Introduction – CDSA and CDSA².⁰™

This Support Guide is intended to help clinicians understand and use the CDSA & CDSA².⁰ Profile, a select set of fecal biomarkers aimed at identifying key processes that influence both gastrointestinal and overall health.

The test report is organized so that the clinician may move through the results in a logical order that enhances clinical utility.
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Organization of the Biomarker Review

In the following sections,

- Each biomarker is first identified and described
  - Biomarker Key Points
- The candidate patient population for the biomarker is described
- The comparator or existing gold standard for the biomarker is presented (if established), along with performance characteristics of the fecal biomarker when appropriate
- The interpretation of the fecal biomarker is discussed, including the significance of out-of-range results
- Desirable outcomes and therapeutic recommendations are discussed, indicating how the specific test might benefit patients in a variety of clinical settings

Graphical Representation and Color Coding for Biomarkers

In addition to a numeric result and a stated reference range for each result, all biomarker results on the CDSA & CDSA\textsuperscript{2.0} report are graphically represented and color-coded in the context of a specific reference population by means of standard deviation (SD).

In general, for a 2-tailed test, the green region includes plus or minus 1 SD from the population mean, or 68.2% of all results. The yellow areas include plus or minus 2 SD from the population mean, and encompass 95.4% of the distribution; the red area represents the remainder of the population that falls outside of 2 SD in either direction.

Some biomarkers have established threshold values associated with specific clinical conditions, histopathological findings, or recommended clinical interventions, i.e., medical decision points (MDPs). Biomarkers fecal calprotectin and pancreatic elastase-1 have reference ranges based on a clinically characterized healthy reference population (i.e., not a symptomatic tested population) and cutoff points indicating a normal result, a borderline or weakly positive result, and an abnormal or strongly positive result.

Digestion and Absorption

For proper nutrition and gastrointestinal (GI) function, ingested nutrients must first be broken down (digested, in a biochemical sense), and the products of digestion must then be absorbed through a variety of physical and biochemical processes.

In good health, digestion is accomplished in several steps. First by chewing and other physical processes, and then by the actions of stomach acid and a host of enzymes produced in the pancreas and small intestine, breaking down the three major components of food: complex carbohydrates (starches), proteins, and fats.

Absorption of the resulting products of digestion then occurs by several distinct processes. Damage to, or impairment of any of the processes involved in digestion or absorption results in two main problems: inadequate net absorption of nutrients, producing absolute or relative nutrient deficiencies, and/or delivery of intact nutrients to the colon, where gut microbes may inappropriately digest or ferment nutrients. Such fermentation results in byproducts leading to excessive osmotic loads and gases, leading to abdominal discomfort, diarrhea, flatulence, and other common symptoms.\textsuperscript{2}

Biomarkers of digestion and absorption provide information about nutrient breakdown and entry into the circulation. They ultimately indicate how well the GI tract is performing its basic digestive functions. The biomarkers are:

- Pancreatic Elastase-1 & Chymotrypsin, markers of exocrine pancreatic function
- Putrefactive SCFAs, markers of undigested protein reaching the colon
- Fecal Fat, a marker of fat breakdown and absorption
- Meat and Vegetable Fibers, markers of maldigestion

Maldigestion is defined as impaired breakdown of nutrients, and is often the result of inadequate or impaired digestive enzymes (or gastric acid production), while malabsorption refers to impairments in absorption of the normal end products of digestion.\textsuperscript{3}

When faced with a patient experiencing sub-acute or chronic GI symptoms, it is the task of the clinician to discern which, if any, of these processes is occurring, and then if possible, to identify one or more underlying primary causes. Finally, in many cases, once a primary cause has been identified, a rational and usually simple course of therapy may be prescribed, with the goal of repairing the underlying pathological processes.

In many cases, fecal biomarker testing is useful in discerning whether maldigestion, malabsorption, or both, are present. Such testing is also helpful in identifying the underlying causes, for which treatment may be available.
increases or decreases in intestinal transit times, and is not affected by pancreatic enzyme replacement therapy
• The PE1 reference range was adopted from an FDA-approved kit

Biomarker Key Points
• Noninvasive biomarker of pancreatic exocrine (i.e., digestive) function
• Is not affected by supplemental pancreatic enzymes
• Reflects true pancreatic exocrine function

Fecal PE1 testing can be used for initial determination of pancreatic exocrine insufficiency in suspected patients, as well as the monitoring of pancreatic exocrine function in patients under treatment.

Patient Populations of Interest
Patients in whom PE1 testing may be useful include those with:
• Unexplained diarrhea
• Weight loss
• Other symptoms of maldigestion
• Abdominal pain
  › Including symptoms meeting clinical criteria for irritable bowel syndrome (IBS)
• Low bone density

In addition, pancreatic exocrine insufficiency may occur secondary to:
• Chronic pancreatitis
• Diabetes
• Celiac disease
• Cystic fibrosis
• Inflammatory bowel disease (IBD)
• Excessive alcohol consumption
• Gallstones

There is also evidence that aging populations may exhibit a progressive loss of PE1, since pancreatic exocrine function may decrease with age.

Comparator/Gold Standard Tests
PE1 has a strong correlation with the gold-standard test for pancreatic insufficiency, the secretin-caerulein test. (Caerulein is a synthetic analog for pancreozymin and stimulates pancreatic activity in a similar manner.)

Interpretation

<table>
<thead>
<tr>
<th>Fecal PE1 Value (µg)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 200</td>
<td>Normal exocrine pancreatic function</td>
</tr>
<tr>
<td>100 to 200</td>
<td>Mild-to-moderate exocrine pancreatic insufficiency</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>Severe pancreatic insufficiency</td>
</tr>
</tbody>
</table>

Results are based on Medical Decision Points:
• Fecal PE1 testing may have reduced sensitivity for detecting mild pancreatic exocrine insufficiency in children
• Consumption of vegetarian or vegan diets, or other diets involving decreased meat intake, have been associated with reductions in fecal PE1
• Pancreatic exocrine insufficiency occurs in about 50% of type 1 diabetics, and in about 33% of type 2 diabetics
• Chronic pancreatitis patients may have compromised antioxidant systems

Outcomes and General Therapeutic Considerations
Patients with PE1 results suggestive of exocrine pancreatic insufficiency should undergo further investigation to address the underlying causes of insufficiency. Additionally, complications may result from pancreatic exocrine insufficiency and additional testing may be considered, as shown in the following table:

Additional Testing | Rationale
---|---
Evaluation of fecal fats | Excess fecal fat may be due to:
  • Lack of bile acids (due to liver damage, hypolipidemic drugs, or impaired gallbladder function)
  • Celiac disease
  • Small bowel bacterial overgrowth
  • Other conditions and medications (e.g., Orlistat)

Full nutritional assessment | Defective exocrine pancreatic function may be associated with:
  • Abnormal blood lipids
  • Low levels of minerals (magnesium, zinc, selenium, and calcium)
  • Low levels of fat soluble vitamins (A, D, E, and K)
Supporting the Patient with Evidence of Pancreatic Exocrine Insufficiency

Certain lifestyle, medication, and supplement interventions may be appropriate for patients with abnormal fecal PE1 results suggestive of pancreatic exocrine insufficiency.

Lifestyle Support

- Small, frequent meals (better absorbed)
- Reduce alcohol consumption
- Smoking cessation

Medication/Supplement Support

Support patients with pancreatic exocrine insufficiency by pancreatic enzyme replacement therapy (PERT) at doses appropriate for degree of insufficiency and based on symptom improvement; in some conditions PE1 levels normalize as underlying disorders improve (improved PE1 levels reflect functional improvements, not supplementary enzymes).

Chymotrypsin - CDSA

The Biomarker

Chymotrypsin is one of the numerous digestive enzymes secreted by the exocrine portion of the pancreas. Specifically, it is a protein-digesting enzyme which can be useful when monitoring pancreatic exocrine function in patients with normal stool transit time (degradation of proteases can result in higher chymotrypsin levels in response to diarrhea, and lower levels in response to slow transit time).

Biomarker Key Points

- Noninvasive biomarker of pancreatic exocrine (i.e., digestive) function
- Affected by exogenous supplementation making it an ideal marker for monitoring dosing adequacy
- Altered gut transit may effect chymotrypsin

Patient Populations of Interest

Patients in whom Chymotrypsin testing may be useful include those with:

- Unexplained diarrhea
- Steatorrhea
- Weight loss
- Abdominal distention and flatulence
- Signs of nutrient deficiency
- Other symptoms of malabsorption

Comparator/Gold Standard Tests

The gold-standard test for pancreatic insufficiency is the secretin-pancreozymin test. Unlike PE1, chymotrypsin has not been correlated with the gold standard test, although it has been compared to the 72-hour fecal fat test.

