

CALPRO

CALPROSMART®

CalproSmart® Self-Test Kit for measurement of calprotectin in faecal samples of IBD Patients



1. INTENDED USE

The **CalproSmart Self-Test** is a method for the determination of Calprotectin levels in human stool samples in combination with the dedicated CalproSmart smartphone application. The test is intended as an aid in monitoring the disease level of patients with inflammatory bowel diseases (IBD).

The test is for *in vitro* use.

2. BACKGROUND

Various types of organic diseases in the gastrointestinal tract may cause damage to the intestinal epithelial lining (*mucosa layer*). Such damage may vary from increased permeability of the mucosa to inflammation and ulcerations. The bowel content is rich in bacteria and other microorganisms releasing substances which may be toxic or chemotactic, i.e. they stimulate leukocytes, in particular polymorphonuclear neutrophilic granulocytes (PMN), to migrate into the gut lumen where they release their contents including antimicrobial substances like Calprotectin. This protein constitutes about 60% of total proteins in the cytoplasm of PMNs²⁾ and can be reliably estimated in faecal samples stored for up to seven days at ambient temperature³⁾.

Calprotectin is a 36 kilodalton calcium and zinc-binding protein⁴⁾, produced by PMNs, monocytes and squamous epithelial cells (*except those in normal skin*)^{5,6)}. After binding of calcium, it can resist degradation by leukocytic and microbial enzymes^{3,7)}. By competing with different enzymes for limited, local amounts of zinc, Calprotectin can inhibit many zinc-dependent enzymes⁸⁾ and thereby kill microorganisms or animal and human cells in culture^{9,10)}. Different types of disease, for instance bacterial infections, rheumatoid arthritis and cancer, lead to activation of PMNs and increased levels of Calprotectin in plasma, cerebrospinal fluid, synovial fluid, crevicular fluid, urine or other human materials¹⁾.

It is of special importance that the concentration of Calprotectin in faeces is correlated with the number of PMNs migrating into the gut lumen¹¹⁾, and that it can be detected reliably even in small (*less than one gram*) random stool samples^{3,12)}. Furthermore, organic diseases of the bowel give a strong Calprotectin signal, i.e. elevations are regularly five to several thousand times the upper reference in healthy individuals^{3,13,14,15)}, indicating intestinal inflammation.

Mucosal healing is the optimal goal for IBD treatment, and a test for faecal Calprotectin can tell when this has been achieved. Many IBD patients in clinical remission with normal C-reactive protein (CRP) levels still have on-going inflammation¹⁶⁾, reflected by increased faecal Calprotectin. Such patients have increased risk of relapse within a few months¹⁷⁾. If mucosal healing can be achieved, the risk of relapse and need for expensive medical treatment and/or major abdominal surgery will be reduced^{18,19)}. Normalisation of Calprotectin levels means that mucosal healing has been achieved²⁰⁾. The risk and severity of side effects to treatment should be balanced against the risk of continued inflammation, severe clinical relapse and complications.

The importance of achieving mucosal healing has been focused in many scientific reviews ²¹⁻²⁹⁾ and articles ³⁰⁻³⁵⁾.

Inflammatory bowel diseases i.e. ulcerative colitis and Crohn's disease, may appear from early childhood to late adulthood and the diagnosis is often delayed due to vague symptoms or reluctance to perform endoscopy and biopsy. The CalproSmart Self-Test makes IBD patients able to monitor their disease level at their own homes, ensuring an effective medical treatment regime and for an early warning of relapse.

3. PRINCIPLE OF THE TEST

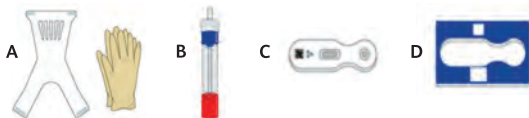
The **CalproSmart Self-Test** is based upon preparation of an extract of faeces using our patented Faecal Extraction Buffer. The level of Calprotectin is determined by testing the extract in a lateral flow immunoassay specific for Calprotectin.

The sample is added to the sample well of the test cassette and is allowed to react with gold-conjugated antibodies which bind the Calprotectin. Calprotectin and conjugated antibody complexes travel together along the membrane and bind to Calprotectin-specific antibodies immobilised on the Test line. This immobilisation causes the Test line to form. Gold-conjugated antibody without any bound antigen is immobilised on the Control line. After ended incubation time the concentration of calprotectin in the sample is calculated by means of the CalproSmart smart-phone application. The colour intensity is proportional with the concentration of Calprotectin in the sample. The test is calibrated using faecal extracts with known concentrations, determined in the CalproLab™ ALP Calprotectin ELISA (*Calpro AS, prod. No. CALP0170*). The reportable range of the test is 77-1500 mg of calprotectin/kg of faeces, with the linear range up to 1100 mg/kg. Between 1100 and 1500 mg/kg the results can be inaccurate.

