). While eradication rates are comparable to Flagyl, this drug is more expensive, and may pose concern for vancomycin-resistant enterococci.[12]
- **Rifampicin** (Rifampin®) – This anti-clostridial antibiotic can be used for longer periods of time but may have side effects.[13]
- **Cholestyramine** (Questran®) – This cholesterol-lowering medication attaches to *C. difficile* toxins and can be used palliatively to decrease diarrhea and cramping. It does not resolve the infection itself; rather it is an adjunctive tool.[14]
- **Lactobacillus** GG – When used with metronidazole and vancomycin, helps eradicate *C. difficile* infection. This probiotic is also effective in reducing the 3 week recurrence rate of *C. difficile* by helping to restore normal flora.[15]
- **Saccharomyces boulardii** – This beneficial fungus has antibacterial activity against *C. difficile*. It transiently colonizes the bowel, and has been proven more beneficial than placebo. This fungus cannot eradicate *C. difficile*; rather it has been found to prevent or shorten the duration of *Clostridium difficile*-associated diarrhea.[16]
- **Prevention** – In lieu of an effective, commercially available human vaccine (currently under development) substantial and adequate infection control measures are crucial in preventing the spread of *C. difficile*.

Where do I find more information about C. difficile*?*

Refer to our website: **www.GDX.net** for detailed information on *C. difficile* which includes: a description of the microbe, sources of isolation, pathogenicity, symptoms and treatment guidelines.

References

1 Riley TV, et al. Community-acquired Clostridium difficile-associated diarrhea. Clin Infect Dis 1995;20(supp 2):S263-265.

2 Hurley BW, Nguyen CC. The spectrum of pseudomembranous enterocolitis and antibiotic-associated diarrhea. Arch Int Med 2002;162:2177-2184.

3 Barbut F, Petit J-C. Epidemiology of Clostridium difficile-associated infections. Clin Microbiol Infect 2001; 7:405-410.

4 Yassin SF et al. Clostridium difficile-associated diarrhea and colitis. Mayo Clin Proc 2001;76:725-30.

5 Bartlett JG. Clostridium difficile: clinical considerations. Rev Infec Dis 1990;12(suppl 2):S243-S251.

6 Package insert: Remel ProstecT™ C. difficile toxin A/B Microplate Assay.

7 Alfa MJ, et al. Characterization of a toxin A-negative, toxin B-positive strain of Clostridium difficile responsible for a nosocomial outbreak of Clostridium difficile-associated diarrhea. J Clin Microbiol 2000;38:2706-14.

8 Brazier JS. The epidemiology and typing of Clostridium difficile. J Antimicrob Chemother 1998;41 (supp C):47-57.

9 Al-Barrak A, et al. An outbreak of toxin A-negative, toxin B-positive Clostridium difficile-associated diarrhea in a Canadian tertiary-care hospital. Can Commun Dis Rep 1999;25:65-9.

10 Malnick SD, Zimbony O. Treatment of Clostridium difficile-associated diarrhea. Ann Pharmacother. 2002 Nov;36(11):1767-75.

11 Gerding DN. Treatment of Clostridium difficile-associated diarrhea and colitis. Curr Top Microbiol Immunol. 2000;250:127-39.

12 Poutanen SM, Simor AE. Clostridium difficile-associated diarrhea in adults. CMAJ. 2004 Jul 6;171(1):51-8.

13 Byrd RP et al. Delayed onset of pseudomembranous colitis after rifampin therapy. South Med J. 1997 Jun;90(6):644-6.

14 Stroehlein JR. Treatment of Clostridium difficile Infection. Curr Treat Options Gastroenterol. 2004 Jun;7(3):235-239.

15 Pochapin M. The effect of probiotics on Clostridium difficile diarrhea. Am J Gastroenterol. 2000 Jan;95(1 Suppl):S11-3.

