Calprotectin Immunoturbidimetric Assay
Instructions for Use (IFU)

For *In Vitro* Diagnostic Use in the United States, Canada, and the European Union

REF **80-CALPHU-IT100**

Rx Only

CE IVD

Manufactured by:
ALPCO
26-G Keewaydin Drive
Salem, MA 03079-4821
Customer service:
Tel: (603) 893 – 8914
Tel: (800) 592 – 5726
e-mail: cs@alpco.com

Authorized Representative:
GeneProof a.s.
Vídeňská 101/119
Dolní Heršpice
CZ – 619 00 Brno
Tel: + 420 730 176 222

Intended Purpose and Use

The Calprotectin Immunoturbidimetric Assay is an *in vitro* diagnostic particle-enhanced immunoassay intended for use by trained laboratory personnel for the quantitative measurement of fecal calprotectin, a neutrophilic protein that is a marker of intestinal mucosal inflammation, in human stool. The Calprotectin Immunoturbidimetric Assay is intended for *in vitro* diagnostic use as an aid in the diagnosis of inflammatory bowel disease (IBD), specifically Crohn's disease (CD) and ulcerative colitis (UC), and as an aid in the differentiation of IBD from irritable bowel syndrome (IBS) in conjunction with other clinical and laboratory findings. The Calprotectin Immunoturbidimetric Assay is used in conjunction with two accessories, the Calprotectin Control Set (80-CALPHU-ITCON) and Calprotectin Calibrator Set (80-CALPHU-ITCAL).

Summary and Explanation of the Test

Calprotectin, a heterodimer comprised of S100A8/A9 monomers, also known as myeloid-related protein (MRP) 8/14 subunits, is an immunomodulatory protein accounting for up to 60% of cytosolic protein in neutrophil granulocytes and macrophages. Calprotectin plays a central role in neutrophil defenses and inflammatory pathologies. Under conditions of intestinal inflammation, calprotectin is released into the gastrointestinal lumen and serves as an accurate biomarker of mucosal inflammation.[1]

Measurement of fecal calprotectin levels provides valuable information that can assist physicians to determine whether to send inflammatory bowel disease (IBD) patients for colonoscopy or treat them for IBS symptoms.[2] Traditionally, IBD activity has been assessed through clinical symptoms, but it has been reported that these recognized activity indexes are subjective and do not correlate as consistently as fecal calprotectin levels with histologic inflammation.[3]

Principles of the Procedure

The Calprotectin Immunoturbidimetric Assay is a particle-enhanced immunoturbidimetric assay. Calprotectin in the sample binds to specific anti-calprotectin antibodies, which are coated on latex particles, and causes agglutination. This agglutination is detected as an absorbance change, with the magnitude of the change directly related to the quantity of Calprotectin in the sample. The actual concentration is then determined by the interpolation from a calibration curve prepared from calibrators of known concentration.

Materials Supplied for 100 tests

The Calprotectin Immunoturbidimetric Assay is provided in the following kit configuration:

Instrument	Catalog No.	Kit size
AU680	80-CALPHU-IT100	R1: 1 x 22.5 mL R2: 1 x 5.3 mL Cal: 80-CALPHU-ITCAL 5 x 1mL * Con: 80-CALPHU-ITCON 2 x 1mL *

* Calibrators and Controls Sold Separately

Composition of Supplied Reagents/Materials

1. **Reagent 1**
   Tris buffer solution with <0.1% sodium azide. Ready to use.

2. **Reagent 2**
   Suspension of latex particles (< 0.5%) coated with anti-calprotectin antibodies with <0.1% sodium azide, Ready to use.

Materials Required but Not Provided

Calibrator for establishing the dose response curve of the Calprotectin Immunoturbidimetric Assay on an analyzer. (80-CALPHU-ITCAL, 5 x 5mL)

Controls for validating the performance of the Calprotectin reagents are provided separately (80-CALPHU-ITCON, 2 x 1mL).

