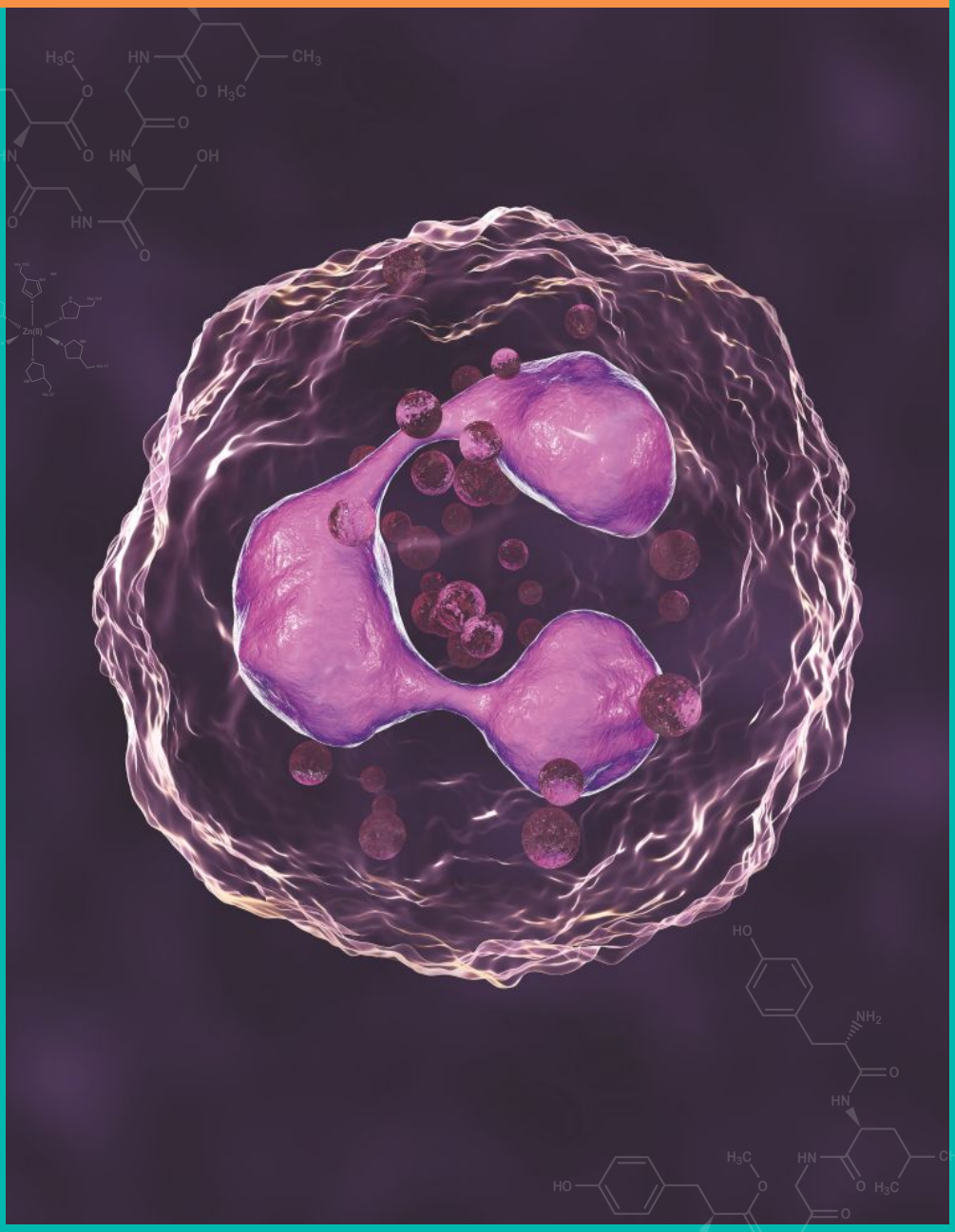


Are Fecal Leukocyte Tests a Waste of Time?

LABORATORY 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 leukocytic cells 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:

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

FLT Costs

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 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.