Interpretation

Low levels of chymotrypsin (< 0.9 U/g) in the presence of normal transit time are indicative of exocrine pancreatic insufficiency. Low chymotrypsin can also result from slowed transit time (constipation).

High levels of chymotrypsin (> 26.8 U/g) suggests a rapid transit time (diarrhea) or may be due to excessive pancreatic enzyme supplementation.

Outcomes and General Therapeutic Considerations

Chymotrypsin is a simple and non-invasive screening test and can assist the physician in assessing exocrine pancreatic insufficiency. Patients with altered chymotrypsin levels should undergo further investigation to determine the underlying causes of their dysfunction; refer to the corresponding Pancreatic Elastase-1 table (on previous page) for information about additional testing.

- Pancreatic dysfunction typically leads to malabsorption, the severity of which is relative to the degree of exocrine pancreatic impairment. Evaluation of absorptive markers will provide valuable insight into the degree of malabsorption present.

Supporting the Patient with Evidence of Pancreatic Exocrine Insufficiency

Certain lifestyle, medication, and supplement interventions may be appropriate for patients with abnormal chymotrypsin results suggestive of pancreatic exocrine insufficiency. Please refer to the corresponding Pancreatic Elastase section.

Putrefactive Short Chain Fatty Acids (SCFA) Total – CDSA & CDSA

The Biomarker

When proteins or their digestion products (oligopeptides and amino acids) reach the distal colon, they are fermented by colonic organisms (a process known as proteolytic fermentation) into the characteristic short-chain fatty acids (SCFAs), isovalerate, isobutyrate, and valerate.

Biomarker Key Points

- Normal protein digestion and absorption is relatively complete in stomach and small intestine
Healthy colonic contents therefore include only small amounts of protein-derived SCFAs.

Protein fermentation can yield a diversity of end products, including SCFAs, amines, phenols, indoles, thiols, sulfur compounds, and branched chain fatty acids.

Though many have shown toxic properties in vitro and in animal models, the relationship between gut health and protein fermentation in humans has not been thoroughly investigated.

Primary colonic SCFAs from protein breakdown are valerate, isovalerate, and isobutyrate.

The result on the CDSA & CDSA report reflects a combined total of valerate, isovalerate, and isobutyrate measurements.

Patient Populations of Interest

Patients with protein maldigestion, or those with abnormally large amounts of protein presented to the distal colon, may demonstrate increased products of colonic protein breakdown in the stool.

Increased fecal presence of putrefactive SCFAs may be present in patients with:

- Hypochlorhydria (diminished hydrochloric acid secretion in the stomach), which is associated with:
  - Advanced age – in roughly 30% of elderly patients, gastric acid secretion is diminished.
  - Use of acid-blocking medications or dietary supplements that produce too high a gastric pH to allow for complete protein digestion in the stomach.
  - Food reactions; elevated gastric pH (less acidic) has been associated with increased risk for food reactions, possibly from hindering protein breakdown.

- Pancreatic exocrine insufficiency or pancreatitis; insufficient pancreatic proteases leave improperly-digested protein fragments that reach the colon.

- Excessive protein intake

- Gastrointestinal bleeding or irritation, mucosal desquamation, and bacterial overgrowth; these conditions result in excessive self-derived proteins in the intestinal lumen.

Comparator/Gold Standard Tests

Currently there is no gold standard assessment for fecal putrefactive SCFAs. Putrefactive SCFAs are utilized as a contributory diagnostic tool. Testing for fecal nitrogen may indicate the presence of protein malabsorption, but fecal nitrogen is difficult to measure and is not in widespread clinical use.

Interpretation

The result for putrefactive SCFAs reflects the sum of fecal valerate, isovalerate, and isobutyrate.

Outcomes and General Therapeutic Considerations

Patients with elevated putrefactive SCFAs should be evaluated for common causes of insufficient protein digestion and/or excessive protein presenting to the colon.

<table>
<thead>
<tr>
<th>Source of Elevated Colonic Products of Protein Breakdown</th>
<th>Possible Causes</th>
<th>Therapeutic Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient Protein Digestion</td>
<td>Hypochlorhydria</td>
<td>- Reduce acid-blocking medications</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Add betaine HCl</td>
</tr>
<tr>
<td></td>
<td>Pancreatic exocrine insufficiency</td>
<td>- Evaluate fecal PE1</td>
</tr>
<tr>
<td>Excessive delivery of protein to colon</td>
<td>High-protein diet</td>
<td>Review protein/carbohydrate intake</td>
</tr>
<tr>
<td></td>
<td>GI irritation/inflammation, bleeding, bacterial overgrowth</td>
<td>Additional testing, e.g., fecal calprotectin, fecal eosinophil protein X (EPX), fecal occult blood, stool culture for beneficial bacteria</td>
</tr>
</tbody>
</table>

Meat Fibers & Vegetable Fibers - CDSA

The Biomarker

Meat and vegetable fibers in the stool have been used to identify adequate digestion and absorption when used in combination with clinical presentation and other biomarkers, such as Pancreatic Elastase-1.

Biomarker Key Points

Meat and vegetable fibers are digested in the upper gastrointestinal tract, therefore the presence of these food fibers are suggestive of maldigestion and malabsorption or increased gut transit (diarrhea).

Patient Populations of Interest

- Patients with signs and symptoms of incomplete digestion including diarrhea, steatorrhea, weight loss, flatulence, abdominal distention, signs of nutrient deficiency and other possible symptoms of maldigestion.

Comparator/Gold Standard Tests

There is no correlation between fecal presence of food fibers and the gold standard for the assessment of digestion and absorption. Used in combination with other digestion and absorption biomarkers, food fibers can help determine insufficiency and monitor treatment progression.
Interpretation

The presence of meat fiber or more than a few vegetable fibers in the stool suggests incomplete digestion (e.g., pancreatic insufficiency, hypochlorhydria).

- Elevated levels can also result from inadequate mastication or hypermotility.

Outcomes and General Therapeutic Considerations

Stool food fibers is a simple screening test and can assist the clinician in assessing digestion and absorption. Patients with increased food fibers in the stool should undergo further investigation into causes, and other biomarkers of absorption and digestion should be evaluated.

Supporting the Patient with Evidence of Maldigestion and Malabsorption

Certain lifestyle, medication, and supplement interventions may be appropriate for patients with the presence of stool food fibers (e.g., pancreatic enzyme therapy). Refer to other biomarkers of digestion and absorption in conjunction with the clinical presentation of the patient to best determine which therapeutic option to select for the patient.

Total Fecal Fats – CDSA & Add-On CDSA

The Biomarker

Under normal conditions, the bulk of dietary fat is digested and absorbed in the small intestine, leaving only small amounts for delivery to the colon and fecal stream. Fecal fat measurements determine the amount of fat in stool, and may therefore identify fat maldigestion, malabsorption, or steatorrhea.

Biomarker Key Points

- The test is a fecal fat extraction method that results in a quantitative value
- Fecal fat extraction methods have been found to correlate with degree of fat malabsorption
- Total fecal fat is made up of long-chain fatty acids (LCFA), cholesterol, triglycerides, and phospholipids

Patient Populations of Interest

Fecal fats should be measured in any patient for whom steatorrhea (passage of pale, bulky, and malodorous stools) may be a symptom of underlying digestive or non-digestive disorders.

Symptoms suggesting evaluation of fecal fat as a means of detecting root causes include:

- Fatigue
- Unexplained anemia
- Nutrient deficiencies
- Unintended weight loss

Comparator/Gold Standard Tests

The 3-day stool collection with total fecal fat determination is the gold standard test for fecal fat. This test is unwieldy and unpleasing for patients and lab personnel. The total fecal fat extraction on a single specimen provides a quantitative value to identify patients that may benefit from the more in-depth 3-day test. Limited research has found extraction methods to correlate with the gold-standard.

Interpretation

Total fecal fat is the sum of fecal triglycerides, long-chain fatty acids, cholesterol, and phospholipids.