Feces sample collection
1. Sample collection tube
2. Transport container

Feces preparation
1. Disposable, breakable sterile inoculation loops or wooden stick
2. Disposable polypropylene screw cap tubes, 14 ml
3. Eppendorf tubes (1 - 1.5 ml)
4. Sensitive digital scale (40-150 mg)
5. Extraction buffer (10-EXBUF-55)
6. Vortex mixer
7. Shaker
8. Centrifuge (1000 – 3000 x *g*)
9. Freezer (-20ºC, -80ºC)
10. ALPCO Stool Extraction Device (30-EZEX-100 in the U.S. and Canada, the Easy 2 Stool Extraction Device (80-ESED2-100) in the E.U. or an equivalent method/device capable of diluting 15 mg of sample 1:100 in a compatible extraction buffer)

An analyzer capable of dispensing two reagents and measuring absorbance at around 800 nm with temperature control (37°C).

Precautions and Warnings

1. For *in vitro* diagnostic use in the United States, Canada, and the European Union.
2. Follow universal precautions. Materials of human origin used in this kit have been tested and confirmed negative for HBsAg and anti-HIV I and II and anti-HCV antibodies. They should be treated as a potential biohazard and handled and disposed of according to local laboratory legislation.

3. Reagents and samples should be allowed to reach room temperature (18-28ºC) before starting the test.
4. Warning: do not interchange components from the different kit lots. Satisfactory performance of the test is guaranteed only when components from the same batch of the Calprotectin Immunoturbidimetric Assay are used.
5. The ALPCO Stool Extraction Device should not be used for the extraction of liquid/watery stool samples. Samples of this consistency should be extracted using the manual weighing extraction method.
6. For information on hazardous substances included in the kit and actions to take in the event of direct contact or ingestion, refer to the Safety Data Sheet available at www.alpco.com or upon request.
7. Dispose of unused reagents according to local and federal regulations.

Storage and Shipment Conditions

The Calprotectin Immunoturbidimetric Assay, Calprotectin Immunoturbidimetric Assay – Calibrators, Calprotectin Immunoturbidimetric Assay – Controls should be stored at 2-8°C. DO NOT FREEZE. The Calprotectin Immunoturbidimetric Assay, Calprotectin Immunoturbidimetric Assay – Calibrators, Calprotectin Immunoturbidimetric Assay – Controls are stable when stored as instructed until the expiration date on the label. Studies at elevated temperatures up to 37˚C have shown the product is stable for up to 30 days. The product is shipped in insulated packaging including ice bricks to protect it from extremes and prevent freezing.
.

Reagent Stability

Unopened Reagents
Store at 2-8ºC. Unopened reagents are stable until the expiration date printed on the label.
Opened / Reconstituted Reagents
Store at 2-8ºC. Opened and reconstituted reagents are stable for up to 7 days.

Specimen Collection

Loose or liquid stool samples are acceptable as normalization to stool weight is part of the calculation of the result. Submission of stool samples from diapers should be avoided unless the sample submitted can be taken from a portion of the stool which is not in contact with the diaper material.

Sample requirements:

1. 1-5 g stool in a screw-top clean vial. No preservative is necessary or indicated.
2. Sample transport: Stool specimen should be received by the laboratory within 4 days of collection. Temperature during shipment should not exceed 28ºC. Samples must be stored at 2-8ºC upon receipt and extracted or frozen within 14 days of collection.
3. Sample storage: Samples may be stored at 2-8ºC for up to 14 days before extraction and testing. If samples will not be tested within 14 days, freeze samples at -20ºC or -80ºC.

Freezing at -80ºC is recommended for long-term storage. Stool samples may be subject to no more than 3 freeze/thaw cycles.

Reagent Preparation

Reagents 1 and 2 are ready to use. Mix by inverting at least 10 times.

Sample Preparation – Extraction Procedure

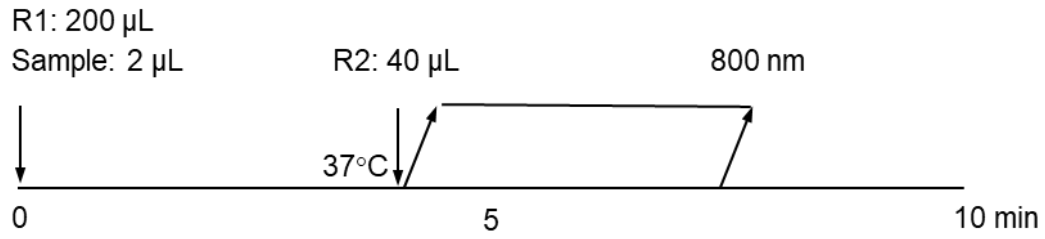
Stool samples can be extracted using either the Easy Stool Extraction method (see Instructions for Use provided with catalog number 30-EZEX-100 in the U.S. or 80-ESED2-100 in the EU) or the Manual Weighing/Standard Extraction procedure described below:

Important: The Easy Stool Extraction method should not be used for the extraction of liquid stool samples.