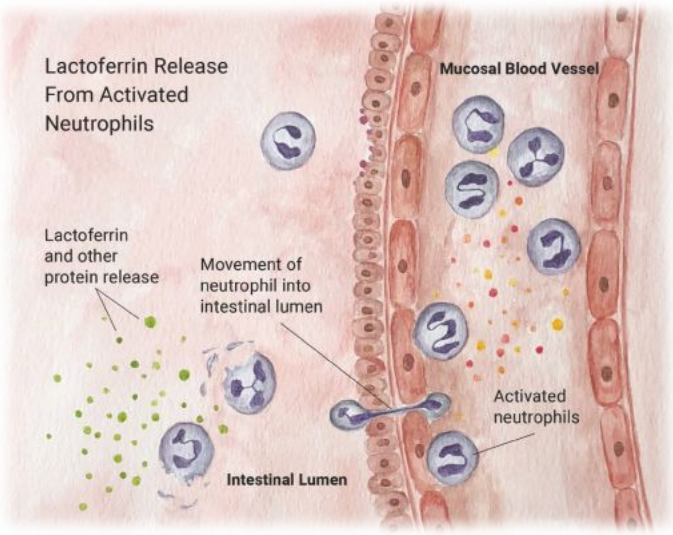
Fecal Biomarkers

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, 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.

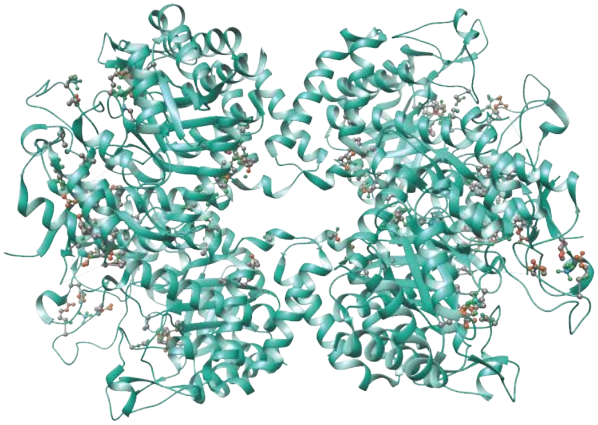
Bacterial pathogens such as *Salmonella, Shigella, Campylobacter,* and *C. difficile* cause inflammatory diarrheas resulting in fecal lactoferrin levels substantially higher than background levels. Many peer-reviewed and unpublished studies have demonstrated the accuracy of fecal lactoferrin as a biomarker 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

Lactoferrin offers many advantages over FLT as an indicator of intestinal inflammation. Four important advantages are stability, reduced time to result, lower labor costs and specimen flexibility. The lactoferrin glycoprotein is a relatively stable molecule. This 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*.



Lactoferrin molecule

Personnel costs are lower with lactoferrin assays. Technical expertise is not a requirement for accurate interpretation of test results. A lateral flow test can provide results within ten minutes. Lateral flow assays are flexible as they can be used with either liquid, semisolid or solid fecal samples with no known interfering substances.

Lactoferrin Performance Testing

The *LEUKO EZ VUE*® test is an FDA-cleared, lateral flow device based on the detection of elevated fecal lactoferrin levels. It is used to detect acute inflammatory diarrheas caused by infectious agents such as bacteria. The lateral flow format is 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 FLT, *LEUKO EZ VUE*® test and lactoferrin tested at a reference laboratory.[18] The *LEUKO EZ VUE*® test and lactoferrin reference test performed off-site were identical and gave increased performance over FLTs.

In a Mayo Clinic study, 168 fresh stool specimens were tested by both the *LEUKO EZ VUE*® test and FLT.[19] Thirty specimens tested positive by *LEUKO EZ VUE*® test 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 *LEUKO EZ VUE*® test as markers of inflammation in children infected with diarrhea-inducing *E. coli*.[20] In 99 samples, all were lactoferrin positive with only 11 having high numbers of fecal leukocytes. The results supported the use of *LEUKO EZ VUE*® test 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.[21] 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.

The *LEUKO EZ VUE*® test as been evaluated favorably in a number of studies, especially when compared to FLTs.

Patients with moderate to severe diarrhea were evaluated using FLTs, lactoferrin and multiplex PCR for pathogen detection.[22] 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.[22]

The Take-Away

In summary, the fecal lactoferrin assay in the form of *LEUKO EZ VUE®* lateral flow device offers significant advantages over FLTs. This assay provides clinicians and their patients with a timely result contributing to more appropriate therapy while simultaneously decreasing healthcare costs.

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