BÜHLMANN fCAL® turbo

Immunoturbidimetric fecal CALPROTECTIN Assay

Unique in Speed, Quality, Flexibility

Reliable and Non-Invasive Diagnostic Biomarker for IBD

Calprotectin is the biomarker of choice for IBD

High diagnostic accuracy with AUC of 0.929 for discrimination of IBD from IBS

Excellent sensitivity of 91.1% at cut-off 80 µg/g

BÜHLMANN fCAL assays are highly referenced with more than 75 peer reviewed scientific articles

BÜHLMANN fCAL® turbo is FDA 510(k) cleared. (K190784). For *in vitro* Diagnostic Use.

CALEX® Cap: FDA 510(k) cleared. (K191718). For *in vitro* Diagnostic Use.

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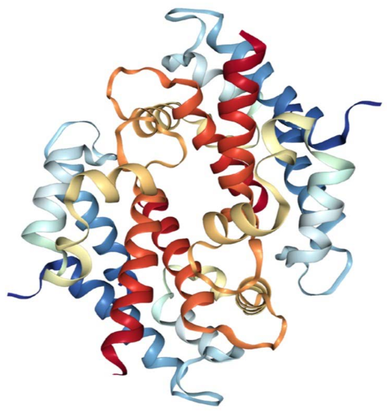
In vitro Assay for the Aid in Diagnosis of IBD

Calprotectin and Inflammatory Bowel Disease IBD

Calprotectin is a very abundant heterodimeric calcium binding protein belonging to the S100 family. It is derived predominantly from the cytosolic fraction of neutrophils and to some extent from monocytes and activated macrophages. It has been shown to be extremely useful as an aid in diagnosis of inflammatory bowel disease (IBD).



Crystal structure of human calprotectin, adapted from Korndoerfer et al.,2007.

IBD includes Crohn's disease (CD) and ulcerative colitis (UC). IBD is a chronic disease with forms involving lower bowel parts or the entire GI tract, and causing symptoms like abdominal pain, diarrhea, fever and weight loss.

BÜHLMANN fCAL® turbo Sets New Standard in the Industry

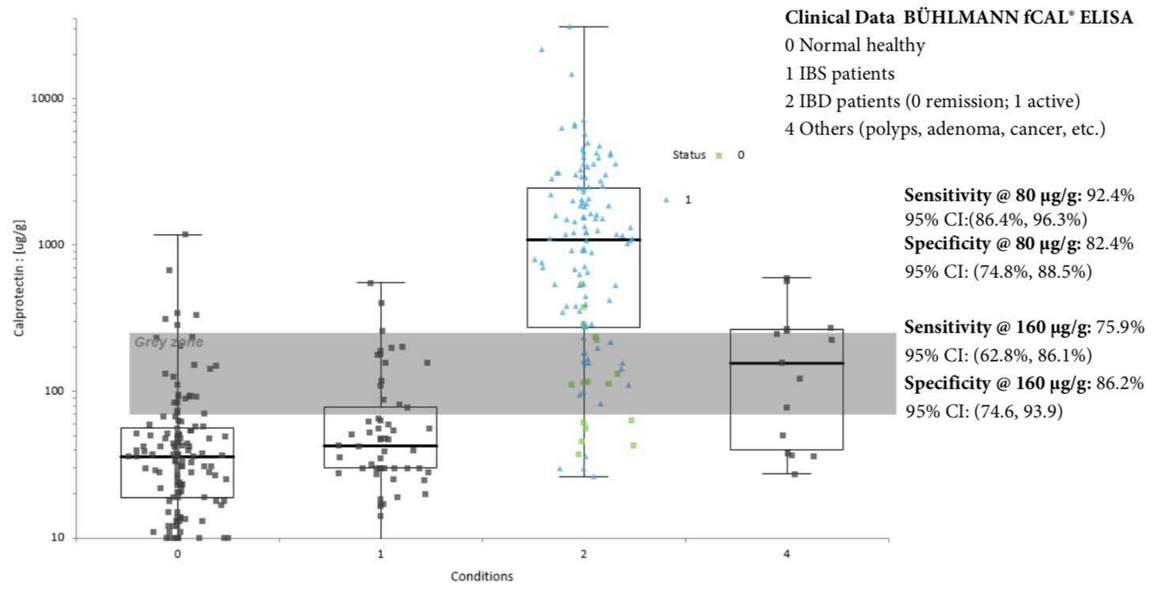
The BÜHLMANN fCAL® turbo is an *in vitro* diagnostic assay intended for the quantitative measurement of fecal calprotectin, a neutrophilic protein that is a marker of intestinal mucosal inflammation, in human stool. The BÜHLMANN fCAL® turbo aids in the diagnosis of inflammatory bowel disease (IBD), specifically Crohn's disease (CD) and ulcerative colitis (UC) and aids in the differentiation of IBD from irritable bowel syndrome (IBS) in conjunction with other laboratory and clinical findings.