Outcomes and General Therapeutic Considerations

Fecal fat may be elevated in situations of fat maldigestion, such as:

- Pancreatic exocrine insufficiency (inadequate lipase production or delivery)
  - Causes include chronic pancreatitis and cystic fibrosis
- Bile salt insufficiency (inadequate solubilization of fats for digestion)
  - Causes include liver damage, hypolipidemic drugs, impaired gallbladder function
- Hypochlorhydria (inadequate stomach acid)
  - Causes include aging and gastric acid-lowering drugs
- Small intestinal bacterial overgrowth and resulting acidic small-intestinal pH (impairment of small intestinal digestive enzymes)
- Use of medications designed to impair intestinal lipase activity (Orlistat, Xenical, Alli), or use of synthetic fat-like products indigestible by normal lipase (Olestra)
- Elevated fecal fat may be associated with deficiencies in fat-soluble nutrients, so consider nutritional assessment of essential fatty acids, fat-soluble vitamins, and minerals
Fecal fat may also be elevated in situations of fat malabsorption, such as:

- Intestinal dysbiosis
- Intestinal parasites
- Gastric bypass, ileal resection, or other surgeries that limit absorptive surface area

Fecal fat may be elevated in patients with:

- Irritable bowel syndrome (often as a symptom of pancreatic exocrine insufficiency)
- Inflammatory bowel disease
- Food intolerances
- Celiac disease
- Excessive alcohol intake
- Chronic use of non-steroidal anti-inflammatory drugs (NSAID)

Supporting the Patient with Elevated Fecal Fat Levels

Depending on root causes, patients with elevated fecal fat levels can be supported as follows: 2,51-53,58-60

<table>
<thead>
<tr>
<th>Suspected Cause of Elevated Fecal Fat</th>
<th>Support Measures</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic exocrine insufficiency</td>
<td>Supplementary plant or pancreatic digestive enzymes</td>
<td>Lipases increase fat digestion</td>
</tr>
<tr>
<td>Disorders of bile formation/transport (cholestasis)</td>
<td>Bile salts or cholangues, taurine or glycine, Diet changes</td>
<td>Enhance intestinal fat solubilization</td>
</tr>
<tr>
<td>Lipase inhibitors (orlistat, Xenical, Alli) or synthetic fat consumption (Olestra)</td>
<td>Discontinue these products</td>
<td>Permit normal fat digestion</td>
</tr>
</tbody>
</table>

Inflammation and Immunology

Interactions between the immune system and the GI tract are being recognized as of growing importance, not only in GI physiology and pathophysiology, but also in their influences on systemic health and disease.

Biomarkers of GI inflammation and immunology provide information about the GI tract's interactions with, and responses to, the outside world. They indicate how well the GI tract is maintaining its role as a barrier, as well as whether the GI tract is undergoing pathological responses to external or internal challenges. The biomarkers are:

- **Calprotectin & Lactoferrin**, markers of neutrophil-driven inflammation
- **Eosinophil Protein X**, a marker of eosinophil-driven inflammation and allergic response

**Calprotectin – CDSA² & Add-On CDSA**

**The Biomarker**

Calprotectin is a protein produced in abundance by neutrophils, the ubiquitous immune system “first responders.” When neutrophils accumulate at sites of inflammation, they release increased amounts of calprotectin in a way that closely correlates with findings on endoscopy and histology, and are thus useful in quantifying the degree of intestinal inflammation. 61,62

This property makes calprotectin useful for differentiating inflammatory from non-inflammatory disease processes, e.g., distinguishing irritable bowel syndrome (IBS) from inflammatory bowel disease (IBD). 63

**Biomarker Key Points**

- Calprotectin is described in the literature as a useful non-invasive screening tool for identifying which patients may benefit from endoscopy for suspected IBD
- Calprotectin is used in diagnosing IBD (Crohn’s disease and ulcerative colitis) and for quantifying degree of inflammation
  - Thus calprotectin may be useful for monitoring treatment and assessing for relapse in patients with known IBD
- Calprotectin is FDA-cleared to differentiate IBS from IBD 63

**Patient Populations of Interest**

Patients with symptoms consistent with IBS should have fecal calprotectin testing done as a means of ruling out significant inflammation; those with positive Rome criteria and normal calprotectin (< 50 mcg/g) have virtually no chance of having IBD. 1,64
Rome III Diagnostic criteria for IBS

Recurrent abdominal pain or discomfort** at least 3 days/month in the last 3 months associated with two or more of the following:

1. Improvement with defecation
2. Onset associated with a change in frequency of stool
3. Onset associated with a change in form (appearance) of stool

* Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis

** "Discomfort" means an uncomfortable sensation not described as pain

Comparator/Gold Standard Tests

The gold-standard comparator test for determining presence and degree of intestinal inflammation is endoscopy with biopsy and histology; fecal calprotectin correlates closely with this approach.

Interpretation

The expected values for fecal calprotectin are shown here:

<table>
<thead>
<tr>
<th>Calprotectin Concentration (micrograms/g stool)</th>
<th>Interpretation</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;= 50</td>
<td>Normal (no active GI inflammation)</td>
<td>None</td>
</tr>
<tr>
<td>&gt; 50 to 120</td>
<td>Borderline, suggestive of low-grade inflammation</td>
<td>Re-evaluate in 4–6 weeks</td>
</tr>
<tr>
<td>&gt; 120</td>
<td>Abnormal</td>
<td>Determine source of inflammation and repeat as clinically indicated</td>
</tr>
<tr>
<td>&gt; 250</td>
<td>Associated with high risk of clinical relapse in IBD61</td>
<td>Adjust therapy accordingly</td>
</tr>
</tbody>
</table>

Results are based on Medical Decision Points, not standard deviations.

- Elevated calprotectin may also result from chronic NSAID use; evaluate such use in all patients with elevated calprotectin
- Per the peer-reviewed literature, calprotectin levels utilizing the reference range of < 50 mcg/g applies to patients ages 4 and older62,70
- Pediatric reference ranges for ages < 4 years old have not been fully established, and may normally be higher, especially in infancy68-72

Outcomes and General Therapeutic Considerations

Calprotectin is a simple, reliable, and non-invasive test that is useful in:

- Selecting patients with abdominal symptoms who may require further diagnostic procedures73
- Aiding in distinguishing between IBD and IBS74
- Selecting/screening patients for endoscopy, especially children in whom general anesthesia might be required for invasive study61
- Determining disease activity and risk of relapse in IBD75
  > Therapy can then be initiated before inflammation reaches critical intensity
- To monitor IBD treatment response and to determine when a full clinical remission has been achieved76
- To evaluate efficacy in trials of new treatments for IBD

Supporting the Patient with Elevated Fecal Calprotectin Levels

Because inflammatory, infectious, or neoplastic processes may result in an elevated calprotectin level, the cause of a value > 120 mcg/g warrants further investigation—including endoscopy or radiography—based on clinical correlation.

- Assessments to uncover causes of bowel inflammation are found at the end of this section on Inflammation and Immunology
Lactoferrin – CDSA
The Biomarker

Lactoferrin is an iron-binding glycoprotein secreted by most mucosal membranes; it is a major granular component of neutrophils (white blood cells). Liberated from the neutrophils in response to inflammation, lactoferrin binds to iron, impeding microbial growth and facilitating generation of hydroxyl radicals.77

Biomarker Key Points78
Expressed by surface epithelial cells and found in most exocrine secretions, including breast milk, tears, nasal secretions, saliva, intestinal mucus, and genital secretions.

- Lactoferrin is a multifunctional protein with antibacterial and immune modulatory activities and is a component of the first line of host defense
- Its expression is upregulated in response to inflammatory stimuli
- In the gastrointestinal tract, lactoferrin serves as a non-specific marker of inflammation

Patient Populations of Interest
Potential indications for testing include patients exhibiting gastrointestinal symptoms with suspected inflammation. Although clinical cut-offs have yet to be determined definitively, lactoferrin may be useful in assisting the clinician in (1) identifying which patients may need further evaluation for inflammatory bowel disease,79-81 and (2) differentiating between IBD and non-inflammatory irritable bowel syndrome (IBS).82

Comparator/Gold Standard Tests
The gold-standard comparator test for determining presence and degree of intestinal inflammation is endoscopy with biopsy and histology. Fecal lactoferrin has been correlated to histological findings.83

Interpretation
A positive lactoferrin test generally indicates inflammation of the intestinal mucosa.

Outcomes and General Therapeutic Considerations
Lactoferrin is a non-invasive screening test and can assist the physician in stratifying symptomatic patients who require further evaluation; subsequent calprotectin testing can provide additional useful diagnostic information and assist in triage for endoscopic referral.

