Inside Diagnostics

FECAL CALPROTECTIN – A NEW TEST FOR THE ASSESSMENT OF INTESTINAL INFLAMMATION

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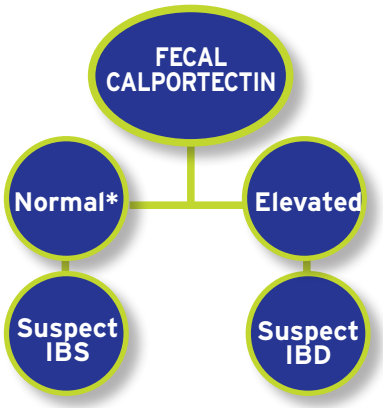
Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are conditions that present with chronic abdominal pain and disrupted bowel habits. Combined, they affect more than 5 million Canadians indiscriminately of age, gender or race[1].

To Scope or Not to Scope

Aside from negatively impacting the quality of life, IBS is relatively benign. It does not lead to any permanent structural damage to the intestines. Dietary and lifestyle changes can usually alleviate the symptoms of this disorder[2]. IBD on the other hand is characterized by intestinal inflammation and ulcers. This organic disease can lead to serious complications including the development of fistulas, abscesses and strictures; sometimes leading to perforation of the bowel[3]. This disease also carries an increased risk of malignancy[4]. Diagnosing IBD typically involves endoscopy and medical imaging which is expensive, invasive and difficult to conduct, especially with young patients.

Helping You Diagnose and Monitor IBD

Calprotectin may be used to confirm the presence of intestinal inflammation before using invasive testing[5]. Fecal Calprotectin is an FDA approved test that offers a non-invasive way to assess for the presence of intestinal inflammation. Calprotectin is a calcium-binding protein found within neutrophils in cases with bowel inflammation. Fecal Calprotectin is detected via an immunoassay of a stool sample. The levels of the protein are high in cases of IBD but not IBS, therefore this test helps differentiate between the two disorders.



CLINICAL UTILITY OF FECAL CALPORTECTIN

- Differentiate between IBS and IBD
- Monitor the effectiveness of IBD therapy
- Detect IBD relapse

** Repeat testing for borderline result is recommended.*

While Fecal Calprotectin is not a substitute for other diagnostic techniques, it could help identify the patients who are most likely affected by IBD[5].

The Fecal Calprotectin test is also appropriate for monitoring of the patients who are being treated for IBD[6]. Elevated levels of Calprotectin could be indicative of disease flare-up, thereby allowing physicians to intervene before the IBD symptoms become severe.

IMPORTANT NOTES

- **To avoid potential false positive results, patients should abstain from using NSAIDs for at least two weeks before taking the test[7].**
- **Repeat testing for borderline results is recommended.**

If you would like to receive more information about this test or order Fecal Calprotectin pamphlets for your patients, please email LifeLabs at contactus@lifelabs.com.

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ROLE OF AMYLASE AND LIPASE TESTING IN PANCREATITIS

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Pancreatitis is an inflammation caused by the injury of pancreatic acinar cells due to activation of digestive enzymes within the pancreatic tissue and is characterized by significant morbidity and mortality.[1,2] Traditionally, pancreatitis has been classified as:

a) **Acute pancreatitis:** A reversible inflammation due to enzymatic necrosis, which leads to acute onset of persistent epigastric and/or periumbilical pain, nausea and vomiting.[1,3] The most common causes of acute pancreatitis include obstruction of the common bile duct by stones (38%) and alcohol abuse (36% of patients). In rare cases acute pancreatitis can be caused by abdominal surgery, extreme hyperlipidemia (triglyceride concentrations > 11 mmol/l),

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congenital anomalies (pancreas divisum), drugs (e.g. azathioprine, steroids, valproate, furosemide), and viral infections (e.g. mumps).

b) Chronic pancreatitis: A progressive inflammation with irregular fibrosis, duct dilation, and irreversible loss of pancreatic parenchyma.[2,4] It presents either as recurrent attacks mimicking acute pancreatitis, or as a constant, disabling abdominal pain. It is often associated with maldigestion and steatorrhea due to pancreatic insufficiency with loss of enzymes, and glucose intolerance or diabetes due to islet damage. Most commonly the cause of chronic pancreatitis is unknown (60-70%), whereas the most common known cause is alcohol (30% of patients). Other, rare causes include drugs (valproate, thiazide), hyperlipidemia, infections (HIV, mumps), autoimmune, genetic (cystic fibrosis), obstruction (cancer), and recurrent acute pancreatitis.