16 Marteau PR et al. Protection from gastrointestinal diseases with the use of probiotics. Am J Clin Nutr. 2001 Feb;73(2 Suppl):430S-436S.

Microbiology

Optimized Parasite Recovery (OPR)

What is Optimized Parasite Recovery?

Optimized Parasite Recovery (OPR) involves combining three stool specimens submitted on consecutive days for intestinal ova and parasite examination. This varies from the traditional approach of using individual samples for parasitic evaluation.

How prevalent are parasites?

Data from analysis shows that parasites are detected in 22% of samples submitted to Genova Diagnostics. This implies that a significant number of individuals suffers from parasite infections, many of whom do not experience overt gastrointestinal (GI) symptoms. This data was derived from over 30,000 samples taken from patients with acute GI symptoms as well as those with few or no GI symptoms. Since a fundamental premise of functional medicine assumes that imbalances in the gastrointestinal environment may influence other physiological functions, health care practitioners should consider parasitology evaluations for patients who do not exhibit specific gastrointestinal symptoms.

How can I be sure that all clinically relevant parasites will be identified?

OPR involves combining multiple stool specimens for intestinal ova and parasite examination, as opposed to only using an individual sample for evaluation. Studies show that by combining stool specimens, the overall detection rate of parasites increases by 3%-6%.[1-4]

How much more accurate is OPR than traditional methods of parasite detection?

A study by Kelton et al. compared the OPR methodology with the traditional individual specimen collection in over 15,000 patients. The results demonstrated an increase in parasite detection from 18% using individual samples, to 22% using the OPR methodology. Throughout the six month study period, there was on average a 5% increase in positivity.[1]

Does pooling of stool samples reduce the recovery of parasitic organisms?

In the study cited above, individual parasite detection rates were calculated to determine if there was an overall increase in detection or a bias towards recovery of certain parasites with the OPR methodology. Prior to pooling, protozoans were the most frequently identified parasite, with helminthes, ova, and larvae making up less than 1% of the total parasite recovery. A review of the data showed that the recovery frequency of specific parasites identified by pooling increased evenly, with the

Turn-around Time 14 days

exception of *Dientamoeba fragilis*, which showed a 2-fold increase in detection frequency. The OPR methodology includes a unique double filtration and spinning methodology. This helps to remove fiber and debris that can otherwise obscure smaller parasites such as *D. fragilis*. With the OPR methodology, helminth identification remained at less than 1%. Overall, pooling of the samples did not affect the detection rate of rarer parasites.[1]

What is the best method for detecting helminthic infections?

Generally speaking, microscopic evaluation for ova is the preferred way to assess helminthic infection. The exception would be when worms are seen visually in the samples. In this circumstance, it is best to collect the worms and separately preserve in a formaldehyde solution (green capped vial in the collection kit) for macroscopic evaluation. If pinworm *(Enterobius vermicularis)* is suspected, then a "scotch-tape flag" is the best method of collection. The female worms migrate outside the rectum to shed their eggs. By placing scotch tape over the perianal region at night, the eggs can be collected on the tape, which is then placed on a glass slide and sent to Genova Diagnostics for microscopic evaluation.[5]

References

1 Kelton J, et al. Impact on detection of intestinal parasites using pooled SAF-preserved samples. Presented at American Society for Microbiology 102nd General Meeting; Salt Lake City, UT, May 2002.

2 Aldeen WE, et al. Comparison of pooled formalin-preserved fecal specimens with three individual samples for detection of intestinal parasites. J Clin Microbiol 1993;31(1):144-45.

3 Long EG, Christie JD. The diagnosis of old and new gastrointestinal parasites. Clin Lab Med 1995;15(2):307-31.

4 Koontz F, Weinstock JV. The approach to stool examination for parasites. Gasterenterol Clin North Am 1996;25(3):435-49.

5 Garcia, LS. Diagnostic Medical Parasitology. 4th ed. Washington DC: ASM; 2001;276.

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