1. Label and weigh (tare) the empty polypropylene tube together with the inoculation loop.
2. Take out 50 to 100 mg of the stool sample by means of the inoculation loop and place it into the pre-weighed tube.
3. Determine the net amount of sample. Break off the inoculation loop and leave the lower part of the loop in the tube. Add Extraction Buffer using 99 times the weight volume (10-EXBUF-50) to the tube and close the tube.
4. Homogenize the sample on a multi-tube vortex by vigorous shaking (at highest speed) for 30 minutes.
5. Centrifuge the extract in the tube for 5 minutes at 3000 x *g*.
6. Decant the supernatant into a fresh labeled tube and continue with the assay procedure or store the extracts at 2-8°C for up to 3 days or at -80°C for up to 14 days. Stool sample extracts may be subject to no more than 3 freeze/thaw cycles. This extraction procedure results in a 1:100 dilution.

Assay Procedure

Calprotectin should be measured according to the specific application parameters for each specific chemistry analyzer. Below is a general example of the assay test scheme and the specific application parameters for the Beckman AU680 analyzer.



Calculate Calprotectin value with the read absorbance change from a calibration curve prepared with calibrators of known concentrations.

Application sheets for use of the ALPCO Calprotectin assay on other automated clinical chemistry analyzers are available upon request.

Assay Calibration and Quality Control

1. 5 levels of Calprotectin Calibrator (80-CALPHU-ITCAL) are provided separately and ready to use. A 6-point calibration is generated using isotonic saline is used as a zero calibrator.

2. We recommend that each laboratory use the Calprotectin Control to validate the performance of the Calprotectin Immunoturbidimetric Assay. A set of normal and abnormal ranges of Calprotectin controls is available from ALPCO (80-CALPHU-ITCON). The range of acceptable control limits should be established by individual laboratories.

Results

Results are printed out in µg/g of calprotectin in stool. Note: Samples with values greater than 1,000 µg/g will require further dilution with isotonic saline which can be performed by the analyzer on-board or performed manually off-line. Diluted samples should be retested, and the results should be multiplied by the dilution factor.

Interpretation of Results

A cut-off study was performed internally and provided the value reported in the table below:

Calprotectin Concentration	Interpretation	Follow-Up
< 50 µg/g	Normal	None
50 - 100 µg/g	Equivocal	Retest in 4-6 weeks
> 100 µg/g	Elevated	Repeat as clinically indicated

Limitations of the Procedure

- False-negative results could occur in patients who have granulocytopenia due to bone marrow depression.
- Some patients who are taking non-steroidal anti-inflammatory drugs (NSAID) will have elevations in their fecal calprotectin levels.[5]
- Patients with IBD fluctuate between active (inflammatory) and inactive stages of the disease. These stages must be considered when using the Calprotectin Immunoturbidimetric Assay.
- The use of proton pump inhibitors (PPIs), microscopic colitis and diverticular disease may also lead to elevated calprotectin level. Patients affected by untreated celiac disease may occasionally show elevated calprotectin values.[6]
- Other intestinal diseases, including many gastrointestinal infections and colorectal cancer, can result in elevated levels of calprotectin. These specimens may test positive with the Calprotectin Immunoturbidimetric Assay. Therefore, a diagnosis of active IBD should be

made only in the context of other diagnostic testing and the total clinical status of the patient.

- Fecal calprotectin is an indicator of neutrophilic presence in the stool and is not specific for IBD.
- The manual weighing extraction procedure is to be used for liquid stool samples. The performance characteristics for the Easy / Easy 2 Stool Extraction Devices have not been established for liquid stool samples.

Expected Values

To verify the low clinical cut-off value (50 µg/g), normal stool samples were obtained from asymptomatic individuals with no abdominal complaints and no history of IBS, IBD or other chronic intestinal disorders; this study cohort was separate from those used to establish estimates of the clinical performance of the test device. The expected result for the "normal"/asymptomatic population is < 50 µg/g (normal).