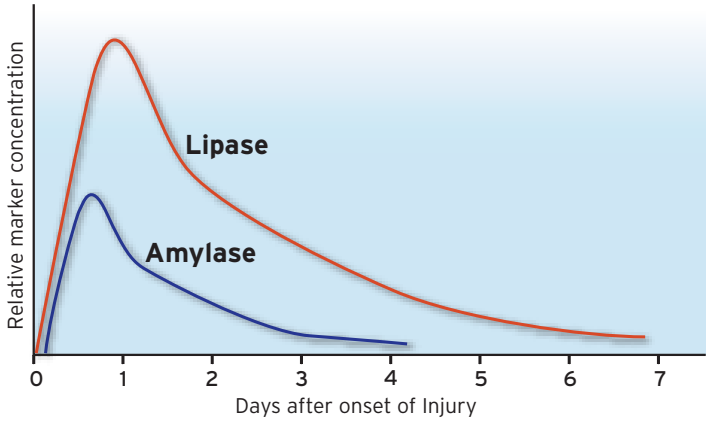
More recently, acute pancreatitis, recurrent attacks and chronic pancreatitis have been regarded as a disease continuum.[4] Due to different underlying pathophysiological processes, the utility of the laboratory and imaging tests varies throughout the disease continuum. This article summarizes the advantages and limitations of testing amylase, lipase or their combination in pancreatitis.

What Diagnostic Tests are Available to Aid Diagnosis of Acute Pancreatitis?

The non-specific clinical picture of acute pancreatitis represents a diagnostic challenge. Laboratory and imaging studies are necessary for prompt and accurate diagnosis, to expedite appropriate treatment and reduce the risk of complications, including the early organ dysfunction.[1,3]

Laboratory testing can confirm the diagnosis of acute pancreatitis. The laboratory tests most commonly used are serum amylase and lipase – pancreatic enzymes released into blood following autodigestion of pancreatic tissue during the inflammatory process.[1,3,5] Blood levels of both enzymes increase markedly within 2-8 hours of symptom onset (Figure 1) and elevations greater than three times the upper limit of normal are typically considered diagnostic.[1] Assays for both enzymes are readily available in the laboratory.

Figure 1. Time-dependent changes in serum amylase and lipase after acute pancreatitis (adapted from reference 5).



Imaging studies to ascertain clinical diagnosis have found that serum amylase has a 81 – 95% sensitivity[3,6]. Factors decreasing amylase sensitivity include late patient presentation as serum amylase levels normalize 2-4 days after symptom onset, (Figure 1), and normal amylase levels reported in up to 30% of patients with alcoholic pancreatitis and other exocrine pancreatic insufficiencies, and in up to 50% of patients with simultaneous hypertriglyceridemia (the latter due to an unidentified circulatory inhibitor of amylase in hypertrigliceridemic serum).[6] The major limitation of serum amylase is its lack of specificity.[5,6] High concentrations of amylase are secreted from salivary glands, and it may also increase in a number of abdominal and extra-abdominal conditions (Table 1).