Supporting the Patient with Abnormal Lactoferrin Results
To further delineate the root cause(s) of an abnormal lactoferrin result, consider additional testing to uncover causes of bowel inflammation, which are found at the end of this section on Inflammation and Immunology.

Eosinophil Protein X – CDSA2.0
The Biomarker

Many inflammatory and neoplastic processes in the gut involve increased activity of eosinophils, white blood cells that normally reside in the lamina propria (connective tissue layer) of the intestinal wall.

When the lamina propria is damaged, eosinophils migrate into the gut lumen, where they degranulate to release a variety of proteins with cytotoxic properties, which contribute to ongoing inflammation and tissue destruction.84 One such protein is eosinophil protein X (EPX), which can be measured in fecal matter.85

Biomarker Key Points
- Fecal EPX offers the practitioner a noninvasive alternative to the invasive gold standard, allowing for better differential diagnosis
- EPX is considered the superior cationic protein for assessment of eosinophil function, because it most accurately reflects the degree of mucosal damage.86
- Baseline EPX levels offer a way to determine and monitor GI inflammation associated with food allergy
  - Significant reduction in EPX after 3 months on an elimination diet has been demonstrated.87
  - Return of EPX to normal levels can be used to indicate clinical efficacy of elimination diets or clinical remission of IBD.86
- Systemic corticosteroids can reduce circulating levels of EPX.88

Patient Populations of Interest
Clinically, elevations in EPX indicate the presence of an IgE-mediated inflammatory process. Patients at risk for having such processes, and in whom fecal EPX may be helpful in making diagnosis and treatment plans, include those with:

- Complaints of GI symptoms related to food intake (possible food allergy)
  - Also for monitoring results of elimination diets or other interventions in patients with known food allergy
- Because food allergy may present with symptoms similar to those seen in IBS, also consider fecal EPX testing in patients with symptoms consistent with IBS
- Concerns about possible parasitic/worm infections (recent travelers with GI symptoms)
• IBD in need of non-invasive monitoring for disease activity and treatment monitoring (consider calprotectin as well)

Comparator/Gold Standard Tests

The gold standard test for quantifying eosinophils in the gut is whole gut lavage. This is an invasive procedure requiring endoscopy, and has limited utility in the office setting. Clinical research indicates significant correlation between whole gut lavage fluids and eosinophil mediators in stool, such as fecal EPX.99

Interpretation

The normal reference range for EPX is < 4.6 mcg/g of stool. Levels above 1.1 may indicate increased eosinophil activity in the gut lumen, which may be suggestive of increased IgE-mediated reactivity associated with allergy or inflammatory states.

• Baseline EPX levels may be used to determine inflammation associated with food allergy
  › Subsequent testing can be used to monitor results of dietary changes

• Elevations of EPX correlate with disease activity in ulcerative colitis and Crohn's disease96

• Serial testing of EPX offers a non-invasive means of evaluating disease activity and for predicting relapses in patients with IBD90

Outcomes and General Therapeutic Considerations

EPX levels above 1.1 mcg/g of stool may be suggestive of elevated eosinophil activity, which may be associated with the following:96,89,91

• IgE-mediated food allergy
• Intestinal parasitic infection (specifically helminths)91-93
• IBD

Less commonly, fecal EPX may be elevated in:84,89,90,94,95

• Atopic dermatitis
• Gastroesophageal reflux disease (GERD)
• Collagenous colitis
• Allergic colitis
• Excessive alcohol intake
• Chronic diarrhea
• Protein-sensitive enteropathy
• Gastrointestinal cancer
• Eosinophilic gastroenteritis (rare)

Conversely, in patients with known food allergy or IBD, acquisition of a normal EPX (< 4.6 mcg/g stool) can indicate the efficacy of treatment, such as elimination diet or remission of IBD.

Supporting the Patient with Elevated Fecal EPX

To further delineate the root cause(s) of an elevated fecal EPX result, consider additional testing to uncover causes of bowel inflammation, which are found immediately below.

Supporting the Patient with Gastrointestinal Inflammation and Immune Reactions

Regardless of the specific findings, the support for patients with evidence of GI inflammation and/or immune reactions involves the following steps:

• Eradicate known pathogens or other infectious agents96
• Consider supporting commensal bacteria with probiotic supplements and dietary changes97-100
• Consider intestinal mucosal and anti-inflammatory support: appropriate nutrients, dietary changes, and botanicals101-108
• Rule out food sensitivities or allergies; consider elimination diet and/or IgG and IgE food sensitivity testing109-111
• Support immune status with supplemental whey protein or increased fiber
• Consider further evaluation of underlying causes as shown in the following table:

<table>
<thead>
<tr>
<th>Tests for Discerning Underlying Causes of Bowel Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal Permeability (IP) Assessment</td>
</tr>
<tr>
<td>Food Antibody Assessment</td>
</tr>
<tr>
<td>Celiac Panel</td>
</tr>
<tr>
<td>ImmunoGenomic™ Profile</td>
</tr>
</tbody>
</table>
Additional Tests

Macroscopic GI biomarkers and occult blood have long been used in the analysis of stool as a diagnostic analyte. When accompanied with other symptoms, these markers may provide information about various GI health conditions. This is a potential indicator of gastrointestinal health and changes may warrant further evaluation. The biomarkers are:

- **Color**, associated primarily with diet and medications
- **Mucus**, normally produced in the GI tract
- **Occult Blood**, which demonstrates presence of microscopic quantities of blood in the stool

**Color – CDSA**

*The Biomarker*

Stool’s characteristic brown color results from the degradation of bile during its course through the intestinal tract. Therefore the color of stool is influenced by what the patient consumed and by the amount of bile, a yellow-green fluid responsible for the digestion of fats. Color is a subjective assessment made by the patient when submitting the test.\(^{112}\)

**Biomarker Key Points**

Stool is the end product of digestion and the color may simply be due to variation of diet, supplementation, or medications. However, it may be due to a GI pathology and warrant a more detailed work-up.\(^{112}\)

**Patient Populations of Interest**

Clinical indication for testing include unexplained changes from the normal brown color of stool, which can indicate the benefit of additional diagnostic testing in conjunction with the patients clinical picture.

**Comparator/Gold Standard Tests**

Stool color is a contributory diagnostic test; currently there is no gold standard comparator test.

**Interpretation**

Changes in stool color can be due to a variety of causes including transit time, diet or medications, bleeding in the GI tract, or impairments in biliary or gallbladder function.

- Changes in bilirubin (e.g., liver or biliary tract disorders) may cause stool to be gray, clay-like, or green.
- Fat malabsorption (or infection by Giardia) can cause stool to be yellow & greasy.\(^{112}\)
- Bleeding in the GI tract can produce varying shades, from bright red (usually lower GI tract), to maroon (upper GI tract), to black/tarry (usually upper GI tract, or swallowed blood from dental procedure or nosebleed). Black stools may also result from consuming bismuth, iron, beets, or licorice.

**Outcomes and General Therapeutic Considerations**

Stool color alone, is unable to define a diagnosis in most cases. However, when used in combination with other GI biomarkers, symptoms, past medical history, dietary changes, and medications, it may help determine the cause of the stool color change.\(^{112}\)

Physical examination and other diagnostic testing will aid in identifying potential GI conditions.

**Mucus – CDSA**

*The Biomarker*

Mucus is a smooth thick substance produced in the digestive tract. Mucus lubricates surfaces and allows materials to pass smoothly.

**Biomarker Key Points**

Mucus can be a normal finding in the stool, however, significant amounts of mucus and/or if accompanied by diarrhea, pain or blood may signify a more significant intestinal condition.\(^{112}\)

**Patient Populations of Interest**

Clinical indication for testing includes unexplained diarrhea, pain, signs of GI inflammation, or blood in stools. Based on the findings and clinical presentation of the patient, it may warrant further testing.

**Comparator/Gold Standard Tests**

There is no gold-standard comparator test for mucus identification in the stool.

**Interpretation**

Excessive mucus in the stool could be associated with an immune response.

**Outcomes and General Therapeutic Considerations**

Clinical implications of excessive fecal mucus include the possibility of an inflammatory GI disorder or malignancy.\(^{112}\) Treatment would be based upon abnormal results of stronger biomarkers in GI assessment.
Occult Blood – CDSA & Add-On CDSA 2.0
The Biomarker

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 and inflammatory bowel disease. Normally stools should be entirely free of blood.