Calprotectin levels were analyzed using the Calprotectin Immunoturbidimetric Assay on 120 samples obtained from apparently healthy individuals with the following demographics:

- Gender: 65 females / 55 males
- Age: 22 to 82 years old, mean age 43.8 years

With a cut-off of 50 µg/g, 117/120 of the samples were normal/negative with the Calprotectin Immunoturbidimetric Assay. Values ranged from <3.9 µg/g to 130.3 µg/g, with 104/120 samples measuring below the lower end of the AMR. The 90% confidence intervals for the lower and upper 95% reference limits of the 120 healthy individuals were determined by the nonparametric quantile method, (N+1)p in accordance with CLSI EP28-A3c, *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, 3rd Edition* using EP Evaluator Version 12. The results are as follows:

Nonparemetric (CLSI C28-A)

Lower Limit (90% CI): 3.9 µg/g (3.9 – 3.9 µg/g)
Upper Limit (90% CI): 67.5 µg/g (20.0 – 130.3 µg/g)
Confidence Ratio: 0.87

Transformed Parametric

Lower Limit (90% CI): 1.6 µg/g (1.3 – 1.9 µg/g)
Upper Limit (90% CI): 20.8 µg/g (17.6 – 24.7 µg/g)
Confidence Ratio: 0.20

Performance Characteristics and Clinical Studies

Summary of Clinical Studies

The clinical performance of the Calprotectin Immunoturbidimetric Assay was evaluated by testing 349 prospectively collected stool specimens from adults suspected of having IBD. Pre-extracted samples from ALPCO were tested at two external sites.

Estimates of the clinical sensitivity, clinical specificity, positive predictive value, and negative predictive value was determined for the Calprotectin Immunoturbidimetric Assay comparing the analytical test results of the prospectively collected stool specimens against the clinical diagnosis of IBD, IBS, or "Other GI conditions" made by the clinical investigator/gastroenterologist. IBD diagnosis was based on endoscopy results and/or histology of biopsies taken during the endoscopy.

- IBS diagnosis was based on the Rome IV criteria and confirmed by negative endoscopy including the colon and terminal ileum.
- Subjects were diagnosed with "Other GI conditions" when they did not meet the diagnostic criteria for IBD and IBS (Rome IV).

Clinical Diagnosis	Number of Subjects
IBD	63
Ulcerative Colitis (UC)	29
Crohn's Disease (CD)	24
Indeterminant/Undefined	10
IBS	105
Other GI conditions	181
Total	349

Estimates of sensitivity, specificity, PPV, and NPV, along with 95% confidence intervals (Wilson score method for sensitivity/specificity and Mercaldo-Wald logit method for predictive value) were calculated for the Calprotectin Immunoturbidimetric Assay as an aid in the diagnosis of IBD (n = 349). The estimates of sensitivity, specificity, PPV, and NPV were calculated considering equivocal results as both positive and negative:

Equivocal results as positive	Calprotectin Immunoturbidimetric Assay Test Result		
Clinical Diagnosis	**> 50**	**≤ 50**	**Total**
IBD	57	6	63
Non-IBD	19	267	286
Total	76	273	349
	Fraction	%	95% CI
Sensitivity	57/63	90.5	80.7 – 95.6
Specificity	267/286	93.4	89.9 – 95.7
PPV	57/76	75.0	65.8 – 82.4
NPV	267/273	97.8	95.4 – 99.0

Equivocal results as negative	Calprotectin Immunoturbidimetric Assay Test Result		
Clinical Diagnosis	**> 100**	**≤ 100**	**Total**
IBD	48	15	63
Non-IBD	7	279	286
Total	55	294	349
	Fraction	%	95% CI

Sensitivity	48/63	76.2	64.4 – 85.0
Specificity	279/286	97.6	94.8 – 98.9
PPV	48/55	87.3	76.5 – 93.5
NPV	279/294	94.9	91.8 – 96.9

Method Comparison

A method comparison was conducted by comparing the Calprotectin Immunoturbidimetric Assay to the ALPCO Calprotectin Chemiluminescence ELISA as the predicate device. 124 stool specimen extracts were tested internally on both the ALPCO Calprotectin Chemiluminescence ELISA and on the Calprotectin Immunoturbidimetric Assay. 17 specimen results were excluded from the analysis due to calprotectin recoveries below the LOQ of the Calprotectin Immunoturbidimetric Assay.