Table 1. Conditions Associated with Increased Serum Amylase and Lipase[3,7]

Amylase	Lipase
1) Intra-abdominal: • Pancreatic: acute pancreatitis, pseudocysts, trauma, cancer • Non-pancreatic: appendicitis, hepatitis, cholecystitis, peptic ulcer, perforated bowel, mesenteric ischemia, intestinal obstruction, peritonitis, abdominal aortic aneurysm, ruptured ectopic pregnancy, fallopian and ovarian cysts, salpingitis.	1) Intra-abdominal • Pancreatic: acute pancreatitis, pseudocysts, trauma, cancer • Non-pancreatic: cholecystitis, peptic ulcer, mesenteric ischemia, abdominal trauma
2) Extra-abdominal: salivary diseases, decreased glomerular filtration, ketoacidosis, pneumonia, cerebral trauma, burns, anorexia nervosa, bulimia, non-abdominal surgery	2) Extra-abdominal: bone fracture, fat embolism
3) Macroamylasemia*	3) Macrolipasemia*
4) Idiopathic hyperamylasemia: familial, non-familial	
5) Drug-induced: azathioprine, sulfonamides, tetracycline, methyldopa, estrogens, furosemide, valproic acid, salicylate, thiazide, calcium	

** Asymptomatic presence of macromolecular complexes of enzyme and immunoglobulins*

Recent studies have shown that lipase demonstrates better clinical performance compared to amylase, with sensitivity of 85-100% that does not decrease with late patient presentation, as serum lipase remains elevated for up to 14 days following symptom onset (Figure 1).[3,6] Lipase also performs better in patients with alcoholic pancreatitis and hypertriglyceridemia.[6] Lipase has better specificity than amylase, but small amounts are present in stomach, intestine, leukocytes, fat cells and milk. Thus, lipase may also be elevated in several conditions other than acute pancreatitis (Table 1). Finally, multiple isoforms of lipase present in serum may interfere with testing.[3]

Studies directly comparing amylase and lipase for the diagnosis of acute pancreatitis have demonstrated that lipase has superior diagnostic accuracy on both day one and day three following symptom onset.[8-10] Furthermore, when both enzymes are tested simultaneously, there is no significant improvement in diagnostic accuracy.[9,11] There is no advantage in serial enzyme measurement, as neither amylase nor lipase levels are related to disease severity or prognosis.[1,3]

What About Amylase and Lipase in Chronic Pancreatitis?

The loss of pancreatic parenchyma in chronic pancreatitis leads to the loss of enzymes and pancreatic insufficiency.[4] Although elevations might exist during the acute pain episodes in the early course of the disease, pancreatic amylase and lipase are typically normal or low in advanced chronic pancreatitis.[2] Thus, monitoring these enzymes in patients with chronic disease does not provide useful clinical information. The treatment goals in chronic pancreatitis, including calming the disease process and relieving pain, correction of metabolic abnormalities, such as diabetes and malnutrition, and management of complications, will dictate a different battery of laboratory tests needed to monitor the patient.

Conclusion

Table 2 summarizes advantages and limitations of enzyme testing in acute pancreatitis. When suspecting acute pancreatitis, it is prudent to take into account the timing of symptom onset, as well as other aspects of the clinical picture. Serum amylase and/or lipase are not useful for monitoring patients with chronic pancreatitis.

Table 2. Advantages and Limitations of Testing Amylase, Lipase or Both for Diagnosis of Acute Pancreatitis

Test	Advantages	Limitations
Amylase	• Sensitive in early diagnosis • Readily available	• Lower sensitivity in late presenting patients, alcoholic pancreatitis and hypertrigliceridemia • Low specificity • No standardized method • Different diagnostic cut-offs used
Lipase	• Sensitive in both early and late diagnosis • Better specificity compared to amylase • Readily available	• No standardized method, technically more difficult method • Different diagnostic cut-offs used
Amylase + Lipase	None	• No improvement in diagnostic accuracy compared to lipase • Increased cost compared to single enzyme testing

POINTS TO REMEMBER

- **Lipase is more sensitive and specific than amylase in diagnosing acute pancreatitis.**
- **Lipase shows better diagnostic accuracy for acute pancreatitis compared to amylase, particularly in late presenting patients, patients with alcoholic pancreatitis and other forms of pancreatic insufficiency, and patients with hypertriglyceridemia.**
- **Ordering both tests provides no significant improvement in diagnostic accuracy for acute pancreatitis.**
- **There is no role for serial monitoring of amylase and/or lipase in patients with chronic pancreatitis.**

