One Step Calprotectin Semi-Quantitative Rapid Test Device (Feces) Package Insert

Specimens: Feces

For professional *in vitro* diagnostic use only.

INTENDED USE

The Calprotectin Semi-Quantitative Rapid Test Device (Feces) is a rapid chromatographic immunoassay for the qualitative detection of Calprotectin in feces.

INTRODUCTION

Calprotectin, first described in 1980[1], is a protein found in the cytosol of neutrophils and macrophages composed of two subunits S100A8 and S100A9. It is released extracellularly in times of cell stress or damage and can be detected within feces and thus can be used as a sensitive marker of intestinal inflammation. It is stable in feces for up to seven days at room temperature and has a homogenous distribution in feces [2], properties which lend it to testing spot fecal samples.

The inflammatory bowel diseases (IBD) [3], Crohn's disease [4] and ulcerative colitis, are chronic relapsing, remitting disorders. Diagnosis along with assessment of disease activity and prognosis present challenges to managing clinicians. Fecal biomarkers, such as fecal calprotectin, are a non-invasive method which can be used to aid the decisions. Fecal protection has been shown to be useful in the diagnosis of IBD, correlates with bowel mucosal disease activity and can help to predict response to treatment or relapse. With growing evidence supporting its use, over the last decade this fecal biomarker has significantly changed the way IBD is managed [5].

PRINCIPLE

The Calprotectin Semi-Quantitative Rapid Test Cassette (feces) detects Calprotectin through visual interpretation of color development on the internal strip. Anti-Calprotectin antibodies are immobilized on the test region of the membrane. During testing, the specimen reacts with anti-Calprotectin antibodies conjugated to colored particles and precoated on the sample pad of the test. The mixture then migrates through the membrane by capillary action, and interacts with reagents on the membrane. If Test line 3 (T3) appears, it indicates that the Calprotectin level in the specimen is below 15ug/g. If the test line 2 and 3 (T2 and T3) appear, it indicates that the Calprotectin level in the specimen is between 15-60ug/g. If all the test lines (T1, T2, T3) appear, it indicates that the Calprotectin level is above 60ug/g. The appearance of control line serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

KIT COMPONENTS

Individually packed Test Devices	Each test contains a strip with colored conjugates and reactive reagents pre-spreaded at the corresponding regions
Tubes with buffer	Phosphate buffered saline and preservative, extract the samples
Package insert	For operation instruction

MATERIALS REQUIRED BUT NOT PROVIDED

Specimen collection container	For specimens' collection use
Timer	For timing use
Centrifuge	For preparation of clear specimens

PRECAUTIONS

•For in-vitro diagnostic use only
•For professional use only
•Use the Test Device only once
•Do not eat, drink or smoke in the area where the specimens or kits are handled.
•Do not use test if pouch is damaged.
•Do not use test kit after expiration date
•Do not mix Sample Collection Tubes from different lots.
•Do not open the Test Cassette foil pouch until you are ready to perform the test.
•Do not spill solution into the reaction zone
•Do not touch the reaction zone of the device to avoid contamination
•Avoid cross-contamination of samples by using a new specimen collection container and specimen collection tube for each sample.
•All patient samples should be treated as if capable of transmitting disease. Observe established precautions against microbiological hazards throughout testing and follow standard procedures for proper disposal of specimens.
•Do not use more than the required amount of liquid.
•Bring all reagents to room temperature (15-30°C) before use.
•Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
•Evaluate the test result after 10 minutes and not beyond 20 minutes.
•Store and transport the Test Device always at 2-30°C (36°-86°F)
•Humidity and high temperature can adversely affect results.

STORAGE AND STABILITY

- The kit should be stored at 2-30°C until the expiry date printed on the sealed pouch.
- The test must remain in the sealed pouch until use.
- **Do not freeze.**
- Cares should be taken to protect components in this kit from contamination. Do not use if there is evidence of microbial contamination or precipitation. Biological contamination of dispensing equipments, containers or reagents can lead to false results.

SPECIMEN COLLECTION AND STORAGE

- The feces specimen must be collected in clean, dry, waterproof container containing no detergents, preservatives or transport media.
- Unscrew the cap of the sample collection device and stick the attached sample collection stick in one go at three different sites into the feces. Only the amount of stool that sticks to the grooves of the sample collection tube
- If the Calprotectin Semi-Quantitative Rapid Test Cassette (feces) is not run within one day of sample collection, the sample collection tube should be stored at 2~8℃, but not longer than 7 days.

PROCEDURE

Bring tests, specimens, buffer and/or controls to room temperature (15-30°C) before use.

1. To collect fecal specimens:
Collect sufficient quantity of feces (1-2 mL or 1-2 g) in a clean, dry specimen collection container to obtain enough virus particles. Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2-8°C if not tested within 6 hours. For long term storage, specimens should be kept below -20°C.
2. To process fecal specimens:
Unscrew the cap of the specimen collection tube， then randomly stab the specimen collection applicator into the fecal specimen in at least 3 different sites to collect approximately 50 mg of feces (equivalent to 1/4 of a pea). Do not scoop the fecal specimen.
3. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
4. Place the cassette on a clean and level surface.
Hold the specimen collection tube upright and unscrew the tip of the specimen collection tube. Invert the specimen collection tube and transfer 2 full drops of the extracted specimen (approximately 80μL) to the specimen well (S) of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.
5. Wait for the colored line(s) to appear. The test result should be read at 10 minutes. Do not interpret the result after 15 minutes.

