

# The Detection of Lactoferrin, ASCA and ANCA in Feces is useful for Assessing Pediatric IBD Patients

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## Introduction:

Diagnostic testing for inflammatory bowel disease (IBD) is increasingly common in the clinical setting. More recent immunoassays include fecal lactoferrin (Lf, a marker of intestinal inflammation when elevated), serum anti-*Saccharomyces cerevisiae* antibodies (ASCA, a marker of Crohn's disease (CD)) and serum anti-neutrophil cytoplasmic antibodies (ANCA, a marker of ulcerative colitis (UC) and UC-like CD). These assays, as a panel, offer an aid for distinguishing irritable bowel syndrome (IBS) from active IBD, monitoring for inactive and active IBD, and for distinguishing CD from UC and other intestinal illnesses.

## Aim:

To evaluate a new diagnostic approach measuring fecal Lf, ASCA and ANCA for the assessment of pediatric IBD.

## Method I:

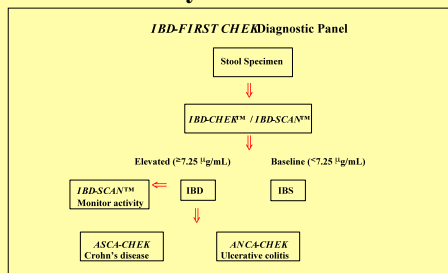
Fecal Lf, ASCA and ANCA levels were determined by enzyme-linked immunoassays that included *IBD-SCAN*™, *ASCA-CHEK*, and *ANCA-CHEK*, respectively. All of the tests were from TechLab. The quantitative ELISA test for lactoferrin uses both a capture and conjugated antibody specific to human lactoferrin. The qualitative ELISA tests for ASCA and ANCA use anti-human immunoglobulin antibody (IgG, IgA, IgA<sub>sec</sub>, and IgM) conjugated to HRP and microwells coated with *Saccharomyces cerevisiae* antigens and neutrophil antigens, respectively.

## Method II:

Fecal specimens were diluted in the kit diluents and results were determined by measurement of the optical density (OD) at 450nm. Results of  $\geq 0.200$  and  $0.150$  were considered positive for the presence of fecal ASCA and ANCA, respectively. The levels of Lf were determined using a standard curve and serial 1:10 dilutions of feces. A cutoff of  $>7.24 \mu\text{g/mL}$  was used to define elevated Lf.

## Subject Population:

A total of 95 fecal specimens was collected at a pediatric IBD clinic from 38 CD subjects, 41 UC subjects and 6 IBS subjects. The control group consisted of 10 healthy subjects. The age range was 2 to 18 years with a male to female ratio of 1.7. Diagnosis and disease activity were assessed clinically.



## Results:

### Lactoferrin Levels for Subject Groups

Group ID	No. of Assessments	Mean Lactoferrin Level (µg/mL)	SE	P Value
Active IBD	60	2321.7	496.0	Active IBD vs Inactive IBD $p < 0.0002$
Inactive IBD	19	1.7	0.4	Inactive IBD vs IBS $p < 0.9$
IBS	6	1.9	1.0	IBS vs active IBD $p < 0.0002$
HC	10	3.4	1.1	HC vs IBS $p < 0.5$

## IBD-FIRST CHEK Panel Results

Total Assessments N=95	Total	Elevated Lactoferrin $>7.24 \mu\text{g/mL}$	Baseline Lactoferrin $<7.24 \mu\text{g/mL}$
Total active IBD	60	60 (100%)	0 (0%)
Total inactive IBD	19	0 (0%)	19 (100%)
Total ASCA positive	10	10 (100%)	0 (0%)
Total ANCA positive	9	5 (55.6%)	4 (44.4%)
Total ASCA and ANCA positive	3	3 (100%)	0 (0%)
Total Active IBS	6	0 (0%)	100% (6)
Total Healthy Controls	10	2 (20%)	8 (80%)

N=95	CD	UC / IBS / HC
Fecal ASCA positive	11	2
Fecal ASCA negative	28	54

N=95	UC	CD / IBS / HC
Fecal ANCA positive	12	1
Fecal ANCA negative	29	53

## Statistical Analysis of Test Results

N=95	ASCA CD vs UC IBS HC	ANCA UC vs CD IBS HC
Sensitivity	28.21%	29.3%
Specificity	96.4%	98.2%
Predictive Pos Value	84.6%	92.3%
Predictive Neg Value	65.9%	64.6%
Correlation	68.4%	68.4%

Group ID	Mean OD	SD	OD Range	P Value
ASCA pos.	1.523	0.796	0.255-2.967	ASCA pos. vs ASCA neg. $p < 0.00001$
ASCA neg.	0.060	0.033	0.040-0.186	ND
ANCA pos.	0.354	0.187	0.198-0.804	ANCA pos vs ANCA neg. $p < 0.0005$
ANCA neg.	0.073	0.030	0.039-0.147	ND

## Conclusions:

- Results show that fecal Lf, ASCA and ANCA were able to resolve 75.3% of subjects.
- Sensitivity and specificity are similar or better than that reported for serum-based ASCA and ANCA tests in the pediatric IBD population.
- Our results show that the combination of fecal Lf, ASCA and ANCA is useful as a noninvasive and inexpensive diagnostic screen for chronic intestinal illnesses.