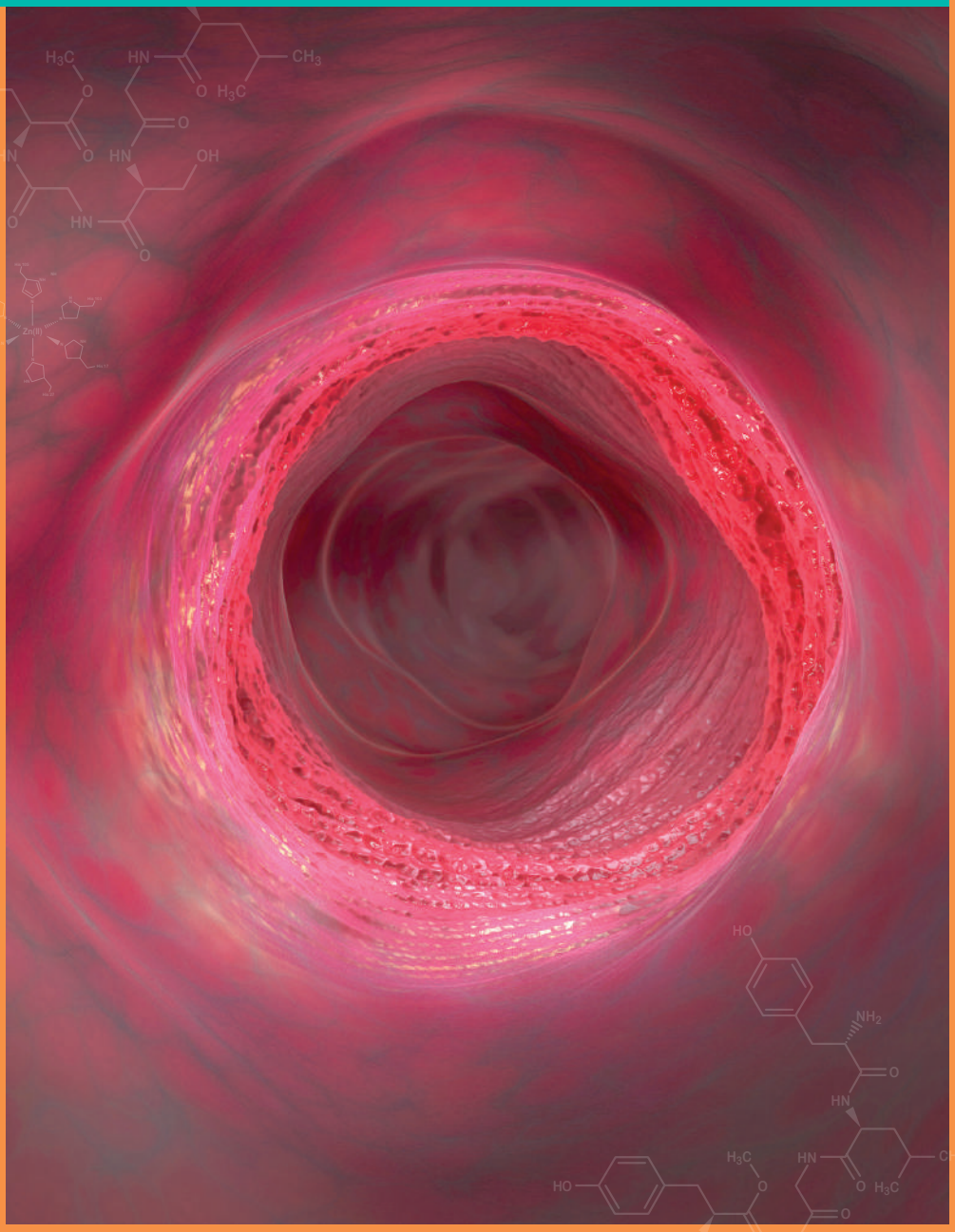


Are Fecal Leukocyte Tests a Waste of Time?

CLINICAL EDITION

Doubts about the utility of fecal leukocyte tests have been publicly voiced.

Doubts about the utility of fecal leukocyte tests using microscopy have been publicly voiced, but detection of leukocyte-release lactoferrin overcomes the challenges. For over a century, fecal leukocytes have been used to diagnose and differentiate between acute inflammatory and non-inflammatory diarrheas. A quantitative cell count from a fecal smear, the fecal leukocyte test (FLT), was originally performed at the patient's bedside as a point-of-care test (POCT) by a trained microscopist.

