Fecal Lactoferrin Testing

A Clinical Aid for Inflammatory Bowel Disease and Irritable Bowel Syndrome
FECAL LACTOFERRIN TESTING IN IBD & IBS

INTRODUCTION

Intestinal inflammation is caused by many different disease conditions including those caused by infectious or noninfectious etiologies. Inflammatory bowel disease (IBD) is an autoimmune disease and is the primary cause of noninfectious intestinal inflammation. IBD can be difficult to distinguish from other intestinal disorders, especially non-inflammatory irritable bowel syndrome (IBS). IBD affects an estimated 2 to 3 million people in the United States. Ulcerative colitis and Crohn’s disease are the primary subgroups of IBD; both involve chronic inflammation. In cases of chronic intestinal illnesses, infectious diarrhea that may involve intestinal inflammation, such as *Shigella*, *Campylobacter*, and toxigenic *Clostridium difficile*, must be ruled out to confirm a diagnosis or a flare of IBD. Although ulcerative colitis and Crohn’s disease differ in disease location and complications, both involve conditions that oscillate between flare and remission. During active disease, activated neutrophils migrate into the intestinal mucosa to engulf bacteria and release neutrophilic proteins like lactoferrin. During this inflammatory response, tissue damage occurs causing many of the symptoms and complications observed with IBD.

IBS is a noninflammatory condition and is much more prevalent than IBD in the U.S., affecting at least 30 million people. In persons with IBS, the intestine appears normal upon endoscopic examination, increased neutrophils are not present in the mucosa, and fecal lactoferrin levels are at baseline. Chronic abdominal pain and diarrhea are symptoms common to both IBS and IBD, making differentiation between the two conditions difficult, and often resulting in unnecessary testing and delayed treatment. Fecal lactoferrin is useful for differentiation between IBD and IBS and for identifying active IBD.

FECAL LACTOFERRIN: A RELIABLE BIOMARKER OF INFLAMMATION

Human lactoferrin is a glycoprotein that is present in the secondary granules of activated neutrophils - a type of white blood cell. During the onset of intestinal inflammation, activated neutrophils migrate from the blood vessels into the intestinal lumen and surrounding tissue engulfing bacteria and releasing granule proteins including lactoferrin (Figure 1). The increased number of neutrophils in the intestinal tissue is a hallmark of active IBD and infectious colitis such as that caused by toxigenic *C. difficile*. Lactoferrin is a biomarker of activated neutrophils and increased levels in feces indicates the presence of intestinal inflammation.

Research studies have shown the link between increased fecal lactoferrin and presence of intestinal inflammation caused by IBD and enteric pathogens. In a study by Langhorst et al., 63 patients (22 Crohn’s disease, 21 ulcerative colitis and 20 IBS controls) were recruited and assessed by endoscopy including biopsy with histology. Stool samples were collected from each patient within 3 days of the examination and fecal lactoferrin was measured quantitatively (µg/g). Lactoferrin levels were significantly increased in moderate to severe IBD and showed higher levels with increasing endoscopy scores (Figure 2).

Lactoferrin is highly stable and is resistant to degradation in feces for 2 weeks at room temperature or refrigerated at 2°C to 8°C, and when frozen -20°C and lower. In an in-house study, a total of 7 stool samples were tested for lactoferrin and then stored refrigerated over a 15 day period and retested on days 9, 11, 14, and 15. Mean lactoferrin levels of each stool sample during the 15 days ranged from 14 to 449 µg/g stool. The percent Coefficient of Variation (%CV) ranged from 3.4 to 38.6 percent and the levels remained stable during the storage period for all samples (TECHLAB in-house data).
Figure 1: Mucosal Inflammation

Phagocytosis of bacteria (e.g. Toxigenic C. difficile)

Toxins A & B

Degranulation of proteins such as Lactoferrin, Myeloperoxidase, Defensins, and Elastase

Mucosal inflammation & tissue damage (e.g. Inflammatory Bowel Disease)

L-selectin

Activated neutrophil: increased expression of granule proteins

Rolling

Sialyl-LewisX

Shedding of L-selectin

Integrin

E-selectin

Release of proinflammatory cytokines

Adhesion

Diapedesis

INTESTINAL LUMEN

MUCOSAL BLOOD VESSEL

Source: TECHLAB®, Inc.