Biomarker Key Points

The Hemosure diagnostic kit uses fecal immunochemical testing (FIT); it has higher specificity than the common guaiac test because of its use of mono- and polyclonal antibodies specific to human hemoglobin. Therefore there are no drug or dietary restrictions prior to collecting the sample in relationship to FIT.

Patient Populations of Interest

Clinical indications include risk factors or suspicion of colorectal cancer or inflammatory bowel disease.

Comparator/Gold Standard Tests

FIT-based diagnosis have been recommended by the American College of Gastroenterology in 2009 as the preferred test for colorectal cancer screening/detection. Further investigation is warranted in the presence of a positive test.

Interpretation

Abnormal amounts of hemoglobin in the stool suggest excessive blood loss. Other biomarkers, testing, and physical exam should be employed to determine the presence of hemorrhoids, peptic ulcer, intestinal polyps, diverticulitis, infection/parasite, inflammatory bowel disease, or malignancy. Other conditions that may yield a positive result should also be explored.

Outcomes and General Therapeutic Considerations

With a positive result, follow-up testing is indicated. The American College of Gastroenterology recommends flexible sigmoidoscopy or colonoscopy for positive fecal occult blood follow-up testing.

Gastrointestinal Microbiome

Biomarkers of the GI Microbiome provide information about the health, function, and diversity of the trillions of microbial cells in the GI tract. They indicate how well the microbiome is performing its shared metabolic functions with the human host. The biomarkers are:

- **Metabolic indicators**, which demonstrate specific and vital metabolic functions performed by the microbiota
- **Bacterial and mycological culture**, which demonstrate presence of specific beneficial and pathological organisms
- **Parasitology**, which demonstrates presence of parasites

Clinically significant imbalances in the GI microbiome (dysbiosis) have been associated with:

- Irritable bowel syndrome (IBS)
- Inflammation
- Inflammatory bowel disease (IBD)
- Immune Modulation
- Metabolic Disorders
- Body weight & fat distribution
- Insulin sensitivity/type 2 diabetes
- Autism
- Other acute and chronic disorders

There are multiple ways of assessing the GI microbiome and its impact on human health.

**Metabolomic** methods involve measuring and interpreting the metabolic products of bacterial activity in the colon; these include determinations of fecal short-chain fatty acids and of enzymes elaborated by gut organisms, such as beta-glucuronidase.
Metabolic Products: Short-Chain Fatty Acids – CDSA & CDSA^2.0

The Biomarker

Commensal gut bacteria anaerobically ferment resistant starch and dietary fiber (including prebiotics) to produce the beneficial short-chain fatty acids (SCFAs) acetate, propionate, and butyrate. In particular, n-butyrate is the obligate fuel source for colonocytes, and inadequate levels are associated with disordered colonic health.\(^{129,130}\)

In the gut, short-chain fatty acids:

- Maintain intestinal barrier function
- Provide fuel for colonocytes (n-butyrate)
- Regulate colonic absorption of water and electrolytes
- Salvage unabsorbed carbohydrates
- Support commensal bacteria

SCFA levels are influenced by many factors, including:

- Diet composition
- Fecal ammonia content (indicative of excessive undigested protein)
- Obesity
- Environment

Biomarker Key Points

- Fecal SCFA concentrations are a metabolomic indicator of the health of the GI microbiome
  - Low concentrations suggest either dysbiosis (abnormal levels or function of gut bacteria) or inadequate substrate for commensal organisms to ferment

- While optimal levels have not yet been identified, higher fecal concentrations of SCFAs have been associated with decreased GI disease in epidemiologic studies

Patient Populations of Interest

Studies have demonstrated distinct differences between healthy populations and those with gastrointestinal disorders when comparing the composition of gut microbiota and SCFA distribution. Lower levels of SCFAs are associated with colorectal diseases in population studies. Altered SCFA production has been seen in IBD and IBS. Fecal SCFA testing is a non-invasive contributory diagnostic tool in evaluating such patients.

Comparator/Gold Standard Tests

There is currently no gold-standard test for assessing SCFA production. SCFAs can also be measured in the blood. All three major SCFAs (acetate, butyrate, and propionate) are present in portal blood at concentrations several times greater than peripheral venous blood indicating the gut as a major source of these fatty acids.\(^{130}\) Because SCFAs are rapidly utilized in the body, it is problematic to correlate blood levels with fecal SCFAs.

Interpretation

Optimal levels have not yet been determined for fecal SCFAs, however, in general, higher levels are considered beneficial.

The relative concentrations of n-butyrate, acetate, and propionate are also reported as percentages of total SCFAs. Low SCFA production is associated with:

- Decreased carbohydrate or fiber intake\(^{128}\)
- Low levels of fecal anaerobic or commensal bacteria (dysbiosis)\(^{73,132,133}\)
- Dysbiosis has been associated in research with inflammatory processes (e.g., IBD),\(^{134}\) and functional bowel disorders (e.g., IBS)\(^{135-137}\)

Outcomes and General Therapeutic Considerations

Low fecal SCFA levels typically indicate disordered metabolic processes in the colonic commensal community, e.g., inadequate amounts of beneficial bacteria or inadequate substrate for those bacteria to produce their beneficial metabolic products, SCFAs.

Diets high in fiber and resistant starch, and relatively low in protein content, may increase SCFA production with resulting reduced risk of colorectal diseases and lowering of cholesterol and blood sugar.\(^{138-146}\)

Supporting the Patient with Low Fecal SCFA Concentrations

Support of patients with low fecal SCFAs is centered on increasing metabolic substrates for beneficial SCFA-producing organisms:

- Prebiotic supplementation
- Increased dietary carbohydrate and fiber intake
- Increased consumption of resistant starch, which is known to increase levels of fecal butyrate\(^{137}\)
- Evaluate and treat abnormalities of commensal gut bacteria
**Beta-glucuronidase**

**The Biomarker**

Beta-glucuronidase is an enzyme that breaks down complex carbohydrates. Additionally, it acts to deconjugate glucuronide molecules from a variety of toxins, carcinogens, hormones, and drugs, which are naturally glucuronidated in the liver to facilitate biliary excretion. Deconjugation of these molecules in the gut permits their reabsorption via enterohepatic recirculation, producing higher than desired blood levels of potentially harmful compounds.

Additionally, many beneficial nutrients are ingested as the glucuronide conjugate of the active molecule, which must be deconjugated in order for the beneficial molecule (the “aglycone”) to be absorbed. Such nutrients include lignans, flavonoids, ceramides, and glycyrrhetinic acid.

Thus, a proper balance of glucuronidase in the gut lumen is essential. Beta-glucuronidase is inducible in colonocytes, but it is also produced by anaerobic gut bacteria (particularly *E. coli*, but also *Peptostreptococcus*, *Bacteroides*, and *Clostridium*).

**Biomarker Key Points**

- Limited research suggests an association between elevated fecal beta-glucuronidase and colon cancer risk.148,149
- Low fecal beta-glucuronidase may also represent a problem, because the enzyme is needed to release the active aglycone forms of many dietary phytonutrients.

**Patient Populations of Interest**

Evaluating beta-glucuronidase may be of interest to clinicians interested in evaluating substances that require deconjugation of glucuronide molecules, such as hormones, vitamin D, toxins, and phytonutrients.

**Comparator/Gold Standard Tests**

There is currently no gold-standard test.

**Interpretation**

Abnormally high levels of this biomarker warrant further investigation; abnormally low levels may diminish the bioavailability of many phytonutrients.

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**Outcomes and General Therapeutic Considerations**

Further evaluation of patients with elevated fecal beta-glucuronidase includes consideration of exposure to and intake of toxins, hormones, and drugs.

**Supporting the Patient with abnormal fecal beta-glucuronidase:**

For patients with *elevated* fecal beta-glucuronidase:

- The following supplements may be helpful:
  - Calcium-D-glucarate
  - Milk thistle
  - Probiotics (*Lactobacilli* and *Bifidobacteria*).

- The following dietary management may be helpful:
  - Increased consumption of vegetables and insoluble fiber

Beta-glucuronidase may be *lower* following antibiotic administration, which may reduce beta-glucuronidase activity due to reduction of gut bacteria.150-153

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**pH — CDSA & CDSA2.0**

**The Biomarker**

Fecal pH indicates the relative acidity or alkalinity of the feces. The pH of the stool should not be confused with stomach pH (GI tract pH fluctuates significantly, depending on location), and therefore is not directly influenced by hydrochloric acid in the stomach.154

**Biomarker Key Points**

Factors that have an impact on stool pH include fiber and food constituent intake,155-157 fermentive processes, bacterial populations, antibiotics,158 and stool transit time.159

**Patient Populations of Interest**

Clinical indications for testing fecal pH include diarrhea or constipation, suspected carbohydrate malabsorption, or small intestinal bacterial overgrowth (e.g. bloating).154,160,161

**Comparator/Gold Standard Tests**

Fecal pH is a standard accepted laboratory procedure, but is a non-specific test. Currently, there is no gold standard comparator test.