INTERPRETATION OF RESULTS

POSITIVE RESULT:

Possible Interpretation of Calprotectin Levels

A Control band (C) and a test band (T3) appears indicates a Calprotectin level is no more than 15ug/g, means normal, little risk of intestinal inflammation

A Control band (C) and two test bands (T3 and T2) appear indicates a Calprotectin level is between 15~60ug/g, means Normal, low risk of IBD

A Control band (C) and three test bands (T1, T2 and T3) appears indicates a Calprotectin level is higher than 60ug/g, means High risk of intestinal inflammation

NEGATIVE RESULT:

Only a Control band (C) appears and no colored band appears in the test region (T) indicates a Calprotectin level is lower than15ug/g.

INVALID RESULT:

No Control band appears. Results from any test which has not produced Control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The intensity of the color in test region (T) may vary depending on the concentration of aimed substances present in the specimen. Therefore, any shade of color in the test region should be considered positive. Besides, the substances level can not be determined by this qualitative test.
2. Insufficient specimen volume, incorrect operation procedure, or performing expired tests are the most likely reasons for control band failure.

QUALITY CONTROL

- Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.
- External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS OF THE TEST

1. The Calprotectin Rapid Test Device (feces) is for professional in vitro diagnostic use. The test should be used for the detection of calprotectin in human feces specimens only.
2. The Calprotectin Rapid Test Device (feces) will only indicate the semi-quantitative level of calprotectin in the specimen and should not be used as the sole criteria for evaluating inflammatory conditions.
3. Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended.

PERFORMANCE CHARACTERISTICS

The One Step Calprotectin Semi-Quantitative Rapid Test Device (Feces) has been evaluated with a leading commercial Calprotectin EIA test using clinical specimens. The results show that relative to leading EIA tests, the One Step Calprotectin Semi-Quantitative Rapid Test Device (Feces) exhibits 100% sensitivity and 97.7% specificity. Results are shown in Table below:

Calprotectin Semi-Quantitative Rapid Test Device vs. EIA

		ELISA			Total
Calprotectin Semi-Quantitative Rapid Test	Result	<15ug/g	15~60ug/g	>60ug/g	
<15ug/g	<15ug/g	84	0	0	84
15~60ug/g	15~60ug/g	2	35	0	37
>60ug/g	>60ug/g	0	0	22	22
Total		86	35	22	143
% Relative Accuracy		94.6%	100%	100%	96.0%
			100%		

Relative sensitivity: 57/57=100% (CI*: 99.73%~100%)
Relative specificity: 84/86 =97.7 % (CI*:94.5%~99.9%)
Relative Accuracy: 141/143 =98.6 % (CI*: 96.7%~99.9%)
*95% Confidence Interval

ANALYTICAL SPECIFICITY AND CROSS-REACTIVITY

The Calprotectin Semi-Quantitative Rapid Test Device (Feces) was evaluated with a total of 10 bacterial and viral isolates. Bacterial isolates were evaluated at a concentration between 10^6 and 10^8 org/mL. Viral isolates were evaluated at a concentration of at least 10^5 $TCID_{50}$/mL

Staphylococcus aureus	*Proteus mirabilis*
Pseudomonas aeruginosa	*Acinetobacter spp*
Enterococcus faecalis	*Salmonella choleraesius*

Rotavirus	*E.coli*
Enterococcus faecium	*Adenovius*

INTERFERING SUBSTANCES

The following compounds have also been tested using the One Step Calprotectin Semi-Quantitative Test Device (Feces) and no interference was observed.

Sample	Concentration	Sample	Concentration
Ascoribic acid	20mg/dL	Aspirin	20mg/dL
Oxalic acid	60mg/dL	Urea	2000mg/mL
Bilirubin	100mg/dL	Glucose	2000mg/dL
Uric acid	60mg/dL	Caffeine	40mg/dL
Albumin	2000mg/dL		

LITERATURE REFERENCES

1. Fagerhol MK, Dale I, Andersson T. A radioimmunoassay for a granulocyte protein as a marker in studies on theturnover of such cells. Bull Eur Physiopathol Respir 1980; 16 Suppl: 273-282.
2. Røseth AG, Fagerhol MK, Aadland E, Schjønsby H. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand J Gastroenterol 1992; 27:793-798.
3. Kono H, Rock KL. How dying cells alert the immune system to danger. Nat Rev Immunol 2008; 8: 279-289.
4. Sipponen T, Björkesten CG, Färkkilä M, Nuutinen H, Savilahti E, Kolho KL. Faecal calprotectin and lactoferrin arereliable surrogate markers of endoscopic response during Crohn's disease treatment. Scand J Gastroenterol 2010; 45:325-331.
5. Ricanek P, Brackmann S, Perminow G, Lyckander LG, Sponheim J, Holme O, Høie O, Rydning A, Vatn MH. Evaluation of disease activity in IBD at the time of diagnosis by the use of clinical, biochemical, and fecal markers. ScandJ Gastroenterol 2011; 46: 1081-1091.

EXPLANATION OFSYMBOLS

	Consult instructions for use		Keep dry
	Temperature limit		Batch code
	Do not re-use		In vitro diagnostics medical device
	Manufacturer		Date of Manufacture
	Use-by date		contains sufficient for <n> tests
			Keep away from sunlight

 Sirin Diagnostics

20/213rd Industrial Sector – 2nd Cell -

ElMatahra Industrial City – Minia – Egypt