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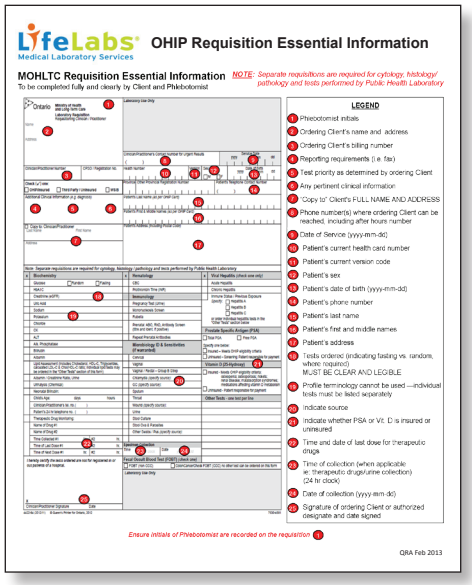
OHIP REQUISITION - ESSENTIAL INFORMATION

Quality and Regulatory Affairs
LifeLabs ON

MOHLTC regulation mandates community laboratories obtain written requests from qualified healthcare practitioners before any tests are performed. Healthcare practitioners have an obligation to provide a signed and legible OHIP requisition to their patients. The information on the OHIP requisition will ensure tests are ordered and reported in a timely manner. Healthcare practitioners may access the essential information that must be provided for the laboratory to perform the analysis on LifeLabs website www.lifelabs.com Look for: 'Healthcare Clients-Ontario' *Click on: Laboratory Services* and then *OHIP Requisition Essential Information folder* (see illustration).



For healthcare practitioners who collect specimens in their facility, LifeLabs will provide a package of information and charts that will assist in collection and submission of quality specimens.

As an Ontario Laboratory Accreditation (OLA) accredited laboratory LifeLabs also requires the phlebotomist who has collected the specimens in the facility, to record their initials on the requisition in the top left hand corner.

POINTS TO REMEMBER

- **Separate requisitions are needed for cytology, pathology and tests performed by the Public Health Laboratory.**

GASTROINTESTINAL PARASITIC INFECTIONS

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Although more common in the developing world, parasitic infections of the intestinal tract remain an important clinical entity in Canada due to a number of factors, including increased international travel to developing countries, increasing numbers of immunocompromised individuals because of chemotherapy, solid organ or hematological transplants, increased crowding in day care centers and nursing homes, consumption of raw or partially cooked foods and contaminated water sources, and increased immigration from developing countries. Parasitic pathogens as etiologic agents of diarrhea in developed countries occur with different frequencies in immunocompetent and immunocompromised patients.

Common Etiologic Agents of Parasitic Intestinal Infections

- **Cryptosporidium —** Cryptosporidium is one of the most common parasitic causes of acute foodborne diarrhea in North America accounting for 8 percent of cases in a 2005 survey from FoodNet.[1] Infection with *Cryptosporidium* species may present as a severe dehydrating but self-limited diarrheal illness in immunocompetent hosts. In immunocompromised hosts it may have a more prolonged and severe course.

Transmission of cryptosporidiosis occurs via spread from an infected person or animal, or from a fecally contaminated environment such as a food or water source. Numerous waterborne community outbreaks have occurred from contaminated drinking or swimming water.[2] Person-to-person transmission is also common, particularly among household members, sexual partners, children in daycare centers and their caretakers, and healthcare workers.

- **Giardia —** *Giardia lamblia* is a flagellated protozoan parasite that is one of the two most common gastrointestinal parasites in North America (Cryptosporidium is the other). *G. lamblia* causes both epidemic and sporadic disease and is an important cause of waterborne and foodborne diarrhea in day-care centers.

