



Evaluation of the *LEUKO EZ VUE*TM for Measuring Fecal Lactoferrin as a Marker of Intestinal Inflammation

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INTRODUCTION

Infectious diarrheal diseases represent one of the primary causes of morbidity throughout the world. Inflammatory infections like *C. difficile* disease often involve tissue damage and dissemination, requiring immediate medical attention. Noninflammatory cases, on the other hand, do not cause extensive mucosal damage and tend to be more self-limiting; although in some instances (e.g., giardiasis), treatment still is needed. The biomarker, fecal lactoferrin, is a stable glycoprotein that is expressed by neutrophils. During intestinal inflammation, the infiltration of leukocytes into the lumen increases levels of fecal lactoferrin over baseline. The aim of our study was to compare a new immunochromatographic assay (*LEUKO EZ VUE*TM) for the detection of elevated fecal lactoferrin as an indicator of fecal leukocytes.

METHODS

In our study, we compared a new immunochromatographic test, *LEUKO EZ VUE*TM, with the *LEUKO-TEST*, a latex agglutination test to measure elevated lactoferrin as a marker for fecal leukocytes and an indicator of intestinal inflammation. There were 234 fecal specimens submitted from patients suspected of inflammatory diarrhea that were tested by immunoassay followed by resolution of discrepant results using microscopy for the presence of fecal leukocytes. Additional studies were done to assess stability of the marker, reproducibility of results and testing of potential interfering substances.

TECHLAB® *LEUKO-TEST* – Latex agglutination assay for detection of human lactoferrin – visual agglutination indicates a positive result.

Microscopic examination – Methylene blue stained fecal smear for leukocytes – Greater than 1 cell observed per high field (100x) indicates a positive result.

TECHLAB® *LEUKO EZ VUE*TM – Immunochromatographic test for detection of human lactoferrin.

1. Sample is diluted 1:50 and passed through a filter into the sample well.
2. Test cassette is incubated 10 minutes.
3. A visual line in the results and control window indicates a positive result.



RESULTS

Table 1. Comparison of the *LEUKO EZ VUE*TM to the *LEUKO-TEST*

N=234	<i>LEUKO-TEST</i> positive	<i>LEUKO-TEST</i> negative
<i>LEUKO EZ VUE</i> TM positive	43	35
<i>LEUKO EZ VUE</i> TM negative	3	153

A total of 234 fecal specimens submitted for fecal leukocyte testing were evaluated by the *LEUKO EZ VUE*TM and *LEUKO-TEST* assays. There were 38 specimens providing discrepant results that were further tested by microscopy.

Table 2. Comparison of the *LEUKO EZ VUE*TM to the *LEUKO-TEST* with resolution of discrepant results using microscopy for fecal leukocytes

N=234	<i>LEUKO-TEST</i> / microscopy positive	<i>LEUKO-TEST</i> / microscopy negative
<i>LEUKO EZ VUE</i> TM positive	63	15
<i>LEUKO EZ VUE</i> TM negative	3	153

Of the 35 specimens that tested *LEUKO EZ VUE*TM positive and *LEUKO-TEST* negative, 20 were confirmed positive for fecal leukocytes using microscopy. All three *LEUKO EZ VUE*TM negative but *LEUKO-TEST* positive specimens were positive by microscopy.

Table 3. Summary of Test *LEUKO EZ VUE*TM Test Performance

Test Comparison (95% confidence intervals) ¹	Percent Positive Agreement	Percent Negative Agreement	Overall Agreement	Number
<i>LEUKO EZ VUE</i> TM vs. <i>LEUKO-TEST</i>	94% (88 – 97%) ¹	84% (79 – 88%) ¹	84% (79 – 87%) ¹	234
<i>LEUKO EZ VUE</i> TM vs. <i>LEUKO-TEST</i> / Microscopy	96% (86 – 99%) ¹	91% (86 – 98%) ¹	92% (90 – 94%) ¹	234

*LEUKO EZ VUE*TM showed a higher overall agreement when compared to the combined results generated by the *LEUKO-TEST* and microscopic examination. The majority of discrepant results were *LEUKO-TEST* negative and *LEUKO EZ VUE*TM positive that were confirmed positive by microscopy.

RESULTS

Table 4. Specimens tested by the *LEUKO EZ VUE*TM at 3 clinical labs

Test Site (20 samples)	Site #1	Site #2	Site #3
% Correlation to Control site	90%	95%	100%

A total of 10 lactoferrin-positive and 10 lactoferrin-negative were sent to 3 separate clinical labs for reproducibility testing. All 3 test sites correlated 90% or better with the results generated at the control site. All of the lactoferrin-positive specimens stayed positive and all of the lactoferrin-negative specimens remained negative over the 15 day test period when stored refrigerated.

Table 5. Analysis of potential interfering substances

Test Substances for spiking	Results for spiked pooled positive specimen	Results for spiked pooled negative specimen
Mucous	Positive	Negative
Fecal Fat	Positive	Negative
Mylanta [®]	Positive	Negative
Pepto-Bismol [®]	Positive	Negative
Imodium [®]	Positive	Negative
Kaopectate [®]	Positive	Negative
Bilirubin	Positive	Negative

A total of 4 lactoferrin-positive and 4 lactoferrin-negative fecal specimens were pooled separately and spiked with potential interfering substances. No interference was noted with any of the selected substances.

CONCLUSIONS

- The *LEUKO EZ VUE*TM is both highly sensitive and specific for elevated fecal lactoferrin as an indicator of intestinal inflammation.
- The 10-minute format allows for a rapid assessment for fecal leukocytes.
- Results are simple to interpret and reproducible.
- Lactoferrin is a stable biomarker for intestinal inflammation.
- The *LEUKO EZ VUE*TM outperformed the *LEUKO-TEST* and represents an improved assay for intestinal inflammation.

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