As clinics, where samples are taken, and laboratories, where fecal specimens are tested, have grown further apart, doubts about the current utility of the FLT have been voiced. *Are FLTs now a waste of time?*

False-Negatives With FLTs

When assaying with FLTs, technicians can only detect and count intact leukocytes which have been stained with methylene blue. These fragile cells can rupture and degrade during transportation to off-site laboratories due to physical and temperature abuse. If not promptly counted, there is the potential for false-negatives in FLTs due to the degradation of the leukocytes.

Also, toxins released by some enteric pathogens such as *Clostridioides difficile* can lyse neutrophils. A study published in 2006 concluded that the fecal leukocyte test had poor sensitivity and was not a good predictor of *C. difficile*-associated diarrhea, which accounts for more than 25% of all antibiotic-associated diarrheas.[1]

As far back as 1977, Pickering et al. reported a lack of correlation between fecal leukocytes and the recovery of enteric pathogens in feces.[2-3] The American College of Gastroenterology recommended the use of FLTs in 1997 despite their acknowledgments that the assay exhibited low sensitivity (40%) which was reported in a large systematic review with meta-analysis published the previous year.[3-5] In a 2004 performance assessment involving 205 patients, results did not distinguish between infectious and noninfectious diarrhea, detection of an invasive or noninvasive pathogen by stool culture, or response to antimicrobial therapy when evaluated by FLTs.[6] They concluded that the FLT does not change patient management and summarized with the following statement:

FLT Costs

"The fecal leukocyte test was only **20%** better than a coin toss."[6]

Gupta et al. published a 100-year history of the stool cellular exudate test—also known as the FLT.[3] The authors highlighted the limitations and excessive costs of the assay. From 2012 through 2016, the Centers for Medicare and Medicaid Services spent an average of $329,000 per year on approximately 58,000 fecal leukocyte assays. This translated to a cost of roughly $5.69 per assay. In 2018, the Medicare midpoint reimbursement for a fecal leukocyte test was $5.27.

Originally conceived as a bedside test to be performed within 15 minutes after patient donation, laboratories are obliged to offer 24-hour service because only fresh stool samples are fit for analysis. Additionally, Medicare beneficiaries represent only 17% of the U.S. population, so the overall use and costs of the FLTs may be significantly greater when labor costs for trained personnel and equipment time are calculated.[3]

The costs to the participating laboratories conducting FLTs may be higher than the Medicare reimbursement.

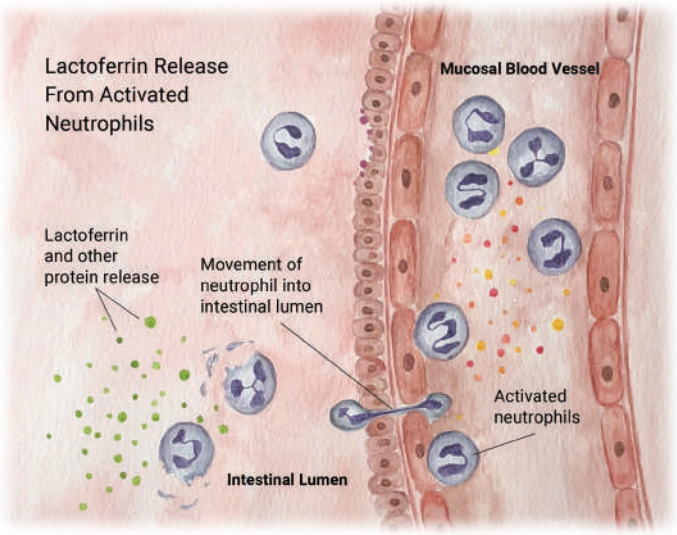
Fecal Biomarkers

Enter fecal biomarkers. Fecal biomarkers such as albumin, α-1-antitrypsin, elastase, secretory IgA, calprotectin and lactoferrin were examined in clinical research studies for use as diagnostic aids to differentiate between acute inflammatory diarrheas from non- or minimally inflammatory ones. The most promising biomarkers were calprotectin and lactoferrin, both of which have been developed into valuable clinical tools. When compared to calprotectin, lactoferrin has been proven to have broader clinical applications.

Lactoferrin is a glycoprotein which is relatively stable in various bodily fluids and fecal specimens. It is found in mucosal secretions such as tears, saliva, vaginal fluids, urine, breast milk and colostrum. It is also found in leukocytes; neutrophils which are part of the host innate defense system. The amount of lactoferrin in the feces of a healthy intestine is consistent, exhibiting a stable baseline concentration. The detection of elevated levels of lactoferrin above the normal baseline can serve as a diagnostic tool for differentiating inflammatory from noninflammatory diarrheas.