4. MATERIALS

4.1. Reagents and components supplied with the kit

- A EasySampler** Disposable paper samplers for collection of stool samples and nitrile gloves.
- B Extraction device** Faecal extraction device, prefilled with 5 ml Calpro faecal extraction buffer. The device is designed to collect 10 mg of faecal sample and give a 1:500 extract.
- C Rapid test cassettes** Tests individually sealed in aluminium foil with desiccant bag. The cassettes are disposable
- D Support frame** Rectangular plastic frame with an opening for placement of the rapid test cassette, labelled with a design that allows the smartphone app to identify the position of the control and test line and read the test result.



4.2. Materials and equipment required but not supplied

Paper tissue - sheet or towel

5. STABILITY AND STORAGE

When stored unopened at 2 – 8°C, kit reagents are stable up to the expiry date stated on the label.

Avoid exposure to high temperature and direct sunlight.

6. REGISTRATION AND DOWNLOAD OF THE APPLICATION

CalproSmart is available as standalone solution and integrated into other host apps (3rd party apps). If you are using the CalproSmart app as standalone, please refer to section 6.1. If you are using CalproSmart together with a 3rd party/host app, please refer to section 6.2.

6.1. CalproSmart standalone application

Upon registration of the patient at the hospital, the clinic user (treating clinician or gastro nurse) enters patient's name, date of birth and e-mail address into the system. Once completed, the patients will receive following e-mail:

.....

Hi (*Patients name*),

An new account has been created for you at calprosmart.com.

You now need to set your password by visiting the following web-page:

http://calprosmart.com/password_reset/.../

This page will expire after 24 hours, you can however request another password reset at any time.

Thanks,

The Calpro team.

.....

Once completed setting the password, the patient can download the CalproSmart app. Download the app from App Store or Google Play by searching for “CalproSmart”

or by scanning the QR code to the right and selecting the appropriate operating system. Ensure that the logo is identical to the one shown to download correct version of the app.



When the patient logs on using their credentials for the first time, they will have to accept the EULA (End User Licence Agreement) in order to use the app.

6.2. CalproSmart integrated into a 3rd party/ host application

For patients using a host app, all registration and password setup is done in the host application. Please refer to the host application Instruction for Use (IFU) for information on download, setup, and registration of the host app. To measure Calprotectin through the integration with a host app, you will need to download the CalproSmart DeepLink app. You can find it by searching for “CalproSmart DeepLink” on App Store or Google Play or by scanning the QR code to the right and selecting your mobile phone operating system. Ensure that the logo is identical to the one shown to download correct version of the app.



Keep in mind that you cannot access the app directly, but you will have to go through the host app, and both apps must be installed on the phone.



7. PREPARATION

Unpack and prepare 1 sampler, 1 pair of nitrile gloves, 1 prefilled extraction device, 1 rapid test and 1 support frame.

7.1. EasySampler

Unpack one sampler and one pair of nitrile gloves from the plastic bag. Unfold the sampler, and inspect visually for any damage.

7.2. Test cassette

Remove the ready-to-use rapid test cassette from the aluminium pouch just prior to analysis. Inspect visually for any damage to the cassette or membrane in the test window.

8. PERFORMING THE CALPROSMART SELF-TEST

The test procedure is explained in the enclosed instructions for use (IFU) and in the CalproSmart application, where every step of the procedure is explained both through images and text. The app will also contain a link to an instructional video.

Log in to the CalproSmart app on your device, before preparing the test. Internet connection is required during login to the app.

- ❗ **Important:** If you are using the CalproSmart standalone solution, you will log in by clicking on the CalproSmart icon on your mobile phone and log in with your credentials.
- ❗ **Important:** If you are using CalproSmart through a 3rd party/host app, you will log in through the host app by clicking the button to measure your Calprotectin levels. Please refer to host app IFU for more details.
- ❗ **Important:** Let the app guide you! For each instruction in the app, press next, perform the steps as shown in the app to make sure that all steps are performed in timely manner.
- ❗ **Important:** Remember to use the timers (3 minutes shaking time for the extraction device and 15 minutes incubation time for the test cassette) inside the app. The timers start automatically when pressing next at the step before the timer function.