Benefits

- High diagnostic accuracy for discrimination of IBD from IBS with AUC of 0.929 obtained in a clinical study with 248 adult and pediatric patients.
- Excellent sensitivity at a cut-off 80 µg/g of 91.1%: minimize false negatives in IBD diagnosis!
- Stable and consistent standardization (same as BÜHLMANN fCAL® ELISA) since 2006
- Narrow clinical grey zone between 80 µg/g and 160 µg/g
- More than 75 clinical, peer-reviewed publications
- The BÜHLMANN fCAL® turbo and BÜHLMANN fCAL® ELISA are the only global assays with IVD registrations in the US (FDA), Canada (HC), Europe (CE-IVD), Japan (PMDA), China (SFDA), Australia (TGE), Brazil (ANVISA) etc.

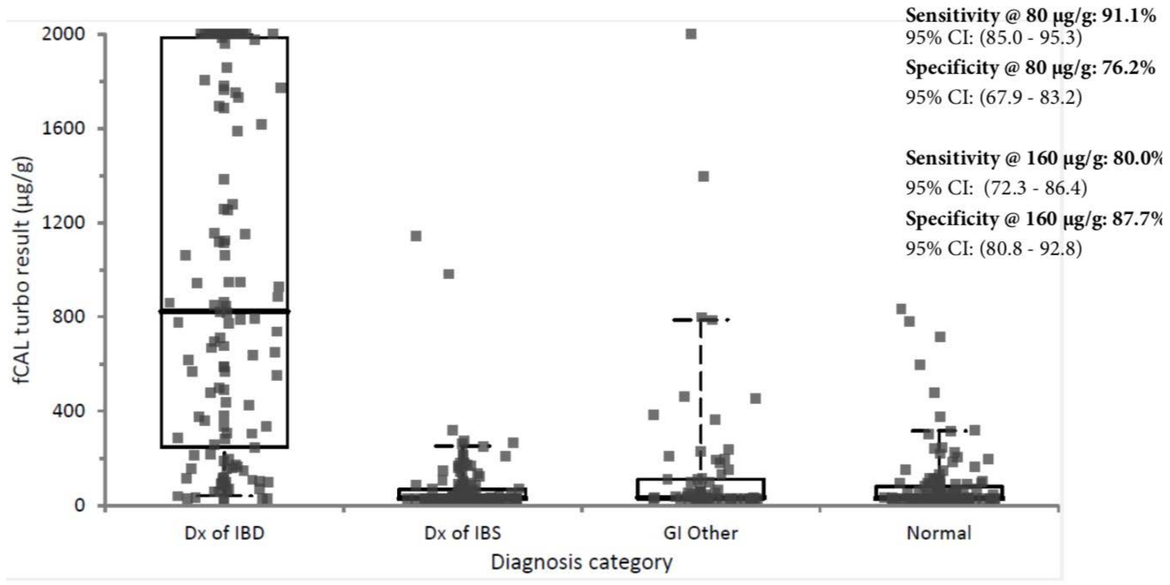
Determination of Cut-off

Multiple studies were performed for the determination of cut-offs for the BÜHLMANN fCAL® ELISA.



