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LEUKO EZ VUE® for elevated fecal lactoferrin is simpler and more sensitive than microscopy for fecal leukocytes

Acute infectious diarrheal diseases represent one of the primary causes of morbidity throughout the world. Inflammatory infections like toxigenic *C. difficile*, *Salmonella*, *Shigella*, *E. histolytica*, and *Campylobacter* often involve tissue damage, requiring immediate medical attention.[1] Noninflammatory cases are typically mild and do not cause mucosal damage, as with giardiasis, cryptosporidiosis, infections by rotavirus and norovirus, and functional disorders like lactose intolerance in irritable bowel syndrome.[1,2] The fecal biomarker, lactoferrin, is a stable glycoprotein that is expressed by activated neutrophils, a primary cell-type of leukocytes. During intestinal inflammation, the infiltration of activated neutrophils into the intestine increases fecal lactoferrin levels.[3-9] Multiple studies have linked elevated fecal lactoferrin to intestinal inflammation.[6-9]

Fecal lactoferrin is a stable biomarker for intestinal inflammation

The *LEUKO EZ VUE*® test is an immunochromatographic test for the qualitative detection of elevated fecal lactoferrin as a marker for fecal leukocytes and an indicator of intestinal inflammation. The *LEUKO EZ VUE*® test is a second generation diagnostic that offers improved performance over the first generation *LEUKO-TEST*, a latex agglutination assay for elevated levels of fecal lactoferrin. The *LEUKO EZ VUE*® test offers improved performance because latex agglutination may give difficult-to-read reactions with fecal specimens. This second generation test overcomes the problems of microscopy by utilizing immunochromatographic technology and provides results in 10 minutes. Lactoferrin is very stable and is not degraded during infections by the toxins of pathogens such as *C. difficile*. The assay can be used with liquid, semi-solid, and solid fecal specimens.

The performance of the *LEUKO EZ VUE*® test has been evaluated in a number of studies. In initial studies, the *LEUKO EZ VUE*® test was compared to the first generation *LEUKO-TEST*. Discrepant findings were resolved by comparison to microscopy for fecal leukocytes using methylene blue stained fecal smears. Three clinical sites in the U.S., including a medical center in the Midwest and a large healthcare center in the East, were involved in the clinical studies. A total of 375 fecal samples were analyzed. The *LEUKO EZ VUE*® test had positive, negative, and overall agreements of 93%, 80%, and 83%, respectively. These results were presented in part at the annual 2008 Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) and at the 2008 Infectious Disease Society of America meeting.[10,11]

In another study performed at a Midwestern hospital, a study involving 42 specimens was performed comparing fecal WBC smears, the *LEUKO EZ VUE*® assay, and lactoferrin levels tested at a reference laboratory.[12] The results demonstrated that the *LEUKO EZ VUE*® assay detected a higher number of positive results that compared identically with the reference method, indicating increased performance of fecal lactoferrin detection over microscopic examination for fecal leukocytes.

The *LEUKO EZ VUE*® test offers more flexibility in specimen handling when compared to microscopy. In a study done at the Mayo Clinic by May et al.,[13] 168 fresh patient stool specimens were tested by the *LEUKO EZ VUE*® test and by microscopy for fecal leukocytes. Of these, 30 were positive for elevated fecal lactoferrin, 12 by both lactoferrin and microscopy and 1 by microscopy only. The authors concluded that the discrepant 18 lactoferrin-positive,

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microscopy-negative were likely a result of lysed and degraded cells. Detecting elevated lactoferrin offers an advantage over microscopy by not requiring intact cells, allowing for longer specimen storage time prior to testing. Specimens being tested for lactoferrin may be stored for up to 2 weeks at room temperature.

The potential for false-negative results by microscopy has been reported by other groups. In a study by Granville, L. A. et al.,[14] 205 adult inpatients were identified as having specimens submitted for microscopy for fecal leukocytes. Discharge codes were retrieved and patients were divided into 2 groups. Patients in Group 1 had inflammatory gastrointestinal (GI) diseases (enteric infections, intestinal inflammation, bloody stool and acute vascular insufficiency, etc.). Patients in Group 2 included those with noninflammatory causes (no lower GI disease, impaction, IBS, constipation, etc.). Fecal specimens were examined within 1 hour of being received into the lab using microscopy and methylene blue stain for counting neutrophils. Using a cut-off of 1 cell per high-power field (HPF), only 32% of patients with infectious gastroenteritis were positive. In addition, patients with intestinal inflammation confirmed by endoscopy had undetectable neutrophils by microscopy. Reasons considered for the poor performance included degeneration of cells during transit and distribution of cells within the stool specimen. The authors concluded that microscopy for fecal leukocytes using a cut-off of 1 cell/HPF was about 20% better than a coin toss for detecting inflamed mucosa. Based on increased sensitivity and ease of use, the *LEUKO EZ VUE*® test offers an improved method for detecting fecal leukocytes.

In a study done in Lima, Peru, fecal leukocytes and fecal lactoferrin were evaluated as markers of inflammatory responses in children infected with different pathotypes of diarrhea-causing *Escherichia coli*.[15] The study was done to learn more about the inflammatory response elicited by *E. coli* in symptomatic children compared to children who carried these pathotypes asymptomatically. Fecal smears stained with methylene blue were evaluated by skilled technicians for the presence of leukocytes. The *LEUKO EZ VUE*® test was used to determine fecal lactoferrin. There were 626 diarrheal episodes, with 72.7% considered mild, 25.6% considered moderate, and 1.8% considered severe. In 99 selected samples analyzed for fecal lactoferrin, 11 samples had high numbers of fecal leukocytes and all were lactoferrin-positive. There were 88 samples with lower levels of leukocytes and 83 of these were positive for lactoferrin. The presence of inflammation, as noted by fecal leukocytes and fecal lactoferrin, was associated with enterotoxigenic *E. coli*, indicating that this pathogen was associated with more inflammation than previously recognized.