8.1. Collection of faecal sample



- 1 Put up the toilet seat before EasySampler is mounted and dry off the toilet bowl so the EasySampler can be mounted on a clean and dry surface.
- 2 Unfold the EasySampler. Remove the two tape protections from the EasySampler and mount them on the back of the bowl.
- 3 Remove the remaining tape protection and mount the front part of the EasySampler at each side in mid position of the bowl, leaving space to dispose toilet paper in front of EasySampler. It is important that the EasySampler is mounted so it creates a sample area.

- ❗ **Important:** The EasySampler must **NOT** come in contact with toilet water or urine.
- 4 Finally put down the toilet seat. EasySampler is now ready for use. Have a bowel moment.



Prepare

2

Mix

3

Incubate

8.2. Extraction using the extraction device



- 1 Put on the nitrile gloves. Turn the white cap counterclockwise and pull the cap and stick straight up. Place the tube with the blue adapter steady, e.g. in a cup or in a rack. Insert the end of the rod into the stool sample so that both grooves in the stick are completely filled. If possible, insert the rod repeatedly in different places in the stool. Avoid filling the grooves with air bubbles. Also avoid grains, fiber, etc.
- 2 Hold the tube with the blue cap (*adapter*) and press the stick with the stool through the hole in the blue adaptor. Excess stool is wiped off in the funnel insert. Turn the white cap clockwise onto the blue adapter until you feel a click and it stops. Ensure that the tube is completely closed, and shake it vigorously for 3 minutes



Prepare



Mix

3

Incubate

8.3. Applying the sample on the test cassette

Preparation: Please read the test protocol carefully *before* performing the assay.



- 1 Place the rapid test in the support frame.
- 2 Remove the red cap and throw away the first drop. Wipe the tip of the device with paper to ensure the tip is dry.

- 3 Hold the device vertically above the test cassette and apply 2 drops (*equivalent to 80 μ l*) onto the rapid test. Incubate in room temperature for 15 minutes.

Important: Normally you will see the applied liquid move in the cartridge window. If this is not observed within 30 seconds, add one additional drop of extract to obtain normal flow.

8.4. Performing the measurements



- 1 Hold the Smartphone horizontally over the support frame with the embedded rapid test.
- 2 The picture is taken automatically when the barcode, Test line and Control line is identified by Smartphone application.
- 3 Once the test has been measured, you will see the results:
 - If you are using the CalproSmart standalone app, the final image as can be seen above to the right will appear, showing you the results as both traffic light and quantitative value. The results will automatically be sent to the CalproSmart web portal, where the treating physician/team can view the results.
 - If you are using a host app with CalproSmart integrated, the right screen will appear for 15 seconds, and after this you will be redirected to the host app where you can continue to see the results. For more information on how the results will appear in a host app, please refer to the host app IFU.
- 4 The result is given as a traffic light, which reflects the patient's disease state (*green (0-200 mg / kg) = mild disease state, yellow (200-500 mg / kg) = moderate disease state, red (over 500 mg / kg) = severe disease state*).

8.5 Disposal



- 1 After completing the test, remove the gloves. Put the used extraction device, rapid test cassette and gloves into the empty plastic bag of the sampler and dispose. Wash your hands to prevent contamination from sample.

Important: Do NOT discard the support frame! This should be used for all tests in CalproSmart kit. In the event of discarding or losing the frame, an additional support frame is included in the kit as a spare.

- 2 Remove EasySampler from the Bowl and flush it out in the toilet.

Important: When using water-saving toilets use full flush. Repeat it until the amount of water are 10-15 litre. Tape leftovers on the bowl can easily be removed with denatured spirit.

9. INTERPRETATION OF RESULTS

Degree of disease activity	Traffic light colour	Calprotectin concentration
Mild disease activity	Green 	< 200 mg/kg
Moderate disease activity	Yellow 	200-500mg/kg
Severe disease activity	Red 	> 500 mg/kg

Note: Diagnosis should not be established based on a single test result. Although the diagnosis should mainly be based on clinical history and symptoms, the CalproSmart test results could be a valuable aid in deciding further examination like endoscopy.



10. QUALITY CONTROL

- The Control and Test lines should be clear and well defined. If the lines are missing, or the quality of the lines is not acceptable, an error message will appear and a new test should be performed.
- If the Calprotectin level is below the detection limit, a test line will not be seen, and the results will be reported as <77 mg/kg (green zone).

- If the bar code is damaged, it will not be detected by the software and a message stating “looking for barcode” will appear at the top of the screen. If barcode is not found within 2 minutes, the test will terminate without results
- If the test cassette has expired, the error message will appear when scanning the test cassette without any results being registered.

11. LIMITATIONS OF THE PROCEDURE

Procedure: It is important to follow the instructions carefully to get an accurate result.