**Interpretation**

A normal fecal pH is associated with a mildly acidic stool (often a result of SCFA production), which encourages beneficial
bacteria and discourages intestinal pathogens that prefer a more neutral pH.\textsuperscript{154}

- Abnormally low fecal pH of < 6.1 (stool acidity) may be related to malabsorption of carbohydrates (including lactose),\textsuperscript{154,160} or to small bowel bacterial overgrowth.\textsuperscript{161} Osmotic diarrhea is another possible cause of a low fecal pH,\textsuperscript{162} such as from using osmotic laxative agents.

- Abnormally high fecal pH of > 7.9 (stool alkalinity) may be due to hypochlorhydria,\textsuperscript{154} slow transit time/constipation, antibiotics,\textsuperscript{154} inadequate dietary fiber,\textsuperscript{163} or a high protein/low carbohydrate diet.\textsuperscript{40}

### Outcomes and General Therapeutic Considerations

No direct clinical action is necessary; however, follow-up with identifying and addressing a possible cause is recommended.

### Secondary Bile Acids – CDSA\textsuperscript{2.0} & Add-on CDSA

#### The Biomarker

Bile acids are the end products of hepatic cholesterol metabolism and are responsible for fat emulsification, aiding lipid absorption and digestion in the small intestine. Primary bile acids, chenodeoxycholic acid (CDCA) and cholic acid (CA) are derived from cholesterol. Once they enter the colon, they are acted upon by anaerobic bacteria to produce the secondary bile acids lithocholic acid (LA) and deoxycholic acid (DCA). CDCA is modified into LA and CA is modified to DCA.

#### Biomarker Key Points

- Secondary bile acids have been shown to have carcinogenic and mutagenic properties.\textsuperscript{164-170}

- The specific bacteria involved in primary bile acid deconjugation to secondary bile acids include \textit{Clostridium}, \textit{Enterococcus}, \textit{Bacteroides} and \textit{Lactobacillus}.\textsuperscript{171}

- Diet can have a significant impact: Increased dietary fiber can reduce secondary bile acids, while high saturated fat and high meat diets are associated with elevated levels.\textsuperscript{170,172}

- Secondary bile acids have been associated with increases of oxidative stress and DNA damage.\textsuperscript{170,173,174}

- Secondary bile acids have been shown to have carcinogenic and mutagenic properties.\textsuperscript{164-169}

- LCA is thought to be more toxic than DCA, partly due to its inhibitory effects on glutathione-S-transferase (GST) in colocytes.\textsuperscript{160-166}

#### Patient Populations of interest

- Clinical indications for testing include: IBS, IBD; history of cholecystectomy; gallstones; presence of risk factors or suspicion of CRC; suspicion or evidence of increased intestinal permeability; impaired intestinal motility.

### Comparator/Gold Standard Tests

Currently, there are no comparator gold standard test for secondary bile acids; LCA and DCA are utilized as a contributory diagnostic tool.

#### Interpretation

Elevated LCA/DCA ratio has been associated with:

- Colorectal cancer\textsuperscript{175-178}
- Gallstone formation\textsuperscript{179}
- Cholecystectomy\textsuperscript{180,181}

Elevated secondary bile acids have also been associated with:

- Impaired gallbladder function or cholesterol gallstone formation\textsuperscript{182,183}
- Inflammation within colonic mucosa, which is thought to also compromise intestinal permeability\textsuperscript{174,184}

Low secondary bile acids may result from:

- Broad-spectrum antibiotics\textsuperscript{185}
- Reduced cholesterol intake or absorption

### Outcomes and General Therapeutic Considerations

Measurement of secondary bile acids can help guide treatment of patients with GI disorders or give insight into a more serious GI pathology which would require further testing. Certain dietary recommendations and supplement interventions may be appropriate for patients with abnormal secondary bile acids.

#### Dietary support:

- Fiber and probiotics can help reduce an elevated bile acid ratio. Fiber reduces the concentration of secondary bile acids in the stool.

- Resistant starch, insoluble fiber contained in wheat bran, legumes and certain vegetables, decreases the level of secondary bile acids. These insoluble fibers enhance short-chain fatty acids production in the proximal colon, thus lowering intestinal pH. A reduction in pH inhibits 7 alpha-hydroxylase activity, which reduces the concentrations of LCA, DCA, and the LCA:DCA ratio.\textsuperscript{186-188}

- Probiotics and prebiotics have been found to reduce the conversion of CDCA to LCA.\textsuperscript{189}

- Lowering saturated fat and meat intake may decrease secondary bile acid levels.\textsuperscript{170}
Bacteriology (Culture)

Culture methods have established clinical utility and defined parameters that have been long recognized as “gold standard” in traditional clinical diagnostics.

Beneficial Bacteria (Culture)

Cultivable gut bacteria include beneficial bacteria such as Bifidobacteria, Lactobacilli, and E. coli, all of which are thought to exert positive local and systemic effects through their anti-inflammatory and immune modulating properties. Conversely, impaired commensal bacterial populations have been associated with intestinal and chronic diseases.

Biomarker Key Points

- Lactobacillus species and E. coli are facultative anaerobes, capable of surviving in an environment with limited oxygen
- Bifidobacteria are obligate anaerobes, and must be grown in anaerobic chambers

Culture is required for determining therapeutic interventions such as sensitivities to pharmaceutical or botanical antibiotics.

Patient Populations of Interest

Bacterial culture remains of great utility in evaluating patients with symptoms of possible gastrointestinal infection or dysbiosis.

Comparator/Gold Standard Tests

Bacterial culture is the gold standard for identification of populations of cultivable organisms.

Interpretation

Results of bacteriology culture are reported as “No Growth,” or growth in one of three categories of bacteria, using a color-coding system:

- No growth (white)
- Non-pathogen (white)
- Potential pathogen (yellow)
- Known pathogens (red)

The standard deviation reporting system is not used for this test; instead, growth is reported as present at one of four semi-quantitative levels of abundance.

Bacteriology

12. Beneficial Bacteria

Lactobacillus species
Escherichia coli
Bifidobacterium

Outcomes and General Therapeutic Considerations

Patients with low growth of beneficial bacteria may benefit from dietary manipulations and supplements.

Dietary changes:

- Increased intake of fiber and whole, complex carbohydrate and resistant starch
  - Introduce high-fiber foods gradually to avoid exacerbation of GI symptoms

Supplements:

To promote and sustain beneficial commensals, consider addition of probiotics (live bacteria) and/or prebiotic supplements (products to support indigenous microbiota) to alter commensal bacteria; for example:

- Probiotics
  - Lactobacillus
  - Bifidobacteria
- Prebiotics
  - Psyllium
  - Oat bran
  - Oligofructose
  - Xylooligosaccharide
  - Inulin
  - Beta-glucan
  - Arabinogalactan

Botanical products may also be used to decrease or modulate gut bacteria.
Additional Bacteria (Culture)

Bacterial culture of fecal material may also yield additional organisms of interest in the assessment of the gut microbiome. Many such organisms constitute part of the normal aerobic or commensal flora, and are not recognized as major pathogens. They are typically readily cultured for identification when they occur at clinically significant levels.

Additional bacteria also include organisms that may be potential pathogens (PP on the report form), or “opportunistic” pathogens. Not usually pathogenic, these organisms may overgrow during periods of perturbation in the gut environment and their presence on culture may be an indicator of imbalance or dysbiosis. The significance of these organisms must be interpreted in the context of a specific patient’s clinical presentation (e.g. symptoms, immunosuppression, etc.).

Biomarker Key Points

Additional or opportunistic pathogens are typically restrained and controlled by balanced levels of commensal organisms, but their overgrowth may occur when commensal bacterial populations are impaired by:

- Infection with overt pathogens or parasites
- Poor diet
- Antibiotic use
- Lowered gut immunity

Patient Populations of Interest

Patients whose management might benefit from testing for Additional Bacteria include those with symptoms of unexplained or persistent diarrhea, especially those with known or suspected imbalance of the normal gut flora (dysbiosis), and those who are immunosuppressed.