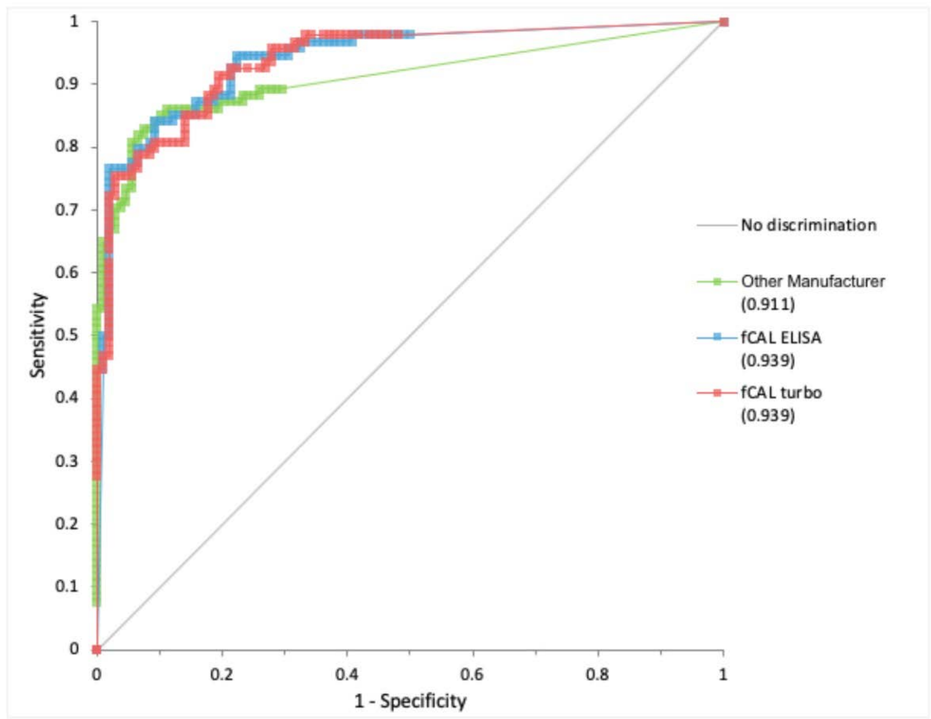
Validation of Cut-off

The cut-off was validated in a study using 478 evaluable study subjects. The boxplot below was generated for BÜHLMANN fCAL® turbo test results by diagnostic category for all subjects.



Clinical Study

A clinical study with a total of 248 adult and pediatric patients was performed to assess the ability of the BÜHLMANN fCAL® turbo to discriminate between patients with IBD and other non-inflammatory GI disorders, including IBS. A subset of this population (94 IBD vs 108 IBS) that included only samples that were run with another manufacturer's assay were used to create the ROC curve below to provide a competitive comparison.



ROC curve comparison, 94 IBD vs. 108 IBS subjects:

- **Red curve** = BÜHLMANN fCAL® turbo; AUC = 0.939
- **Blue curve** = BÜHLMANN fCAL® ELISA; AUC = 0.939
- **Green curve** = Other Manufacturer; AUC = 0.911

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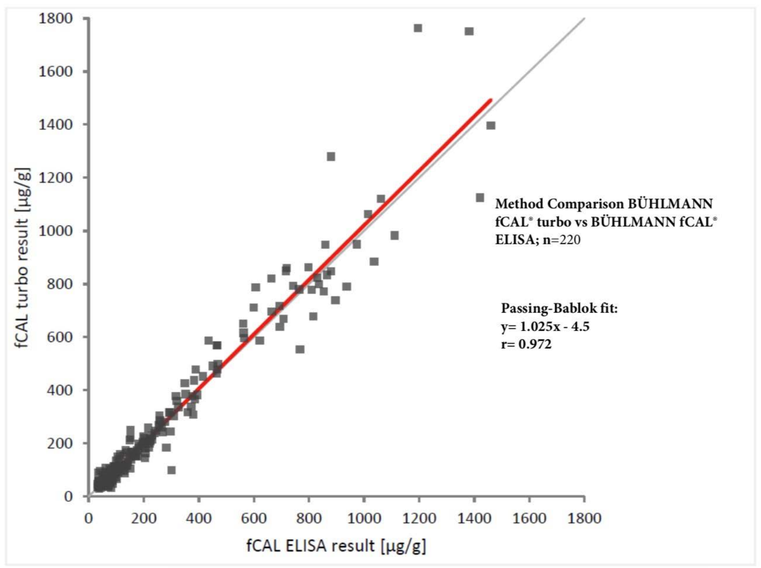
Use the Established Reference

The BÜHLMANN fCAL® turbo standardization is based on the well established BÜHLMANN fCAL® ELISA (K181012), which is implemented globally, robust, validated and highly published worldwide. Our numerous years of experience with the complex protein and our large scientific network will guarantee continuous quality of the assay.

Focus on what really matters - accurate results you can stand behind.