Acceptance Criteria: Slope: 1.0 ± 0.1

Correlation (r2): ≥ 0.90

Results:

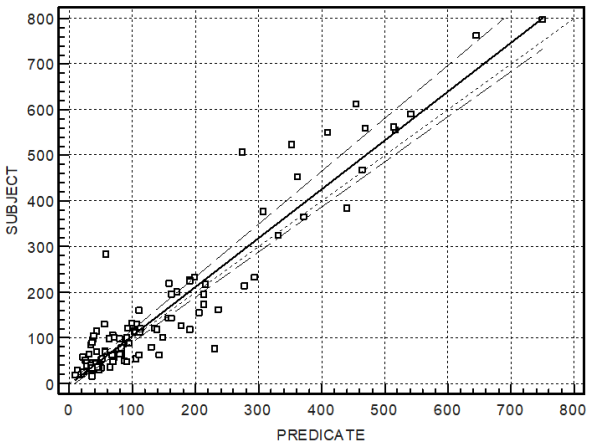
Sample ID	[CALP] µg/g by ALPCO ELISA	[CALP] µg/g by Immunoturbidimetric method
2D	43.9	69.5
4D	198.6	232.3
5D	191.3	227.6
16D	332.2	324.2
17D	205.5	153.9
18D	99.9	130.9
20	43.6	113.9
21	69.1	106.7
22	34.5	83.4
23	57.4	71.1
24	38.4	95.3
25	72.4	101.4
26	63.1	97.0
27	39.3	103.8
28	36.2	88.6
29	57.4	69.1
30	56.1	128.9
S2	15.2	19.3
S5	42.1	31.3
S6	92.7	121.8
S7	107.8	128.8
S8	171.2	199.2
S9	372.3	365.5

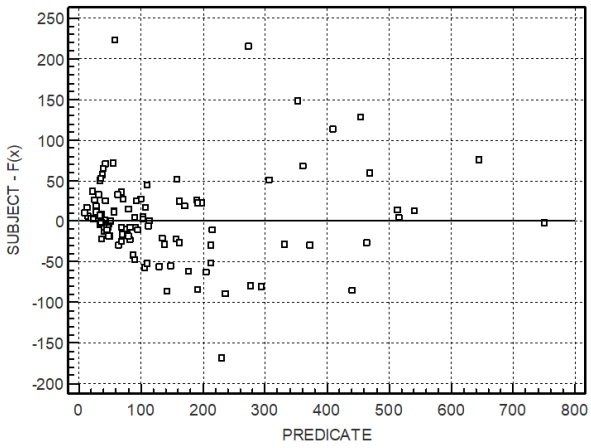
Sample ID	[CALP] µg/g by ALPCO	[CALP] µg/g by Immunoturbidimetric method
121	10.1	18.2
123	92.1	88.1
178	148.4	100.7
222	34.2	40.5
223	110.8	63.0
319	177.5	125.5
343	294.2	231.5
871	129.9	79.5
951	191.6	117.8
981	52.1	53.5
994	19.3	20.6
996	82.7	77.6
999	64.5	36.0
1012	541.7	589.2
1101	230.9	75.2
1102	236.9	161.3
1107	514.4	561.0
1114	51.1	33.3
1115	161.8	143.1
1157	110.4	158.9
1163	87.8	49.1
1173	142.5	62.9
1176	90.9	46.3

Sample ID	[CALP] µg/g by ALPCO ELISA	[CALP] µg/g by Immunoturbidimetric method
S10	106.3	53.5
S11	464.9	467.5
35	34.8	29.7
36	103.9	113.8
37	113.6	118.8
38	162.1	195.1
39	307.7	377.3
40	37.5	45.7
41	362.0	452.2
42	22.0	57.3
43	13.6	27.9
44	48.0	44.3
45	31.6	63.6
46	51.8	52.2
47	43.1	42.3
48	40.6	40.6
49	26.1	50.8
50	40.6	27.8
51	28.2	45.9
196c	80.8	98.0
218c	191.5	224.2
220c	104.5	111.3
223c	83.5	63.6
240c	517.0	554.9
253c	28.4	37.9
261c	410.1	548.9
322c	454.5	611.9
323c	750.3	797.4
324c	78.3	70.6
352c	47.6	39.8
366c	157.2	142.9
368	644.7	762.0
388c	41.6	43.4
25	40.5	32.5
109	37.5	34.9
113	135.1	120.6
109	37.5	34.9
113	135.1	120.6
114	91.0	98.7