Figure 2: Endoscopic Pictures (top) & Histologic Slides (bottom) of Intestines

Endoscopic Pictures (top) & Histologic Slides (bottom) of Intestines

<table>
<thead>
<tr>
<th>Score</th>
<th>Endoscopic Pictures</th>
<th>Histologic Slides</th>
</tr>
</thead>
<tbody>
<tr>
<td>No acute inflammation</td>
<td>Score = 0</td>
<td>Mean lactoferrin = 22±7 μg/mL</td>
</tr>
<tr>
<td>Mild inflammation</td>
<td>Score = 1</td>
<td>Mean lactoferrin = 62±12 μg/mL</td>
</tr>
<tr>
<td>Moderate inflammation</td>
<td>Score = 2</td>
<td>Mean lactoferrin = 270±127 μg/mL</td>
</tr>
<tr>
<td>Severe inflammation</td>
<td>Score = 3</td>
<td>Mean lactoferrin = 739±254 μg/mL</td>
</tr>
</tbody>
</table>

Source: Langhorst et al. 2006. Comparison of CRP, clinical activity indices, and fecal lactoferrin with disease status in ileocolonoscopy of patients with IBD and IBS. Digestive Disease Week (DDW). Los Angeles, CA.
Conditions associated with intestinal inflammation

Many different conditions cause inflammation, but two diseases, in particular, are often associated with intestinal inflammation: IBD and *Clostridium difficile* infection (CDI). IBD is the primary cause of noninfectious intestinal inflammation and can be difficult to distinguish from other intestinal disorders, especially IBS. CDI is the primary cause of infectious intestinal inflammation in hospitalized patients. IBD patients often become infected with CDI presenting the challenge of a differential diagnosis.

IBD patients with active disease may present with symptoms similar to IBS, making it difficult to diagnose in the early stages of disease. In suspected cases of ulcerative colitis and Crohn’s disease, colonoscopy and barium x-ray examinations are the most commonly used techniques for confirmation of intestinal inflammation and ulceration. There are an estimated 25 to 30 million persons in the U.S. with IBS, accounting for the majority of office visits to the gastroenterologist. It is thought that only about 10% of these people ever seek medical treatment. Even so, the 2.4 to 3.5 million visits to the gastroenterologist for IBS not only overwhelm many smaller medical practices but put an excessive strain on the already burdened healthcare system. Although the prevalence of IBD is much smaller, ranging from 1.5 to 2 million patients in the U.S., these patients oscillate between active and inactive disease for life, needing continued medical treatment and management of disease. Cases of IBD occur not only in adults but also can affect children. Diagnosis typically involves multiple clinical assessments and a combination of expensive and invasive diagnostic procedures such as barium X-rays, colonoscopy, and biopsy for histological analysis.

Challenges in diagnosing and managing IBD

IBD may mimic other chronic intestinal illnesses such as diarrhea-predominant IBS. Both the number of patients requiring a gastroenterologist and the associated medical costs are driving a growing need for rapid diagnostic tests to assess chronic gastrointestinal noninfectious illnesses. Many IBD patients require a combination of diagnostic procedures for distinguishing a flare from infectious diarrhea like *C. difficile* from noninfectious IBS. Due to the combination of expense and invasiveness of current assessment procedures and the constant need for repeat patient evaluations, rapid diagnostic procedures like immunoassays and lateral flow tests are being used more often. The more common serum diagnostic tests are the serological assays for antibodies to *Saccharomyces cerevisiae* (ASCA) that is an indicator of Crohn’s disease, and for antibodies to neutrophil cytoplasmic antigens (ANCA) that indicate ulcerative colitis. In a clinical study that included both adult and pediatric IBD patients with the testing being done at 5 different test centers, the sensitivity of the ASCA serum assays ranged from 39 to 44% and showed a specificity of 87%. The sensitivity and specificity for the ANCA serum assays range from 31% to 63% and 75% to 100%, respectively. The poor sensitivity for IBD along with the associated expense for the testing, discourage many medical centers from utilizing these assays as a first-step assessment tool. The serum assays in combination don’t typically increase the sensitivity but significantly increase the specificity to 95% to 100% for both Crohn’s disease and ulcerative colitis. Another approach is detecting fecal ASCA as an indicator of Crohn’s Disease. This FDA-cleared test has a specificity of 91% and overall correlation to CD of 77%. A more common approach is to use all of these diagnostics for more complicated cases of IBD.