Comparator/Gold Standard Tests

This test uses bacterial culture. Identification of cultured bacteria is via Vitek-MS using Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF). The MALDI-TOF mass spectrometry platform utilized for the rapid identification of bacteria and yeast from pure cultures on the CDSA & CDSA2.0 Profile report relies on the most extensive FDA-cleared library of microbial targets available on the market, which can accurately identify approximately 200 different additional bacterial species.

Interpretation

Additional bacteria are reported using the same conventions as for Bacteriology Culture, above.

<table>
<thead>
<tr>
<th>*NG</th>
<th>NP</th>
<th>PP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Growth</td>
<td>Non-Pathogen</td>
<td>Potential Pathogen</td>
<td>Pathogen</td>
</tr>
</tbody>
</table>

- No growth (white)
- Non-pathogen (white)
- Potential pathogens (yellow)
- Known pathogens (red)

The standard deviation reporting system is not used for this test; instead, growth is reported as present at one of four semi-quantitative levels of abundance.

The presence of Potentially Pathogenic organisms (PP) at higher levels, or of Pathogenic bacteria (P) at any level, should trigger increased concern. Such levels may be of clinical relevance in patients with bacterial gastroenteritis including:

- ‘Travelers’ diarrhea\textsuperscript{190-194}
- Food poisoning\textsuperscript{195}
- IBD\textsuperscript{196}
- IBS\textsuperscript{197}

Presence of opportunistic or potentially pathogenic organisms may also indicate intestinal microflora imbalance or dysbiosis, poor diet, antibiotic use, or lowered gut immunity.

Outcomes and General Therapeutic Considerations

Once identified, pathogenic and potentially pathogenic/opportunistic bacteria may be decreased through pharmacological and botanical treatments, and by restoring a healthy intestinal microflora balance.

Pharmacological treatments are best utilized subsequent to standard methods of diagnosis, including sensitivity profiles. When opportunistic bacteria are found, they are automatically cultured and the sensitivity of pharmaceuticals and active botanical ingredients are assessed. Agents marked Sensitive or “S” have been
shown to be effective treatments in culture/sensitivity studies and may be used as indicated.

**Mycology (Culture)**

The Biomarker

The fungal kingdom includes yeasts and molds. Yeasts are single-celled organisms in the fungal kingdom; 1500 species have been described to date. All humans house fungal colonies in their colons, typically without detriment. Yeasts are likely to be normal members of the human microflora and certain strains (e.g., *Saccharomyces boulardii*) have been shown to:

- Reduce symptoms of diarrhea in children
- Prevent re-infection with *C. difficile*
- Reduce bowel movements in patients with diarrhea-predominant IBS
- Reduce the incidence of antibiotic-, traveler’s- or HIV-associated diarrhea

**Biomarker Key Points**

- Fungi are detected in fecal samples by standard culture techniques
- Pathogenic and potentially pathogenic (opportunist) fungi are associated with gastrointestinal symptoms, especially in immune-compromised people
- Fungal infections can produce imbalances of GI microorganisms (dysbiosis)

**Patient Populations of Interest**

Patients at increased risk of significant fungal infections (mycosis) include people with:

- Compromised immunity
  - Those on corticosteroids
  - Those with diabetes
  - The very young or very old
  - Yeast overgrowth syndrome (clinical presentation does not have the severity described in conventionally recognized fungal infections)

**Comparator/Gold Standard Tests**

This test uses fungal culture, which is the gold standard. Identification of cultured yeast/fungi is via Vitek-MS (MALDI-TOF). The MALDI-TOF mass spectrometry platform utilized for the rapid identification of bacteria and yeast from pure cultures on the CDSA & CDSA

Interpretation

Fungal culture is reported as Mycology (Culture) on the test report, using the same conventions as for Bacteriology Culture, above.

- No growth (white)
- Non-pathogen (white)
- Potential pathogens (yellow)
- Known pathogens (red)

The standard deviation reporting system is not used for this test; instead, growth is reported as present at one of four semi-quantitative levels of abundance. The example shows growth of a non-pathogen (NP) at the +1 level of growth.

When fungi are cultured at pathogenic or potentially pathogenic levels, sensitivities of pharmaceuticals and active botanical ingredients are assessed. Agents marked Sensitive or “S” have been shown to be effective treatments in culture/sensitivity studies and may be used as indicated.

**Outcomes and General Therapeutic Considerations**

Once identified, treatment for pathological yeast species and yeast overgrowth should be directed at eliminating the offending species and restoring microbial balance. Treatments may be pharmaceutical or botanical.

**Supporting the Patient with Abnormal Fungal Culture**

In supporting patients with yeast problems, consider the degree of infection, the patient’s overall immune status, diet (carbohydrate/sugar intake), and offer support for a potentially impaired immune system.

**Parasitology**

The Biomarker

Though prevalence data for intestinal parasitic infection in the US is limited, one survey found one-third of 5792 fecal specimens to be positive for parasites, with positivity peaking seasonally between July and October. According to the American Association for Clinical Chemistry (AACC), the most common parasites in the United States include: *Cryptosporidium, Entamoeba histolytica*, and *Giardia*.

A single fecal specimen tested for parasites by O&P may detect approximately 90% of GI parasite infections.
The parasitology test uses two complementary methodologies:

1. Microscopic examination of fecal specimens for ova and parasites (O&P), the gold standard of diagnosis for many parasites

2. Enzyme immunoassay (EIA) for the identification of Cryptosporidium, Entamoeba histolytica, and Giardia lamblia.

Note that the macroscopic for worms add-on test may be considered if visual inspection of the sample raises suspicion for worms. The microscopic examination is currently the gold standard for detecting helminthic infection. The macroscopic exam is performed prior to the microscopic exam to detect intact, adult worms. Most nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms) to a lesser degree, are primarily diagnosed by ova in the stool, which would be identified during the microscopic O&P exam. The cestodes (tapeworms) would be the most likely candidate for the macroscopic evaluation for worms.

Biomarker Key Points
- EIA is a biochemistry-based test that detects immunogenic macromolecules such as toxins or organism-specific antigens
- EIA is widely recognized for its diagnostic utility for detection of pathogenic antigens

Patient Populations of Interest
- Excessive or persistent diarrhea
- Stools containing blood or mucous
- Severe abdominal pain (chronic or subacute)
- Nausea and vomiting

Common Parasitic Protozoans

<table>
<thead>
<tr>
<th>Parasitic Protozoans</th>
<th>Symptoms</th>
<th>Therapeutic Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blastocystis hominis</strong></td>
<td>Watery or loose stools, diarrhea, abdominal pain, anal itching, weight loss, constipation, fatigue, and excess flatulence have been reported in persons with Blastocystis infection. Many people are asymptomatic.</td>
<td>The clinical significance of Blastocystis spp. is controversial, although there is increasing evidence that it may be a pathogen in some individuals with symptoms meeting criteria for Irritable Bowel Syndrome.***</td>
</tr>
<tr>
<td><strong>Cryptosporidium spp.</strong></td>
<td>Patients will be symptomatic or present with diarrhea varying from mild to severe, abdominal cramping, weight loss, anorexia, nausea, vomiting, flatulence, malaise, and mild fever.</td>
<td>Most people who have healthy immune systems will recover without treatment. Diarrhea can be managed by drinking plenty of fluids to prevent dehydration. Immunosuppression increases infection severity.</td>
</tr>
<tr>
<td><strong>Dientamoeba fragilis</strong></td>
<td>Patients will be asymptomatic or present with diarrhea, nausea, and vomiting; abdominal tenderness is possible.</td>
<td>There is no consensus as to best clinical practice; goal is eradication of parasite.</td>
</tr>
<tr>
<td><strong>Entamoeba coli, Entamoeba histolytica and Entamoeba dispar</strong></td>
<td>Several protozoan species in the genus Entamoeba colonize humans, but not all of them are associated with disease. Entamoeba histolytica is well recognized as a pathogenic amoeba, associated with intestinal and extra-intestinal infections. Only about 10% to 20% of people who are infected with E. histolytica become sick. A severe form of E. histolytica is associated with stomach pain, bloody stools, and fever (may resemble ulcerative colitis). E. dispar is non-pathogenic.</td>
<td>Only one antibiotic is used in non-symptomatic E. histolytica infection; two antibiotics if patients are symptomatic.</td>
</tr>
<tr>
<td><strong>Giardia lamblia</strong></td>
<td>Patients can be asymptomatic. If symptomatic, will present with acute to chronic diarrhea with bloating, intestinal malabsorption, and steatorrhea. Giardiasis has been associated with agammaglobulinemia, chronic pancreatitis, achlorhydria, and cystic fibrosis.</td>
<td>Several prescription drugs are available to treat giardiasis.</td>
</tr>
</tbody>
</table>
Because of the diversity of presentations in patients with GI parasites, it may be useful to test for parasites in patients with such symptoms who:

- Have unexplained persistent headache and fatigue
- Have been exposed to a parasitic outbreak at daycare or school
- Have traveled outside of the US
- Have consumed untreated water

**Comparator/Gold Standard Tests**

Microscopic examination of fecal samples for ova and parasites is the gold standard test for such examinations, and continues to have the highest proven diagnostic and clinical utility for parasite detection.