Sample ID	[CALP] µg/g by ALPCO	[CALP] µg/g by Immunoturbidimetric method
1183	37.1	14.7
1186	112.4	111.7
1187	36.1	34.2
1192	23.7	25.3
1195	352.6	522.7
1196	213.0	195.7
1263	277.8	214.1
1289	70.7	56.2
1304	213.4	173.9
1396	274.1	505.1
1404	139.3	117.2
1407	215.4	216.3
1412	158.0	218.1
1413	469.1	558.1
966	440.3	383.1
1272	47.7	30.0
1273	69.8	46.3
1276	95.3	87.9
1277	58.2	282.2
1284	80.7	64.7
1285	45.5	34.7
1287	68.8	62.5
994*	13.7	6.2
158*	13.5	1.9
1032*	20.8	8.5
1035*	16.6	4.8
1104*	9.4	9.2
1402*	12.2	-1.5
1410*	14.4	4.0
1411*	10.3	0.1
19D*	29.0	2.6
20D*	0.0	10.4
S1*	15.3	3.1
S3*	14.1	2.8
S4*	26.5	10.5
31*	23.3	7.6
32*	22.6	10.7
33*	7.4	3.2
34*	21.3	10.6

* Excluded from analysis





Variable X : PREDICATE
Variable Y : SUBJECT

Sample size = 107

PREDICATE
Lowest value = 10.1000
Highest value = 750.3000
Arithmetic mean = 145.9822
Median = 90.9000
Standard deviation = 149.4568
Standard error of the mean = 14.4485

SUBJECT
Lowest value = 14.7000
Highest value = 797.4000
Arithmetic mean = 157.0682
Median = 97.0000
Standard deviation = 171.9584
Standard error of the mean = 16.6238

-- REGRESSION EQUATION ----------

Y = -2.8508 + 1.0702 X

Intercept A : -2.8508
95% CI : -8.5136 to 1.9838

Slope B : 1.0702
95% CI : 0.9888 to 1.1577

Cusum test for linearity

No significant deviation from linearity (P>0.10)

Variable Y : SUBJECT

Variable X : PREDICATE

Sample size = 107

Correlation coefficient r = 0.9492 P<0.0001

95% Confidence interval for r = 0.9263 to 0.9651

Conclusion: The analytical method comparison (Passing and Bablok) between the Immunoturbidimetric assay and the ELISA shows a slope of 1.0702 (95% CI: 0.9888 to 1.1577) and an intercept of -2.8508 (95% CI: -8.5136 to 1.9838). The correlation coefficient (r^2) was 0.90.

Performance characteristics

Precision

The precision of the Calprotectin Immunoturbidimetric Assay was evaluated on an AU680 analyzer. Seven samples were tested with 2 replicates with two run per day for 20 days for a total of 80 replicates per sample. Results are summarized below.

Sample	Mean (µg/g)	n	Within Run		Between Run		Between Day		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
1	14.4	80	1.7	12.1%	1.5	10.2%	1.4	9.9%	2.2	15.0%
2	37.6	80	2.2	5.9%	1.9	5.1%	1.5	3.9%	2.5	6.7%
3	81.3	80	2.1	2.6%	2.2	2.7%	2.3	2.8%	3.1	3.9%
4	173.8	80	3.3	1.9%	4.2	2.4%	6.5	3.7%	7.5	4.3%
5	414.5	80	4.2	1.0%	6.7	1.6%	9.2	2.2%	10.8	2.6%
6	622.5	80	5.5	0.9%	6.8	1.1%	13.8	2.2%	15.1	2.4%
7	854.8	80	9.0	1.1%	9.5	1.1%	12.5	1.5%	15.6	1.8%

Lot-to-Lot Reproducibility

The lot-to-lot reproducibility of the Calprotectin Immunoturbidimetric Assay was evaluated using three lots of reagents and one lot of calibrators. The testing was performed on one clinical analyzer. Seven stool specimen extracts covering the assay reportable range were tested with 5 replicates with one run per day for 5 days for a total of 75 replicates per sample.