Clinical Performance Characteristics

BÜHLMANN fCAL® turbo in discriminating IBD from non-IBD – IBS and other GI-related disorders, at 80 µg/g and 160 µg/g clinical decision points:

IBD vs. non-IBD	Clinical decision point			
	80 µg/g		160 µg/g	
Sensitivity (95% CI)	91.1%	(85.0%, 95.3%)	80.0%	(72.3%, 86.4%)
Specificity (95% CI)	74.3%	(67.7%, 80.1%)	85.1%	(79.5%, 89.8%)
PPV (95% CI)	70.3%	(62.9%, 76.9%)	78.3%	(70.4%, 84.8%)
NPV (95% CI)	92.6%	(87.4%, 96.1%)	86.4%	(80.9%, 90.9%)
ROC AUC (95% CI)	0.916	(0.884, 0.947)		

BÜHLMANN fCAL® turbo in discriminating IBD from IBS at 80 µg/g and 160 µg/g clinical decision points:

IBD vs. IBS	Clinical decision point			
	80 µg/g		160 µg/g	
Sensitivity (95% CI)	91.1%	(85.0%, 95.3%)	80.0%	(72.3%, 86.4%)
Specificity (95% CI)	76.2%	(67.9%, 83.2%)	87.7%	(80.8%, 92.8%)
PPV (95% CI)	79.9%	(72.7%, 85.9%)	87.1%	(79.9%, 92.4%)
NPV (95% CI)	89.2%	(81.9%, 94.3%)	80.9%	(73.4%, 87.0%)
ROC AUC (95% CI)	0.929	(0.898, 0.960)		

Sample Carry-over

The sample carry-over was established according to the CLSI guideline EP10-A3. No statistically significant sample carryover with the BÜHLMANN fCAL turbo test on Roche cobas® 6000 c501 instrument was detected.

	Day 1 [%]	Day 2 [%]	Day 3 [%]	Day 4 [%]	Day 5 [%]	Mean [%]
Adjusted carry-over coefficient	0.80	-0.13	0.64	0.17	-0.70	0.16

Sample Carry-over Study Results

Reproducibility: Multi-site precision evaluation study

Reproducibility was established according to the CLSI guideline EP05- A3 by performing measurements at three laboratory sites. Eight pooled stool specimen extracts with calprotectin concentrations covering the measuring range of the test and clinical decision points were tested over five days, in one run per day, with five results generated per run. One reagent lot was used in the study.

ID	Mean [µg/g]	n	Within-run (Repeatability)		Between-day		Between-site		Total (Reproducibility)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
S01	47.2	75	3.6	7.6%	2.4	5.0%	0.0	0.0%	4.3	9.1%
S02	91.1	75	3.5	3.8%	3.5	3.8%	2.8	3.1%	5.7	6.2%
S03	185.4	75	5.1	2.7%	2.7	1.4%	5.5	3.0%	7.9	4.3%
S04	276.9	75	6.4	2.3%	4.5	1.6%	9.7	3.5%	12.5	4.5%
S05	674.5	75	12.9	1.9%	1.2	0.2%	22.8	3.4%	26.3	3.9%
S06	1519.6	75	25.3	1.7%	17.8	1.2%	62.3	4.1%	69.6	4.6%
S07	3343.8	75	54.6	1.6%	35.6	1.1%	100.0	3.0%	119.4	3.6%
S08	5475.6	75	72.1	1.3%	35.8	0.7%	154.2	2.8%	173.9	3.2%

Reproducibility Study Results
within-run, between-run and between-day variance component estimates

Between-Lot Precision

Between-lot precision was established according to the CLSI guideline EP05-A3. Eight pooled stool extracts with calprotectin concentrations covering the measuring range of the test and clinical decision points were tested over five days, in one run per day, with five results generated per run. Three reagent lots were used in the study.