Utility of lactoferrin testing in routine diagnostics

A developing approach for assessing and managing patients with IBD is the use of fecal biomarkers. Studies have shown fecal lactoferrin as a useful biomarker for active IBD. Lactoferrin is a sensitive indicator of mucosal inflammation and is highly stable in feces, making it suitable as a clinical aid to diagnosing intestinal inflammation. Measurement of fecal lactoferrin may be helpful in distinguishing IBS from active...
IBD during the initial assessment of patients suffering from chronic intestinal illnesses. Clinical studies have shown that lactoferrin levels of healthy controls are similar to IBS patients but increased in patients with active IBD (Figure 3). IBD patients with active disease may present with symptoms similar to IBS, making it difficult to diagnose in the early stages of disease. In suspected cases of ulcerative colitis and Crohn’s disease, colonoscopy and barium x-ray examinations are the most commonly used techniques for confirmation of intestinal inflammation and ulceration. Lactoferrin testing is not intended as a replacement for these methods, but rather as a first step to help physicians screen out IBS patients, quickly identify likely IBD patients for further testing, and assess inflammation levels in diagnosed IBD patients.

**Utility of lactoferrin testing in IBD patient monitoring and treatment**

Measuring the level of fecal lactoferrin may be used as an aid in identifying disease activity in response to medical treatment. In a recent clinical study by Lamb et al., 117 Crohn’s disease patients who underwent surgery for resection were monitored using fecal lactoferrin. Results showed that lactoferrin levels for the patients who responded to bowel resection and did not suffer any complications had levels that returned to baseline similar to levels observed with healthy controls (Figure 4). Considering the speed and minimal cost for noninvasive fecal testing, fecal lactoferrin offers a valuable aid to the gastroenterologist for the management of medical treatment. Coupling lactoferrin results with physician’s assessment and additional testing leads to a confident diagnosis for optimizing treatment. Even though these rapid tests do not replace the traditional diagnostic methods like endoscopy, they serve as additional tools for the gastroenterologist when assessing patients suspected of having intestinal inflammation.
Pediatric IBD patients

The management of pediatric IBD patients is more challenging than the management of adult patients. Approximately 20% of IBD patients are diagnosed in childhood. A study of 1370 children at six pediatric centers found a median age at diagnosis of 10 years. Confirmed diagnosis of children under three years of age was rare (6%), and those children were more likely to have a first-degree relative with IBD. Several studies of Crohn’s patients have indicated that pediatric patients are more likely to have a familial history of the disease, have severe disease and have a higher rate of surgery. Monitoring disease activity is crucial in preventing surgery and maintaining quality of life for these patients.

For pediatric patients, many methods of monitoring disease activity are difficult for both the parents and the child. Parents may be reluctant for their child to undergo repeated colonoscopy given the risk of the procedure. Routine laboratory measurement of serum biomarkers requires phlebotomy which is invasive and often provokes severe anxiety. Several clinical indices are dependent on the patient being able to describe their symptoms. The rate of diagnosis of Crohn’s disease in younger children is increasing. Patients less than five years of age may not be able to communicate symptoms such as abdominal discomfort. Recent studies have shown fecal biomarkers to be useful in assessing pediatric IBD. Fecal lactoferrin testing is a non-invasive approach which causes less stress for parents and children and offers a sensitive and specific indicator of active disease.

Several studies have highlighted the effectiveness of fecal lactoferrin in diagnosing and monitoring pediatric IBD. A study by Walker et al. demonstrated that fecal lactoferrin levels are significantly elevated in pediatric
patients with ulcerative colitis or Crohn’s disease compared to healthy controls. Moreover, fecal lactoferrin levels correlate with disease activity. Patients with active disease have higher levels of lactoferrin when compared to patients with inactive disease. During the course of the study, a subset of patients who were in remission experienced a disease flare. Fecal lactoferrin levels were elevated in these patients prior to symptoms, suggesting that these levels can indicate a patient who is at risk for a disease flare. Further studies by Pfefferkorn et al. and Joshi et al. further demonstrate the difference in fecal lactoferrin levels between pediatric patients with IBD compared to healthy children or children with non-IBD functional diarrheas.

Preventing disease flare also decreases the likelihood of pediatric patients developing skeletal abnormalities. Children with Crohn’s disease are more likely to have lower bone mineral density and shorter stature as an adult. Since treatment with infliximab is linked to increasing bone mineral density, systemic inflammation is believed to be linked. Monitoring lactoferrin levels can help in the appropriate therapy for maintenance of remission during growth and development.