Acceptance Criteria: Inter-assay (between run): ≤ 15% for samples ≥ 30µg/g

Intra-assay (within run): ≤ 15% for samples ≥ 30µg/g

Total: ≤ 15% for samples ≥ 30µg/g

Sample	Mean (µg/g)	n	Within Run		Between Day		Between Lot		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
1	14.3	75	1.8	12.3%	1.5	10.3%	1.6	11.3%	2.8	19.6%
2	38.4	75	1.9	4.9%	1.4	3.6%	1.8	4.6%	2.9	7.6%
3	86.0	75	2.6	3.1%	2.5	2.9%	4.0	4.6%	5.4	6.2%
4	173.4	75	3.6	2.1%	3.9	2.3%	5.0	2.9%	7.3	4.2%
5	416.5	75	5.0	1.2%	7.8	1.9%	10.1	2.4%	13.8	3.3%
6	626.3	75	7.1	1.1%	9.5	1.5%	11.4	1.8%	16.5	2.6%
7	869.8	75	7.5	0.9%	8.9	1.0%	11.3	1.3%	16.3	1.9%

Site-to-Site Reproducibility

The site-to-site reproducibility of the Calprotectin Immunoturbidimetric Assay was evaluated using one lot of reagents and one lot of calibrators. The testing was performed on three different AU680 clinical analyzers at three different sites. Seven stool specimen extracts covering the assay reportable range were tested with 5 replicates with one run per day for 5 days for a total of 75 replicates per sample.

Acceptance Criteria: Inter-assay (between run): ≤ 15% for samples ≥ 30µg/g

Intra-assay (within run): ≤ 15% for samples ≥ 30µg/g

Total: ≤ 15% for samples ≥ 30µg/g

Sample	Mean (µg/g)	n	Within Run		Between Day		Between Site		Total	
			SD	CV	SD	CV	SD	CV	SD	CV
1	11.2	75	1.8	15.7%	1.7	14.9%	1.8	16.2%	3.0	27.1
2	35.0	75	2.1	6.0%	1.7	4.8%	3.2	9.2%	4.2	11.9
3	77.3	75	3.2	4.1%	2.6	3.3%	3.3	4.3%	5.3	6.8%
4	168.5	75	2.9	1.7%	6.1	3.6%	6.7	4.0%	9.5	5.6%
5	445.7	75	4.7	1.0%	5.8	1.3%	9.6	2.2%	12.2	2.7%
6	643.8	75	6.4	1.0%	10.9	1.7%	8.9	1.4%	15.5	2.4%
7	782.2	75	14.3	1.8%	15.2	1.9%	21.2	2.7%	29.7	3.8%

Matrix and Aqueous Linearity

The matrix linearity and aqueous linearity of the analytical measuring range of the Calprotectin Immunoturbidimetric Assay were evaluated in accordance with CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. Eleven levels of the linearity set were prepared by diluting a sample containing > 1,000 µg/g of calprotectin. Linearity was tested on the AU680. Data analysis show that the Calprotectin Assay was linear throughout its analytical measured range (AMR) of 11 to 1,000 µg/g with a slope of 0.97, y-intercept of -7.9.

Analytical Sensitivity

The LOB, LOD, and LOQ of the Calprotectin Immunoturbidimetric Assay was determined according to CLSI EP17-A2 on the AU680 analyzer. The LOB, LOD, and LOQ were determined to be 2.2 µg/g, 3.9 µg/g, and 11.0 µg/g respectively.

Accuracy/Recovery

Seven extracted stool samples containing various concentrations of calprotectin across the analytical measuring range of the assay were spiked with native calprotectin. The stool sample extracts were mixed with the spiking material at a ratio of 9 parts sample to 1 part spiking material. Recovery was calculated compared to the baseline result. The spiked sample recoveries were within 10% of the expected values. The results of the recovery study is summarized below.

Specimen Extract	Mean Baseline Result (µg/g)	Spiked Value (µg/g)	Expected Post-Spike Result (µg/g)	Observed Post-Spike Result (µg/g)	Recovery
1	40.9	50.0	86.8	85.5	98.5%
2	55.6	50.0	100.0	103.9	103.9%
3	110.9	50.0	149.8	159.5	106.5%
4	230.8	105.0	312.7	313.9	100.4%
5	379.2	105.0	446.3	431.8	96.8%
6	452.3	105.0	512.0	494.8	96.6%
7	658.8	105.0	697.9	666.7	95.5%

Prozone/Hook Effect

Samples with fecal calprotectin concentrations up to 20,000 µg/g were tested on the Calprotectin Immunoturbidimetric Assay on the AU680 clinical analyzer. The prozone detection feature is utilized on the AU680. The Calprotectin Assay shows prozone/hook sample tolerance up to 20,000 µg/g of fecal calprotectin.

