A New Test for the Detection of White Blood Cells in Stool Samples

Paul Guthrie, Editor

Microscopic examination of a stained fecal smear has been used for many years as a diagnostic tool for the detection of intestinal inflammation. The typical procedure requires fresh stool (tested within 1 hour of collection, or refrigerated for up to 4 hours) and is performed as follows:

1. Using a cotton tipped swab or two wooden applicator sticks, gently roll out a drop of feces on a glass slide. Repeat this for a second slide. If the specimen contains mucous or blood, sample from this area, it's more likely to contain WBC's. Make as thin a smear as possible without using excessive force, as this will rupture WBC's, making identification difficult. Let smears air dry.

2. Stain both slides using the Wrights stain procedure.

3. Examine stained smears under high power (40X) objective noting the average number of WBC's per high power field.

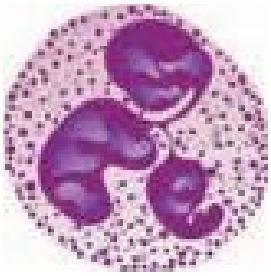
A new rapid test, *LEUKO EZ VUE*™ is commercially available for the detection of white blood cells (WBC) in the stool. The test measures lactoferrin, a glycoprotein component of neutrophilic granules in leukocytes. Testing for fecal lactoferrin is much more sensitive than examination for fecal leukocytes because it does not require intact cells for detection.

PRINCIPLE OF FECAL LACTOFERRIN TESTING

Diarrheal diseases can be classified into inflammatory and non-inflammatory categories. Non-inflammatory diarrheas include those caused by viruses are, for the most part, effectively treated with simple oral rehydration therapy. Inflammatory diarrheas, on the other hand, tend to be more serious and need to be followed up by more extensive testing and/or treatment. Infectious causes for this type of diarrhea are due to pathogens such as *Shigella*, *Salmonella*, *Campylobacter jejuni*, and *Clostridium difficile*. Non-infectious causes of inflammatory bowel disease (IBD) include ulcerative colitis and Crohn's disease, both of which are highly inflammatory and are diagnosed by ruling out infectious agents and other potential causes of inflammation. In all inflammatory diarrheas, fecal leukocytes are found in feces in large numbers.

The determination of fecal leukocytes by microscopy has disadvantages. Microscopy is not standardized. Due to rapid deterioration of WBCs in feces, specimens must be examined shortly after collection to be accurate. Some enteric pathogens, such as *Clostridium difficile*, produce toxins that lyse leukocytes and other cells. As a result, leukocytes may not be visible late in the infection even though there is severe inflammation. The method of collection also affects the sensitivity of the test. Cup specimens are often hard to collect but they are more sensitive for leukocytes than swab specimens, which tend to destroy the morphology of the leukocytes.

The *LEUKO EZ VUE*™ overcomes the problems of microscopy by utilizing immuno-chromatography technology and providing results in less than 15 minutes. The assay detects elevated levels of lactoferrin in fecal samples. Lactoferrin is very stable and is not degraded during infections by the toxins of pathogens such as *C. difficile.* The method utilizes rabbit anti-lactoferrin antibodies that are conjugated directly to gold particles. The *Membrane Cassette* contains two stripes of immobilized antibodies. One stripe contains anti-lactoferrin antibodies. The other, representing a control stripe, contains anti-IgG antibodies. The diluted sample and gold conjugate migrate by capillary action when the sample is added to the well. If elevated lactoferrin is present in the sample, gold conjugate-lactoferrin complexes form and are captured by the immobilized anti-



lactoferrin antibodies in the stripe. The lactoferrin-conjugate-antibody complexes appear as a single red line in the test portion of the *Results Window*. In the control stripe, conjugate binds to the immobilized anti-IgG antibodies, demonstrating correct migration of the sample and conjugate along the membrane. The conjugate-anti-IgG antibodies appear as a single red line in the control portion of the *Results Window*.

PROCEDURE

For the up to date procedure, please see product insert

LIMITATIONS OF FECAL LACTOFERRIN TESTING

1. Fecal leukocyte/lactoferrin testing has been shown to be a poor predictor of C. difficile toxin positivity.
2. This test should not be performed on hospitalized patients admitted more than 3 days. Multiple studies have demonstrated that fecal leukocyte/lactoferrin testing does not reliably detect infectious or noninfectious gastroenteritis in this patient group and will give misleading results.
3. Human breast milk contains very high levels of lactoferrin and infants whose diet includes breast milk will have positive fecal lactoferrin. If intestinal inflammation is suspected in a breast milk fed infant, the stool should be examined for leukocytes by microscopy.

IMPLEMENTATION OF FECAL LACTOFERRIN TESTING

At Bronson Methodist Hospital where the author is employed, the Microbiology Senior Technologist Bridget Yager performed a three way comparison study between the fecal WBC smear, the new *LEUKO EZ VUE*™ lactoferrin method, and lactoferrin tested at reference laboratory. The results between the in-house and reference lab lactoferrin matched perfectly. When compared to the smear exam for WBC's, it became apparent that the sensitivity of the Leuko EZ was much higher than the smear method.

42 samples tested	Smear	Leuko EZ
POSITIVE	5	17
NEGATIVE	37	25
Total	**42**	**42**

With the implementation of the *LEUKO EZ VUE*™ test, orders for fecal WBC are "converted" to fecal lactoferrin, and a notice explaining the rationale for the new test is included in the patient report. The fecal WBC test has been retained. However it is only used for testing on breast fed infants. As expected, the supply costs for the new test are higher than the glass slides and stain used for the fecal WBC exam. However, the Medicare Reimbursement is also higher, being $22.33 for lactoferrin and only $5.96 for the smear.

REFERENCES

Package insert, *LEUKO EZ VUE*™ distributed by Inverness Medical, Princeton, New Jersey 08540, 1-800-257-9525

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