- **Cyclospora —** *Cyclospora cayetanensis* has been identified in both sporadic cases and as a cause of outbreaks of diarrhea with the source of infection identified, for example, imported fruit or vegetables. The clinical course of diarrhea is often lengthy, with symptoms lasting a number of weeks, and can be accompanied by fatigue and malaise.

- **Entamoeba histolytica —** Intestinal amebiasis is caused by *Entamoeba histolytica*. Amebiasis is a worldwide disease, but developing countries have significantly higher prevalence rates because of poorer socioeconomic conditions and sanitation levels. In developed countries amebiasis is mainly seen in immigrants from and travelers to endemic countries. Institutionalized patients and men who have sex with men are also at increased risk of infection.

Clinical infection usually has a subacute onset, usually over one to three weeks. Symptoms range from mild diarrhea to severe dysentery producing abdominal pain and bloody diarrhea.

When to Send Stool for Testing for Ova and Parasites

There are several possible indications for sending stool for testing for ova and parasites:[3]

1. Persistent diarrhea (associated with Giardia, Cryptosporidium, and *Entamoeba histolytica*)
2. Persistent diarrhea following travel
3. Persistent diarrhea with exposure to infants in daycare centers (associated with Giardia and Cryptosporidium)
4. Diarrhea in a man who has sex with men (MSM associated with Giardia and Entamoeba histoloytica) or a patient with HIV infection or other risk factors causing immunosuppression (associated with a variety of parasitic pathogens)
5. A community waterborne outbreak (associated with Giardia and Cryptosporidium)
6. Bloody diarrhea (associated with intestinal amebiasis).

Specimen Collection, Processing and Reporting

If after one stool specimen no parasites are seen, up to three specimens should be sent on consecutive days for parasite examination since parasite excretion may be intermittent in contrast to bacterial pathogens. Stool samples should be taken prior to starting any anti-parasitic treatment. Ingestion of mineral oil, bismuth, or barium should be avoided prior to stool collection as they may interfere with recovery of parasitic pathogens.[4] Freshly passed stool is collected into a container with SAF preservative, and should be transported to the lab at room temperature within 72 hours of collection.

From the preserved specimen, microscopic examination is done on a concentrated sample and on a direct smear. Fecal concentration is performed for detection of protozoan cysts, helminth eggs and larvae in small numbers. A Kinyoun Haematoxylin stain is performed for detection of protozoan cysts and trophozoites.[5]

Once specimens are examined, any helminth eggs or larvae are reported as are any pathogenic protozoa that are detected. The pathogenic protozoa of clinical importance include those listed above (*Entamoeba histolytica, Giardia lamblia, Cryptosporidium species, Cyclospora cayetanensis*), along with *Microsporidia, Dientamoeba fragilis, Balantidium coli* and *Isospora belli*.[6]

Of note, it is not possible to microscopically distinguish between pathogenic *E. histolytica* and the non-pathogenic *E. dispar*, therefore when seen, the organism is reported as *Entamoeba histolytic/dispar.* If further testing is needed, an unpreserved stool specimen should be submitted in a sterile container and antigen testing to distinguish between *E. histolytica and E. dispar* can be done at the Public Health laboratory.

Overall, parasitic pathogens remain an important etiology of clinically important intestinal symptoms and proper testing and examination of stool specimens is paramount in ensuring recovery and detection of these pathogens.

POINTS TO REMEMBER

- **Up to three stool samples taken on consecutive days should be examined for the presence of ova and parasites.**
- **Stool must be collected and transported to the lab in a container with SAF preservative.**
- **Some of the organisms with potential to cause clinically important diarrhea which may require treatment include *Entamoeba histolytica, Giardia lamblia, Cryptosporidium species, Cyclospora cayetanensis, Microsporidia, Dientamoeba fragilis, Balantidium coli* and *Isospora belli.***
- **On initial examination, the pathogenic E. histolytica and the non-pathogenic E.dispar cannot be distinguished, therefore if further testing is required, antigen testing can be done at the Public Health Laboratory on an unpreserved stool specimen.**

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