Interfering Substances

Interfering substances were spiked into various concentration of calprotectin in stool extracts at a 10% of the total specimen volume. Recovery of the test samples showed less than 10% difference

from expected values. The list of interfering substances at the concentrations listed below showed no interference in the Calprotectin Immunoturbidimetric Assay.

Pharmaceuticals		
Trade Name	***Active Components***	***Concentration in Stool***
Ferro-Gradumet	Iron (II) sulfate	0.11 mg/50 mg stool
Prednisone	Prednisone	0.31 mg/50 mg stool
Imurek	Azathioprine	0.19 mg/50 mg stool
Pentasa/Asacol	Mesalamine; 5-ASA	5.21 mg/50 mg stool
Prevacid	Lansoprazol	0.18 mg/50 mg stool
Vancocin	Vancomycin	2.0 mg/50 mg stool
Sulfamethoxazole	Sulfamethoxazole	1.6 mg/50 mg stool
Trimethoprim	Trimethoprim	0.35 mg/50 mg stool
Cipro	Ciprofloxacin	1.25 mg/50 mg stool
Nutritional Supplements		
Trade Name	***Active Components***	***Concentration in Stool***
Vitamin E	DL-α Tocopherol Acetate	0.3 mg/50 mg stool
Multiple Vitamin	A, B1, B2, B3, B5, B6, B8, B9, B12, C, D, E, and minerals	1.06 mg/50 mg stool
Biotin	B7	1750 ng/50 mg stool
Rheumatoid Factor (RF)		500 IU/50 mg stool
Human anti-mouse antibody (HAMA)		0.241 µg/50 mg stool
Hemoglobin		7.5 mg/50 mg stool
Microorganisms		
Escherichia coli		1.5×10^7 cfu/mL
Salmonella enterica subsp. enterica		1.5×10^7 cfu/mL
Klebsiella pneumoniae subsp. pneumonia		1.5×10^7 cfu/mL
Citrobacter freundii		1.5×10^7 cfu/mL
Shigella flexneri		1.5×10^7 cfu/mL
Yersinia enterocolitica subsp. enterocolitica		1.5×10^7 cfu/mL

Ancillary Devices

The pre-filled EasyStool Extraction Device (Product# 30-EZEX-100 for U.S. and Canada), the Easy 2 Stool Extraction Device (80-ESED2-100), and the Extraction Buffer, 4X (Product# 10-EXBUF-55, -225) for manual stool processing have been validated for use with the Calprotectin Immunoturbidimetric Assay. Please contact ALPCO regarding compatibility of additional extraction devices and buffers.

Symbols Glossary

REF	Product reference or catalog number
LOT	Batch or lot number
Rx Only	Prescription device
CE	European Conformity mark
IVD	In Vitro Diagnostic medical device
	Temperature limitation: store at 2 to 8°C
	Expiration or use by date
	Consult instructions for use
EC REP	European authorized representative

References:

1. Manz et al. (2012). Value of Fecal Calprotectin in the Evaluation of Patients with Abdominal Discomfort: An Observational Study. BMC Gastroenterol. PMID: 22233279.
2. Tibble et al. (2000). A simple method for assessing intestinal inflammation in Crohn's disease. Gut, 47(4), 506-513. PMID: 10986210.
3. Lobaton et al. (2012). A New Rapid Test for Calprotectin Predicts Mucosal Healing in Crohn's Disease and Ulcerative Colitis. Journal of Crohn's and Colitis.
4. Oyaerta, Matthijis et. al. (2017). Analytical performance and diagnostic accuracy of six different fecal calprotectin assays in inflammatory bowel disease. Clinical Chemistry and Laboratory Medicine, pp. 1564–1573.
5. Tibble JA et al. High prevalence of NSAID enteropathy as shown by a simple fecal test. Gut 1999;45:362-366.
6. Montalto M. et al: Faecal calprotectin concentrations in untreated coeliac patients. Scand J. Gastr., 2007; 42: 957-961.

Instructions for Use (IFU) Revision 3.1
Revision Date: 15Jan25