Both grooves in the rod of the extraction device should be completely filled. If possible, insert the rod repeatedly in different places in the stool. Avoid filling the grooves with air bubbles. Also avoid grains, fiber, etc. For very liquid stools, insert the rod deep into the sample to ensure the grooves are completely filled. It is not a problem if the grooves are overfilled. The extra material will be scraped off as you insert the rod back in the extraction device.

Extraction device should be held vertically over the application well, when the 2 drops of extract are put onto the rapid test. Tilting the extraction device may cause wrong volume of the drops.

⚠ Important: Wrongly preformed procedure, wrong amount of collected faecal sample or wrong volume of extracts put onto the rapid test cassette, could all cause false positive or negative results.

The user of CalproSmart Self-Test MUST consult a physician before changing medication on the basis of the results, unless previously agreed upon with treating physician.

12. CONTRAINDICATIONS

- False negative results could occur in patients who have granulocytopenia due to bone marrow suppression
- Patients treated with azathioprine can also have granulocytopenia resulting in false negatives.
- Some patients taking non-steroidal anti-inflammatory drugs (*NSAID*) will have elevations in their fecal calprotectin levels
- Results may not be clinically applicable to children less than 2 years of age, as they often have increased fecal calprotectin levels
- Other intestinal diseases, including many gastrointestinal infections and colorectal cancer can result in elevated levels of calprotectin.
- Fecal calprotectin is a non-specific indicator of inflammation in the gut. Elevated levels do not necessarily mean that the patient has active IBD. The full clinical picture always needs to be evaluated.

13. PRECAUTIONS AND WARNINGS

In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore, the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analysers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.

- ***Only for in vitro diagnostic use.***

- Read the Instructions for Use carefully before performing the test.
- Avoid splashing or skin contact with the liquid, as this can irritate the skin and eyes. If the liquid comes into contact with the eyes, rinse well with clean water. Should NOT be consumed. KEEP OUT OF THE REACH OF CHILDREN!
- Ensure that the extraction device is held vertically over the application well, when the 2 drops of extract are put onto the rapid test. Tilting the extraction device may cause wrong volume of the drops.
- Do NOT read the same test cassette several times. If the time limit for measuring the test is exceeded, the test must be performed from the start with a new extraction device and a new rapid test cassette.
- Ensure that both grooves in the stick of the extraction device always are completely filled. For very liquid samples, insert the rod deep into the sample to ensure the grooves are completely filled.
- Avoid carrying out the test in a dark room.
- Do not interchange reagents of different production lots.
- Do not use reagents from other manufacturers with reagents of this test kit.
- Do not use reagents after expiry date stated on the label
- The extraction buffer contains sodium azide at less than 0.1% (w/v).

14. DISPOSAL CONSIDERATIONS

Fecal extracts are potential contagious and should be treated as hazardous waste. Be sure that the extraction device is completely closed after use, to prevent leakage/spillage to the surroundings. All used component (*except from the EasySampler*) should be placed in the empty plastic bag of the sampler, before closed securely and disposed.

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16. ORDER INFORMATION

CalproSmart Self-Test are available in different kit sizes. For a complete list of all product variants with product code, please visit our web page: www.calpro.no

Symbols Key/Symbolschlüssel/Explication des symboles/ Legenda/Símbolos	
	<i>In Vitro</i> Diagnostic Medical Device/ <i>In Vitro</i> Diagnosticum/ Dispositif médical de diagnostic <i>in vitro</i> / Diganostico in vitro/ Producto para diagnóstico <i>In vitro</i>
	Lot Number/Chargenbezeichnung/Numéro de lot/ Lotto/ Número de lote
	Expiration Date/Verfallsdatum/Date de péremption/ Scadenza/ Fecha de caducidad
	Storage Temperature/Lagertemperatur/ Température de conservation/ Temperatura di conservazione/Temperatura de almacenamiento
	CE Mark/ CE-Zeichen/ Marquage CE/Marchio CE/ MarcaCE
	Catalogue Number/ Katalog Nummer/Référence du catalogue/Numero di codice/ Número de Catálogo
	Contains sufficient for "n" tests/ Ausreichend für "n" Tests/ Contenu suffisant pour "n" tests/ Contenuto sufficiente per "n" saggi/ Contenido suficiente para "n" tests
	Do not reuse/nicht wiederverwenden/ ne pas réutiliser/ non riutilizzare/ no reutilizar
	Consult instructions for use/Gebrauchsanweisung konsultieren/ consulter les instructions d'utilisation/consultare le istruzioni per l'uso/ consultar instrucciones de uso
	This side up/Diese Seite oben/ce côté vers le haut/questo lato in su/ Este lado hacia arriba



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