The key to correctly identifying acute inflammatory infectious diarrhea depends on the ability to measure various biomarker levels above background noise.

Bacterial pathogens such as *Salmonella, Shigella, Campylobacter,* and *C. difficile* cause inflammatory diarrheas resulting in fecal lactoferrin levels substantially higher than background levels. Many peer-reviewed and unpublished studies have demonstrated the accuracy of fecal lactoferrin as a biomarker for inflammatory diarrhea. In 14 different trials, in 12 different locations, >3,000 fecal samples were evaluated.[7-17] The combined data confirmed that lactoferrin was consistently more sensitive and stable than other neutrophil-associated proteins such as lysozyme, myeloperoxidase or elastase.



Lactoferrin Advantages

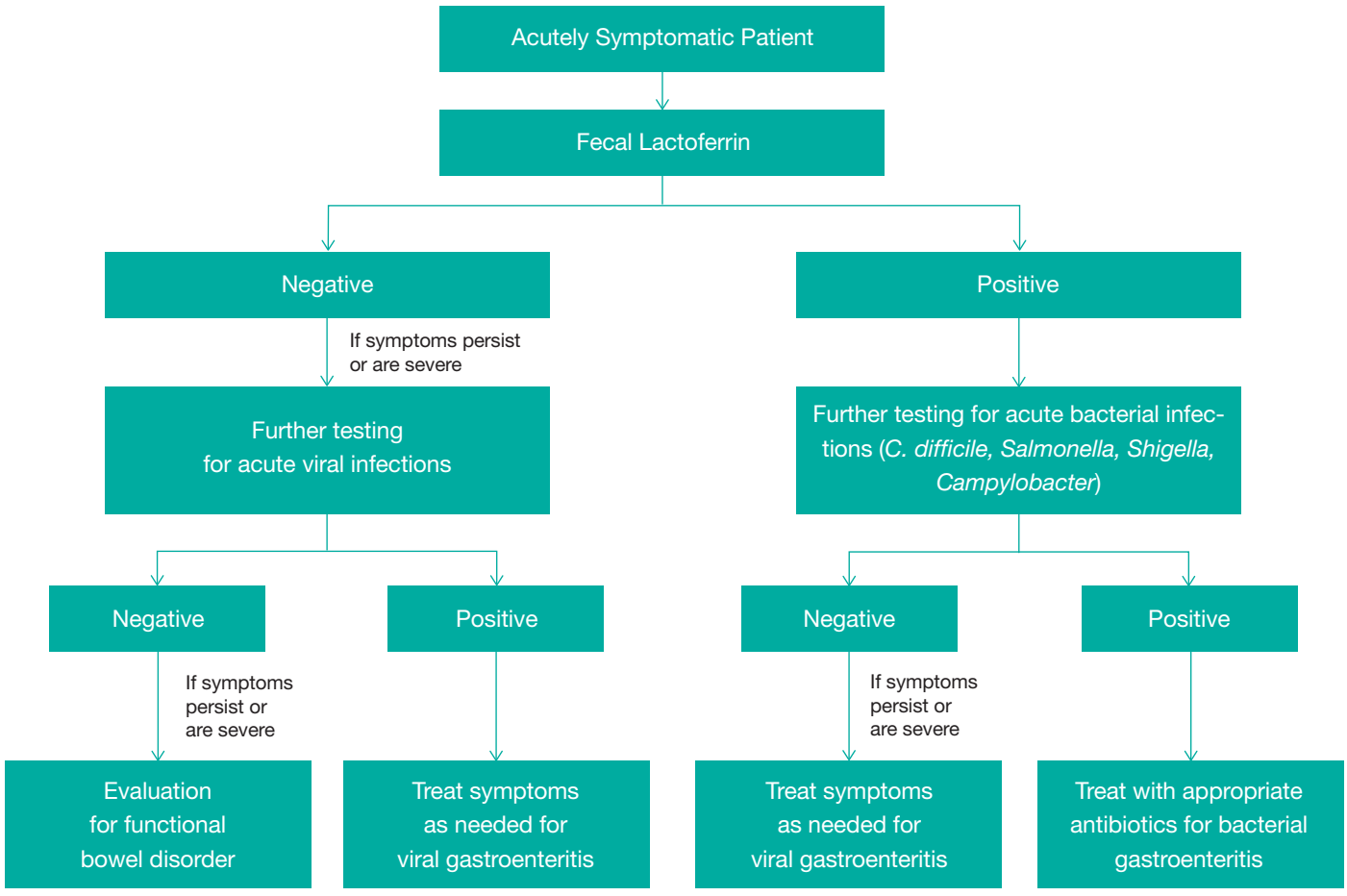
In the intestine, lactoferrin performs several biological functions. It is an antibacterial agent because it sequesters iron, a mineral essential for the survival of many bacteria. Lactoferrin also helps modulate the function of immune cells, regulates cell-to-cell contact in the gut, controls intestinal permeability and serves as a signaling agent between and among