ID	Mean [µg/g]	n	Within-run (Repeatability)		Between-day		Between-lot		Total precision	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
S1	45.2	75	3.22	7.1%	1.36	3.0%	3.70	8.2%	5.09	11.3%
S2	86.4	75	3.69	4.3%	1.19	1.4%	5.66	6.6%	6.86	7.9%
S3	175.8	75	5.04	2.9%	0.29	0.2%	9.90	5.6%	11.11	6.3%
S4	263.9	75	7.55	2.9%	0.00	0.0%	9.98	3.8%	12.52	4.7%
S5	647.4	75	15.47	2.4%	0.00	0.0%	15.28	2.4%	21.74	3.4%
S6	1460.7	75	33.66	2.3%	11.64	0.8%	41.01	2.8%	54.32	3.7%
S7	3234.5	75	71.23	2.2%	8.90	0.3%	130.29	4.0%	148.76	4.6%
S8	5303.1	75	97.42	1.8%	11.18	0.2%	163.87	3.1%	190.97	3.6%

Between-lot Precision Study Results

Established Cut-off for Diagnosis

Calprotectin values below 80 µg/g

Fecal calprotectin concentrations < 80 µg/g are not indicative of active inflammation in the gastrointestinal tract.

Calprotectin values between or equal to 80 and 160 µg/g

Fecal calprotectin concentrations between or equal to 80 and 160 µg/g, also called the gray-zone, are not directly indicative of an active inflammation requiring immediate follow-up with invasive testing. However, the presence of inflammation (alternatively "an inflammatory condition") cannot be excluded.

Calprotectin values greater than 160 µg/g

Fecal calprotectin concentrations > 160 µg/g are indicative of neutrophil infiltrate in the gastrointestinal tract and may signal the presence of active inflammatory disease.

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BÜHLMANN fCAL® turbo, a turbidimetric immunoassay, is a flexible solution to be applied on most clinical chemistry analyzers.

Turbidimetric technology is a milestone in fecal calprotectin quantification. This technology allows very rapid and **flexible random access testing**, and is the ideal solution for high throughput applications in clinical and research laboratories. The fCAL turbo **reduces the hands-on time and time-to-first result dramatically** allowing for rapid reporting of calprotectin results from human stool samples.

BÜHLMANN has developed fCAL turbo applications on a number of manufacturers' chemistry systems (turbo application is currently validated on the Roche cobas c501/c502 in the US) and continuously updates the list of applications. The analysis itself takes ten minutes and covers a range from 30 to 2,000 µg/g. Automatic dilution and re-testing of samples above the measuring range is supported on most manufacturers chemistry platforms.

Save hands on time with random access automation Focus on what really matters-accurate results you can stand behind.

- Time -to-first-result: 10 minutes
- Throughput: 192/hour (throughput is dependent on the specific analyzer)
- Clinical Chemistry Analyzers: random access
- Measuring Range: 30 to 2,000 µg/g (30 to 10,000 with auto-dilution)

Result Interpretation

Calprotectin concentration	Interpretation	Follow-up
< 80 µg/g	Normal	None
80 - 160 µg/g	Gray-zone/ Border-line	Follow-up within 4 to 6 weeks
> 160 µg/g	Elevated	Repeat as needed

Limitations

Test results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.

Fecal calprotectin results may not be clinically applicable to children less than 4 years of age.

Patients taking non-steroidal anti-inflammatory drugs (NSAID) may have elevated fecal calprotectin levels.

Patients with granulocytopenia may have false negative results due to bone marrow depression.

Fecal Extracts on Clin Chem Platforms

Centrifuged extracts are free from undigested diet fibers. The bacterial load is reduced by ~95% to levels comparable to concentrations found in urine samples from subjects with UTI.

Carry-over effects in the high through-put analyzers is minimal and has been determined as <0.5%. Serum contaminations can be excluded.

Turbidimetric reagents are free of interferring compounds. Interactions with other assays are highly unlikely and have not been observed.

References:
Korndoerfer, I.P. et al., 2007, The crystal structure of the human (S100A8/S100A9) heterotetramer, calprotectin, illustrates how conformational changes of interacting alphahelices can determine specific association of two EF-hand proteins, *J Mol Bio*

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BÜHLMANN CALEX® Cap Ordering Codes:

B-CALEX-C50 50 devices
B-CALEX-C200 200 devices
B-CALEX-C500 500 device

BÜHLMANN fCAL® turbo Ordering Codes:

Kit	KK-CAL-U	
Reagent Kit (~200 tests)	B-KCAL-RSET-U.	R1 35 ml, R2 7 ml
Calibrator Kit	B-KCAL-CASET-U.	6 levels, 1 ml each; Ready to use
Control Kit	B-KCAL-CONSET-U.	3 x 2 levels, 1 ml each; Ready to use

FDA cleared 510(k) Nr. K