Although the examination of at least three samples on at least 3 separate days remains the recommendation of the Centers for Disease Control, some literature suggests that approximately 90% of enteric parasite infections may be detected in a single stool sample collected for O&P examination, with small increases in sensitivity and negative predictive values for additional samples. For patients where parasitic infection is suspected or needs to be excluded, at least three samples on three separate days should be submitted for evaluation.

**Interpretation**

The normal result for O&P on microscopic exam is “negative” or “no organisms seen.” Parasitology EIA tests are reported as “Negative” (in range) or “Positive” (out of range) on the report, with each EIA-evaluated organism listed separately (*Cryptosporidium*, *E. histolytica*, and *G. lamblia*).

**Outcomes and General Therapeutic Considerations**

Because parasitic infections typically require pharmaceutical treatment with anti-parasite medications, an accurate and timely diagnosis is essential. In a patient with a high degree of clinical suspicion for parasites, a positive test warrants appropriate interventions.

The table on the previous page lists common fecal parasitic pathogens along with symptoms and therapeutic considerations.

The most up to date information on treatment for parasites may be found by visiting the Centers for Disease Control website: [http://www.cdc.gov/dpdx/az.html](http://www.cdc.gov/dpdx/az.html).

**Bacteria Sensitivity**

When bacterial culture yields pathogenic or potentially pathogenic organisms, the Bacteria Sensitivity section reports the results of in vitro testing for susceptibility.

- **Prescriptive Agents** lists detected organisms along with their relative sensitivity to prescription antibiotics or antimicrobials. Conventional definitions of “susceptible,” “susceptible- dose dependent,” “intermediate,” “resistant” and “no interpretive guidelines established” are used.

- **Natural Agents** lists detected organisms along with their relative degree of growth inhibition by herbal and other natural substances. “High” inhibition indicates a greater ability of the substance to limit microbial growth, while “Low” inhibition suggests less ability to limit growth.

**Mycology Sensitivity**

When fungal culture of stool yields fungal organisms, the Mycology Sensitivity section reports the results of in vitro testing for susceptibility. Prescriptive and Natural Agents are listed and categorized similarly to those reported for Bacteria Sensitivity.

- **Prescriptive Agents** lists detected organisms along with their relative sensitivity to prescription antibiotics or antimicrobials. Conventional definitions of “susceptible,” “susceptible- dose dependent,” “intermediate,” “resistant” and “no interpretive guidelines established” are used.

- **Natural Agents** lists detected organisms along with their relative degree of growth inhibition by herbal and other natural substances. “High” inhibition indicates a greater ability of the substance to limit microbial growth, while “Low” inhibition suggests less ability to limit growth.

**Pathogenic Bacteria – Add-On for Both CDSA & CDSA 2.0**

**The Biomarker**

Pathogenic bacteria are those organisms known to cause distinct human disease processes, and in that way they differ from normal or opportunistic organisms in the human GI microbiome. Pathogenic bacteria are detected on this test by enzyme immunoassay (EIA), which is the standard approach to diagnosis of pathogens.
**Biomarker Key Points**

Organisms for which EIA testing is done are:

- *Helicobacter pylori* (*H. pylori*)
- *Campylobacter* species
- Shiga Toxin-producing *E. coli*
- *Clostridium difficile* (*C. difficile*)

**Patient Populations of Interest**

Pathogenic Bacteria EIA testing will achieve its optimum utility when used in the context of an appropriate differential diagnosis that considers patient symptoms and produces a high index of suspicion for at least one clinically-known syndrome or symptom complex. Testing of non-symptomatic patients is not recommended.

For each organism, symptomatology is as follows:

- *H. pylori*
  - Although prevalence of the organism is 35 to 40% among US adults, most patients remain asymptomatic ("colonized" and not "infected") and therefore do not require testing to document the organism’s presence
  - Indications for testing include upper GI symptoms or pathology such as:
    - Gastritis
    - Duodenal and peptic ulcer disease
    - Gastric lymphoma
    - Gastric cancer (in the patient or a relative)

- *Campylobacter* species
  - Asymptomatic carriage is common; testing of asymptomatic patients may be indicated during outbreaks
  - Indications for testing include:
    - Diarrhea (often bloody)
    - Abdominal cramps
    - Fever
  - Clinically apparent illness typically lasts about one week, but may be serious in immune-compromised hosts

- Shiga toxin-producing *E. coli*
  - Asymptomatic carriage can occur
  - When present, symptoms are variable and may include:
    - Abdominal cramping
    - Watery or bloody diarrhea
    - Vomiting
  - EIA confirms the presence of the pathogenic shiga toxin
  - Instead of routine screening, recommendations for testing now concentrate on clinically relevant populations; specifically, patients with significant diarrhea

- *C. difficile*
  - Asymptomatic colonization can occur; true infection is defined as presence of the organism and/or its toxin in the context of a symptomatic patient
  - When present, symptoms include:
    - Cramping
    - Lower abdominal pain/tenderness
    - Fever
    - Watery diarrhea
    - Nausea
    - Loss of appetite
  - *C. difficile* is a well-known cause of antibiotic-associated diarrhea

**Comparator/Gold Standard Tests**

There is substantial peer reviewed literature indicating the diagnostic utility of the detection of a pathogenic antigen by EIA.

- EIA provides actionable clinical information

**Interpretation**

The positive finding for any pathogen on the report is considered significant.

**Outcomes and General Therapeutic Considerations**

Therapeutic intervention is warranted in any patient in whom the practitioner has a high clinical index of suspicion and in whom a diagnosis of any of these four pathogens is made.

The table on the following page summarizes the organisms, their typical symptoms, and appropriate therapeutic considerations.

*The most up to date information on treatment for pathogenic organisms may be found by visiting the Centers for Disease Control website: [http://www.cdc.gov/foodsafety/diseases/](http://www.cdc.gov/foodsafety/diseases/) and [http://www.cdc.gov/HAI/organisms/cdiff/Cdiff_faqs_HCP.html#a9].*
Pathogen Bacteria by EIA

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Symptoms</th>
<th>Therapeutic Considerations</th>
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<tbody>
<tr>
<td>Campylobacter spp.</td>
<td>Symptoms include: diarrhea (often bloody), abdominal cramps, and fever. Illness typically lasts one week and may be serious in immunocompromised patients. Campylobacter jejuni infection has been associated with the onset of Guillain-Barré syndrome (GBS).</td>
<td>Patients generally recover without any specific treatment. They should drink extra fluids throughout the duration of diarrheal episodes.</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>Symptoms include: cramping, lower abdominal pain/tenderness, fever, watery diarrhea, loss of appetite, and nausea. It is a common cause of antibiotic-associated diarrhea (AAD).</td>
<td>In about 20% of patients, C. diff infection will resolve within 2 to 3 days of discontinuing the antibiotic to which the patient was previously exposed.</td>
</tr>
<tr>
<td>Shiga toxin E. coli</td>
<td>The symptoms vary but may include abdominal cramping, watery or bloody diarrhea, and vomiting.</td>
<td>The infection can be self-limiting. Rehydrate, and consider pre- and probiotics to support infection resolution. Treatment is based on the site and severity of infection, and STEC status.</td>
</tr>
</tbody>
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References

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CDSA Profiles are Especially Ideal for Patients with:

- IBS / IBD
- Autoimmune Diseases
- Weight Issues

CDSA Stool Profiles
- CDSA 2.0
- CDSA
- CDSA with Parasitology

Specimen Requirements
- 5cc stool in each vial:
  - 3 SAF
  - 1 Cary Blair
  - 1 Formalin
- 40 ml stool in capped cup

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