epithelial and immune cells.[17] Due to its various functions in the intestinal lumen, bacterial pathogens causing inflammatory diarrhea trigger a significant increase in fecal lactoferrin, making lactoferrin a highly accurate biomarker for intestinal inflammation.

Abdominal pain, diarrhea, and inflammation are some of the most common complaints seen in primary care and gastroenterology. Determining infectious from non-infectious etiologies directly impacts treatment decisions and patient outcomes. Due to its role in bacterial pathology, lactoferrin can provide valuable information for differential diagnosis. The stability of lactoferrin allows for longer specimen storage prior to testing; up to 2 weeks at room temperature. Detection of lactoferrin does not require intact cells; physical or temperature abuse of the fecal sample are not issues. Unlike fecal leukocytes, lactoferrin is not degraded by toxins produced by pathogens such as *C. difficile*. It is significantly elevated in bacterial infections such as *Salmonella* or *Campylobacter* when compared to norovirus, rotavirus, or healthy patients.[18] Lactoferrin also corresponds to moderate or severe Vesikari and Clark scores of gastroenteritis disease severity, suggesting the role of the biomarker in staging infectious diarrheas.[18]

Lactoferrin offers many practical advantages over fecal leukocyte counts as an indicator of intestinal inflammation. It can be used as part of a diagnostic algorithm to determine the cause of intestinal inflammation in patients with consistent symptoms of diarrhea and abdominal pain. A negative fecal lactoferrin test can quickly rule out non-inflammatory causes and a positive test is suggestive of inflammatory causes that include certain types of bacterial infections as well as other inflammatory disorders.

Diagnostic Algorithm With Fecal Lactoferrin



Lactoferrin Performance Testing

Qualitative fecal lactoferrin assays are FDA-cleared tests based on the detection of elevated fecal lactoferrin levels. They are used to detect acute inflammatory diarrheas caused by infectious agents such as bacteria. The assays are simple to use and interpret, with results available in 10 minutes.

Guthrie et al. ran a study in 2008 in which hospital-acquired specimens were analyzed with side-by-side assays, comparing FLTs, qualitative fecal lactoferrin, and lactoferrin tested at a reference laboratory.[19] The qualitative fecal lactoferrin assays and lactoferrin reference tests performed off-site were identical and gave increased performance over FLTs.

In a Mayo Clinic study, 168 fresh stool specimens were tested by both qualitative fecal lactoferrin assays and FLTs.[20] Thirty specimens tested positive by the qualitative fecal lactoferrin assay only, 12 by both assays, and one by microscopy only. The authors concluded that the 18 discrepant samples not found by FLTs were false-negatives caused by lysed and degraded cells.

Another study compared FLTs and qualitative fecal lactoferrin assays as markers of inflammation in children infected with diarrhea-inducing *E. coli*.[21] In 99 samples, all were lactoferrin positive with only 11 having high numbers of fecal leukocytes. The results supported the use of qualitative fecal lactoferrin assays over FLTs and pointed to the realization that inflammation associated with enterotoxigenic *E. coli* was more common than previously recognized.

Chen et al. found that fecal lactoferrin was correlated with bacterial infection and greater disease severity in children.[22] They noted that the utility of lactoferrin testing went beyond the scope of differentiation between inflammatory bowel disease from irritable bowel syndrome. They recommended lactoferrin as a biomarker for severe dehydration and acute diarrheas associated with *C. difficile, Salmonella, Campylobacter* and other enteric, infectious bacteria.

Patients with moderate to severe diarrhea were evaluated using FLTs, lactoferrin and multiplex PCR for pathogen detection.[18] They found a positive association between lactoferrin, moderate to severe dehydration and detection of pathogens by multiplex PCR. They concluded that lactoferrin was more useful than FLTs.[18]

The Take-Away

In summary, qualitative fecal lactoferrin testing offers significant advantages over FLTs. This provides clinicians and their patients with a timely diagnosis contributing to more appropriate therapy while simultaneously decreasing healthcare costs.

Qualitative lactoferrin testing has been evaluated favorably in a number of studies, especially when compared to FLTs.