A study performed in a medical center in Seoul, South Korea evaluated the utility of fecal lactoferrin and multiplex PCR in patients with moderate to severe diarrhea.[16] A total of 54 patients were included in the study. The results showed that fecal lactoferrin was more useful than microscopy for fecal leukocytes. This conclusion was based on the positive association of fecal lactoferrin with moderate to severe dehydration and the detection of bacterial pathogens by multiplex PCR. These observations corroborate those of Chen et al.[16] who showed that fecal lactoferrin correlated with bacterial infection and greater disease severity in children. The authors noted also that these results extend the utility of fecal lactoferrin testing beyond the scope of differentiation of inflammatory bowel disease from irritable bowel syndrome, to utility as a marker for severe dehydration and acute diarrhea associated with *C. difficile*, *Salmonella*, *Campylobacter*, and other bacterial that cause intestinal disease.

The *LEUKO EZ VUE*® test is easier to perform and more accurate than microscopy for fecal leukocytes

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Key Points

- The *LEUKO EZ VUE*® test offers an easier method for detecting fecal leukocytes compared to microscopy.
- The *LEUKO EZ VUE*® test is more sensitive than microscopy as it is unaffected by lysed and degraded leukocytes.
- Specimens being tested for elevated lactoferrin may be stored at room temperature for 2 weeks before testing.

References

1. Guerrant, R. L., J. M. Hughes, N. L. Lima, and J. Crane. 1990. Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. Rev. Infect. Dis.12:S41-S50.
2. Guerrant, R. L., D. S. Shields, S. M. Thorson, J. B. Schorling, and D. H. M. Groschel. 1985. Evaluation and diagnosis of acute infectious diarrhea. Am. J. Med. 78:91-98.
3. Harris, J. C., H. L. DuPont, and B. R. Hornick. 1971. Fecal leukocytes in diarrheal illness. Ann. Intern. Med. 76:697-703.
4. Koplan, J. P., H. V. Fineberg, M. J. B. Ferraro, and M. L. Rosenberg. 1990. Value of stool cultures. Lancet 2:13-16.
5. Tarr, P. 1991. Microbiology studies. In T. Yamada, D. H. Alpers, C. Owyang, D. W. Powell, and F. E. Silverstein (ed.), Textbook of Gastroenterology, pp. 2523-2538. J. B. Lippincott Company, Philadelphia.
6. Guerrant, R. L., V. Araujo, E. Soares, K. Kotloff, A. A. M. Lima, W. H. Cooper, and A.G. Lee. 1992. Measurement of fecal lactoferrin as a marker of fecal leukocytes. J. Clin. Microbiol. 30:1238-1242.
7. Korzeniowski, O. M., F. A. Barada, J. D. Rouse, and R. L. Guerrant. 1979. Value of examination for fecal leukocytes in the early diagnosis of Shigellosis. Am. J. Trop. Med. Hyg. 28:1031-1035.
8. Langhorst, J. and J.H. Boone. 2012. Fecal Lactoferrin as a Noninvasive Biomarker in Inflammatory Bowel Diseases. Drugs of Today. 48(2):149-161.
9. Choi, S., Park, C., Silva, M., Zaenker, E. and R. Guerrant. 1996. To Culture or Not To Culture: Fecal Lactoferrin Screening for Inflammatory Bacterial Diarrhea. J. Clin. Microbiol. 34:928-932.
10. Pennington, C., J. H. Boone, and D. M. Lyerly. 2008. Evaluation of the *LEUKO EZ VUE*™ for measuring fecal lactoferrin as a marker of intestinal inflammation. ICAAC.
11. Pennington, C., D. M. Lyerly, and J. H. Boone. 2008. Evaluation of the *LEUKO EZ VUE*™ for measuring fecal lactoferrin as a marker of intestinal inflammation. IDSA.
12. Guthrie, P. A new test for the detection of white blood cells in stool samples. 2008. MSCLS Newslinks Volume 21, Number 6.
13. May, A., Roesenblatt, J. and B. Pritt. 2009. Evaluation of the *LEUKO EZ VUE*™ Fecal Lactoferrin Test as a Marker of Fecal Leukocytes. Poster presentation at 109th General Meeting of the American Society of Microbiology, Philadelphia, PA.
14. Granville, L.A., Cernoch, P., Land, G.A. and J.R. Davis. 2004. Performance Assessment of the Fecal leukocyte test for Inpatients. J. Clin. Microbiol. 42:1254-1256.
15. Merado, E. H., T. J. Ochoa, L. Ecker, M. Cabello, D. Duran, F. Barletta, M. Molina, A. I. Gil, L. Huicho, C. F. Lanata, and T. G. Cleary. 2011. Fecal leukocytes in children infected with diarrheagenic *Escherichia coli*. J. Clin. Microbiol. 49:1376-1381.
16. Lee, H. M., S. Lee, B.-I. Lee, D. W. Jekarl, J.-Y. Song, H.- J. Choi, B. K. Kang, E. J. Im, J. S. Kim, J. I. Kim, B.-W. Kim, and H. Choi. 2015. Clinical significance of fecal lactoferrin and multiplex polymerase chain reaction in patients with acute diarrhea. Gut and Liver 9:636- 640.
17. Chen, C. C., C. J. Chang, T. Y. Lin, M. W. Lai, H. C. Chao, and M. S. Kong. 2011. Usefulness of fecal lactoferrin in predicting and monitoring the clinical severity of infectious diarrhea. World J. Gastroenterol. 17:4218-4224.