**Obstetric IBD patients**

IBD is most often diagnosed in the second through fourth decades of life which, for female patients, coincides with their childbearing years. Twenty-five percent of patients conceive for the first time after their diagnosis. Even for patients in remission, pregnancy with IBD can be challenging. A study of 461 pregnant IBD patients and age matched controls indicated that women with IBD were more likely to have a pregnancy complication regardless of disease activity. To ensure a successful pregnancy, close management of IBD during the conception period and pregnancy is essential.

Patients in remission have normal fertility rates and during pregnancy have a low risk for disease flare. For patients with active disease, the majority will continue to have active disease throughout pregnancy. Prior to conception, women with IBD should be assessed for disease activity. Between intervals of colonoscopy examinations, levels of fecal biomarkers such as lactoferrin are an indicator of gastrointestinal inflammation and mucosal healing and can be used to monitor disease activity and response to treatment. For patients with IBD who are attempting to conceive, fecal lactoferrin levels are also useful in differentiating disease flare from symptoms caused by hormonal changes or the added stress of pregnancy.

Pregnant women with IBD are at higher risk for developing gestational diabetes, preterm delivery, and cesarean delivery. Because of the increased risk for pregnancy complications, all pregnant women with IBD should be considered high-risk obstetric patients. The changes that occur during pregnancy can cause disease flares even in well controlled patients. Patients with a suspected disease flare during pregnancy should be evaluated similarly to non-pregnant patients. Stool testing for toxin should be done to exclude CDI since women may be at increased risk of developing the infection in the four weeks prior to or after delivery. Since studies have shown that IBD patients have a higher rate of colonization with *C. difficile*, an immunoassay for the presence of toxin and elevated lactoferrin is useful in determining if the symptoms are caused by an IBD flare or CDI.

Most IBD medications are considered compatible with pregnancy. The exceptions are methotrexate and thalidomide which are absolutely contraindicated during pregnancy and should be discontinued prior to planning conception. For patients on anti-TNF treatment, the timing of doses may need to be adjusted to limit the amount of drug transfer to the fetus during the third trimester. Both infliximab and adalizumab have been shown to cross the placenta during the third trimester and to be present in newborns in high levels. Delaying the introduction of live vaccines for six months is recommended for these infants. Switching or discontinuing medications during pregnancy is not recommended and may cause a disease flare which could be harmful to the pregnancy. However, some women may have concerns about the effects of the medications that they are taking and may choose to discontinue medications during pregnancy. Disease monitoring is crucial for these women to prevent adverse events during the course of the pregnancy. A non-invasive biomarker such as lactoferrin is a good choice to monitor disease activity in this group.
of patients. Careful monitoring and counseling for the prospective mother to help her understand her treatment regimen will help with compliance to treatment. Despite the risks involved in pregnancy for IBD patients, most patients with well-managed IBD will have a successful pregnancy.

**Advantages of lactoferrin testing**

*Lactoferrin testing is reliable.*

Elevated levels of fecal lactoferrin correlate with endoscopic and histologic patterns of intestinal inflammation. Lactoferrin is stable in feces for up to 2 weeks at room temperature and refrigerated, and for much longer periods at ≤-20°C. It is more reliable than microscopy for detecting fecal leukocytes since it does not rely on intact leukocytes which degrade in stool within hours.

*Lactoferrin testing is non-invasive.*

Lactoferrin testing relies only on a fecal sample and thus reduces patient anxiety about more invasive procedures. It provides a patient-friendly first read on inflammation and helps to rapidly identify those relatively few patients who will warrant further investigation. Avoiding unnecessary endoscopy in pediatric patients, who usually require deep sedation or anesthesia for the procedure, is particularly beneficial. Even though these rapid tests do not replace the traditional diagnostic methods like endoscopy, they can serve as an additional tool as determined necessary by the gastroenterologist. Lactoferrin testing is also very well-suited to pregnant women seeking non-invasive detection methods.

*Lactoferrin testing is sensitive and specific for intestinal inflammation*

Lactoferrin testing has a sensitivity of 81.5% and a specificity of 96.5% for differentiating active IBD from IBS. Unlike, white blood cell count, it is specific for measuring intestinal inflammation.

*Lactoferrin testing is cost-effective and covered by most insurance providers*

By ruling out active IBD, lactoferrin testing helps to prevent unnecessary endoscopy, resulting in significant cost savings for both the patient and the health care system. Lactoferrin testing is covered by most insurance plans.