REFERENCES

1 Reddymasu S, Sheth A, Banks DE. Is fecal leukocyte test a good predictor of *Clostridium difficile* associated diarrhea? *Annals Clin Microbiol & Antimicrobials*. 2006;5:9.

2 Pickering LK, DuPont HL, Olarte J, et al. Fecal leukocytes in enteric infections. *Am J Clin Pathol*. 1977;68:562-5.

3 Gupta A, Johnson DH, Agrawal D. Devolution and devaluation of fecal leukocyte testing – A 100-year History. *JAMA Intern Med*. 2018;178:115-1156.

4 DuPont HL. Practice Parameters Committee of the American College of Gastroenterology Guidelines on acute infectious diarrhea in adults. *Am J Gastroenterol*. 1997;92:1962-75.

5 Huicho L, Campos M, Rivera J, Guerrant RI. Fecal screening tests in the approach to acute infectious diarrhea: a scientific overview. *Pediatr Infect Dis J*. 1996;15:486-94.

6 Granville, LA, Cernoch P, Land GA, David JR. Performance assessment of the fecal leukocyte test for inpatients. *J Clin Microbiol*. 2004;42:1254-56.

7 Guerrant RL, Hughes JM, Lima NL, et al. Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. *Rev Infect Dis.* 1990;12:S41-S50.

8 Miller JR, Barret LJ, Kotloff K, Guerrant RL. A rapid test for infectious and inflammatory enteritis. *Arch Intern Med*. 1994;154:2660-4.

9 Scerpella EG, Okhuysen PC, Mathewson JJ, et al. Evaluation of a new latex agglutination test for fecal lactoferrin in Travelers' Diarrhea. *J Trav Med*. 1994;1:4-7.

10 Yong WH, Mattia AR, Ferraro MJ. Comparison of fecal lactoferrin latex agglutination assay and methylene blue microscopy for detection of fecal leukocytes in *Clostridium difficile*-associated disease. *J Clin Microbiol*. 1994;32:1360-1.

11 Croft S, Newcomb-Gayman P, Carroll K. Comparison of the Leuko-Test latex agglutination assay with the methylene blue stain for the detection of fecal leukocytes. Abstract #C396. 94th ASM General Meeting. 1994:560.

12 Thornton S, O'Brien T, Callahan J, et al. Evaluation of a rapid latex test for detection of lactoferrin in stool. Abstract #C397. 94th ASM General Meeting. 1194:560.

13 Manabe YC, Vinetz JM, Moore RD, et al. *Clostridium difficile* colitis: an efficient clinical approach to diagnosis. Ann Intern Med. 1995;123: 835-40.

14 Thielman NM, Fang GD, Guerrant RL. Interleukin-8 (IL-8) secretion by human intestinal epithelial cells in response to *Escherichia coli* enteropathogens. [Abstract] *Clin Res*. 1994:42:286A.

15 Sugi K. Fecal lactoferrin as a marker for disease activity in inflammatory bowel disease. Comparison with other neutrophil derived proteins. *Am J Gastroenterol*. 1996;91:927-34.

16 Silletti RP, Lee G, Ailey E. Role of stool screening tests in diagnosis of inflammatory bacterial enteritis and in selection of specimens likely to yield invasive enteric pathogens. *J Clin Microbiol*. 1996;34:1161-5.

17 Oreby G, Alsoda M, Hamed A, et al. Fecal lactoferrin as a parameter in determining invasive causes of acute diarrhea. *J Med Sci Res.* 2020;3:102-7.

18 Lee HM, Lee S, Lee B-I, et al. Clinical significance of fecal lactoferrin and multiplex polymerase chain reaction in patients with acute diarrhea. *Gut Liver*. 2015;9:636-40.

19 Guthrie P. A new test for the detection of white blood cells in stool samples. *MSCLS Newslinks*. 2008;21(6).

20 May A, Rosenblatt J, Pritt B. Evaluation of the LEUKO EZ VUE™ Fecal Lactoferrin Test as a marker of fecal leukocytes. Poster presentation at the 109th General Meeting of the American Society of Microbiology, Philadelphia, PA. 2009.

21 Merado EH, Ochoa TJ, Ecker L, et al. Fecal leukocytes in children infected with diarrheagenic *Escherichia coli. J Clin Microbiol* 2011;49:1376-81.

22 Chen CC, Chang, CJ, Lin, TY, et al. Usefulness of fecal lactoferrin in predicting and monitoring the clinical severity of infectious diarrhea. *World J Gastroenterol*. 2011;17:4218-24.