**Limitations of lactoferrin testing**

Lactoferrin testing is specific for intestinal inflammation and not the particular disease. Results should be used in combination with other laboratory results and patient history. In addition, patients with low amounts of inflammation having lactoferrin levels near baseline may require repeat testing depending on changes in clinical presentations. Lactoferrin testing is not appropriate for breastfeeding infants. Human breast milk contains 8 to 10 mg of lactoferrin. Breastfeeding infants will have a large amount of fecal lactoferrin from the diet and this will increase the level of fecal lactoferrin over baseline to indicate a positive result.
SUMMARY

Lactoferrin is the basis of the most widely established and accepted diagnostic assay in the U.S. for detecting fecal white blood cells as an indicator of intestinal inflammation. FDA-cleared assays for this biomarker have been available since the early 1990’s. Lactoferrin has been the focus of many publications and well documented clinical studies, supporting fecal lactoferrin as an ideal test for a clinical routine. Diagnosing a patient with IBD or IBS can take years due to the similarity of symptoms and clinical findings. Lactoferrin testing can speed up the diagnosis of these disorders by indicating the presence of intestinal inflammation. Results of a quantitative lactoferrin test can help determine the effectiveness of therapy, predict relapse, and guide treatment decisions.
REFERENCES CITED


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TECHLAB In-house data. Evaluation of the stability of lactoferrin in human feces.


TYPES OF LACTOFERRIN TESTING

Lactoferrin testing formats

Qualitative test formats

Qualitative lactoferrin testing provides a positive or negative result for elevated levels of fecal lactoferrin. It can rapidly and non-invasively 1) distinguish between patients with IBS and active IBD; 2) identify IBD patients with active inflammation; and 3) provide convenient results for smaller clinics with appropriately-licensed in-house laboratories.

The LACTOFERRIN EZ VUE™ test is an immunochromatographic test for the qualitative detection of elevated levels of lactoferrin, a marker for fecal leukocytes and an indicator of intestinal inflammation. The test can be used as an in vitro diagnostic aid to identify patients with an active inflammatory bowel disease (IBD) and rule out those with active noninflammatory irritable bowel syndrome (IBS). IBD, a condition of chronic inflammation, primarily includes ulcerative colitis and Crohn’s disease. Both of these diseases result in elevated fecal lactoferrin. As a result, patients with these diseases test positive in the LACTOFERRIN EZ VUE™ test. In patients with active IBS, the intestine appears normal upon endoscopic examination and there is no indication of inflammation. As a result, IBS patients test negative in the LACTOFERRIN EZ VUE™ test. The LACTOFERRIN EZ VUE™ test is a simple-to-use lateral flow format that provides results in 10 minutes.

The LACTOFERRIN CHEK™ test is an ELISA for the qualitative detection of elevated levels of lactoferrin, a marker for fecal leukocytes and an indicator of intestinal inflammation. The test can be used as an in vitro diagnostic aid to identify patients with active inflammatory bowel disease (IBD) and rule out those with active irritable bowel syndrome (IBS), which is noninflammatory. IBD, which is a condition of chronic inflammation, consists of ulcerative colitis and Crohn’s disease. Both of these diseases result in elevated fecal lactoferrin. IBD patients test positive in the LACTOFERRIN CHEK™ test. In patients with active IBS, the intestine appears normal upon endoscopic examination and there is no indication of inflammation. IBS patients test negative in the LACTOFERRIN CHEK™ test. The LACTOFERRIN CHEK™ test is a simple-to-use microwell format that has a turnaround time of an hour and fifteen minutes.

Quantitative test format

Quantitative lactoferrin testing provides a measurement of fecal lactoferrin. It helps to quickly and non-invasively: 1) distinguish between patients with IBS and active IBD; and 2) assess inflammation levels during IBD treatment to help direct treatment and to aid in predicting relapse.

The LACTOFERRIN SCAN™ test is a quantitative ELISA for measuring concentrations of fecal lactoferrin, a marker of fecal leukocytes. An elevated level is an indicator of intestinal inflammation. The test can be used as an in vitro diagnostic aid to help distinguish patients with active inflammatory bowel disease (IBD) from those with noninflammatory irritable bowel syndrome (IBS). In addition, the test can be used to assess when an IBD patient is in confirmed remission and has responded to treatment.

How to order testing

Both qualitative and quantitative lactoferrin testing can be easily ordered through most laboratories. A stool specimen collected in a cup can be tested. Specimens can be stored at 2-8°C or at room temperature for up to 2 weeks before being tested.