Calprotectin

Universal kit

Order references

Reagents

CALPROGOLD™

REF		CONT
CACOL-B00	*Universal kit*	1 x 18 ml R1 + 1 x 7.5 ml R2
CACOL-H00	*Universal kit*	3 x 18 ml R1 + 3 x 7.5 ml R2
CACOL-L00	*Universal kit*	7 x 18 ml R1 + 7 x 7.5 ml R2

Other necessary products

REF		CONT
CAREK-000	*Calprotectin Calibrators Kit (6 Levels)*	6 x 1 ml
CACOS-002	*Calprotectin Low Control*	1 x 2 ml
CACON-002	*Calprotectin Medium Control*	1 x 2 ml
CACOX-002	*Calprotectin High Control*	1 x 2 ml
SDBUF-B00	*Sample Dilution Buffer*	2 x 70 ml
SDBUF-H00	*Sample Dilution Buffer*	8 x 70 ml
SDBUF-L00	*Sample Dilution Buffer*	16 x 70 ml
CAL0510	*Calpro Easy Extract™*	50 devices

Intended use

CALPROGOLD in vitro diagnostic reagent is used for measuring in human stool, concentration of faecal Calprotectin, a neutrophilic protein that is a marker of mucosal inflammation. CALPROGOLD can be used as an in vitro diagnostic aid in the diagnosis of inflammatory bowel disease (IBD): Chron's disease and ulcerative colitis, to differentiate between IBD from irritable bowel syndrome (IBS), to determine disease activity and monitor response to treatment in patients with IBD.

Medical benefit - Scientific validity

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 PMNs [2)] and can be reliably estimated in faecal samples stored for up to seven days at ambient temperature [3)].
Calprotectin is a 36 kilodalton calcium and zinc-binding protein [4)], 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 enzymes [8)] 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 materials [1)].
It is of special importance that the concentration of Calprotectin in faeces is correlated with the number of PMNs migrating into the gut lumen [11)], 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.
Inflammatory bowel diseases (IBD), 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 CALPROGOLD calprotectin measurement can contribute to an earlier diagnosis of IBD since the test is usually positive in active IBD.
Functional disorders like irritable bowel syndrome (IBS) do not give increased faecal Calprotectin concentrations, but organic abdominal disorders like IBD do. Patients with organic and functional abdominal disorders may have similar symptoms, and clinical examination alone may not be sufficient to give a specific diagnosis. Further diagnostic procedures are complex,

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expensive and may expose the patient to pain and other risks. A test for faecal Calprotectin is a simple, non-invasive, inexpensive and objective method that can help selecting patients for additional examination like endoscopy. Abdominal symptoms are very common both in children and adults and a negative result as measured by the CALPROGOLD calprotectin kit can with high probability rule out inflammatory bowel disorders [13].

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 inflammation [16], reflected by increased faecal Calprotectin. Such patients have increased risk of relapse within a few months [17]. If mucosal healing can be achieved, the risk of relapse and need for major abdominal surgery will be reduced [18,19]. Normalisation of Calprotectin levels means that mucosal healing has been achieved [20]. 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 the focus of many scientific reviews [21-29] and articles [30-35].

Method principle – Instruction for use

The Calprotectin assay is performed using the reagent kit listed in the “Order references” section and the associated calibration kit on the biochemistry analysers. The principle of this immunocolorimetric (PECIA) test includes the following reaction steps: the zero point of the calibration curve is determined with level 1 of the calibration kit. The sample volume is added to the buffer volume R1 in a reaction cell. A 5 minute incubation at 37°C between buffer and sample is necessary to eliminate any non-specific reactions. After the incubation, antiserum R2 is added to buffer R1/sample and a first measurement of the optical density (OD1) is performed at 600 nm (primary wavelength) / 546 nm (secondary wavelength). Subsequently, a 5-minute incubation at 37°C is performed and a second optical density measurement is consecutively performed at the same wavelength (OD2).

Calprotectin present in the test sample reacts specifically with an anti-human calprotectin antibody coated on gold nanoparticles and the colour change induced by the formation of the antigen-antibody immune complex is measured in proportion to the concentration of Calprotectin in the sample.

The calibration curve performed in the non-linear mode must be validated using associated controls (see references in the section " Order references ").

To avoid possible cross-contamination, washes can be programmed into the analyser. Please refer to the detailed application provided by Diagam and the analyser's user manual.

For more information, please refer to the application provided by Diagam. The performance of applications not validated by Diagam is not guaranteed.

Test type	Dilution of the sample*	Reaction diluted sample volume	Buffer Reagent 1 volume	Incubation time	Gold reagent 2 volume	Reading OD 1 (Primary / Secondary)	Incubation time	Reading OD 2 (Primary / Secondary)
ENDPOINT	10x*	20 µl	180 µl	5 minutes	75 µl	600 nm / 546 nm	5 minutes	600 nm / 546 nm

** 10x dilution of controls and samples. Calibrators should not be diluted.*

Note:

- Prior to loading the analyzer, centrifugation is not required for fecal extract made with the Easy Extract™ device but this step can be performed without impacting the determination of Calprotectin concentration.
- Depending on the analyzer, Easy Extract™ tubes can be placed directly on the sample rack. If this is not the case, it is then necessary to transfer their contents into sample cups adapted to the system used.

Warning and precautions

- For in vitro diagnostic use only.
- Must be handled by authorised personnel under the responsibility of a biologist.
- Products of human origin have tested negative for HIV 1 and 2 antibodies, HCV antibodies and HBsAg but should be handled and disposed of as potentially infectious products.
- Environmental hazards: Follow all applicable local regulations for safe disposal.
- These products contain sodium azide. Products containing sodium azide should be handled with care: avoid ingestion and contact with skin or mucous membranes.

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- Sodium azide becomes explosive when in contact with heavy metals such as copper or lead.
- Material Safety Data Sheets are available to professionals on request.

Samples

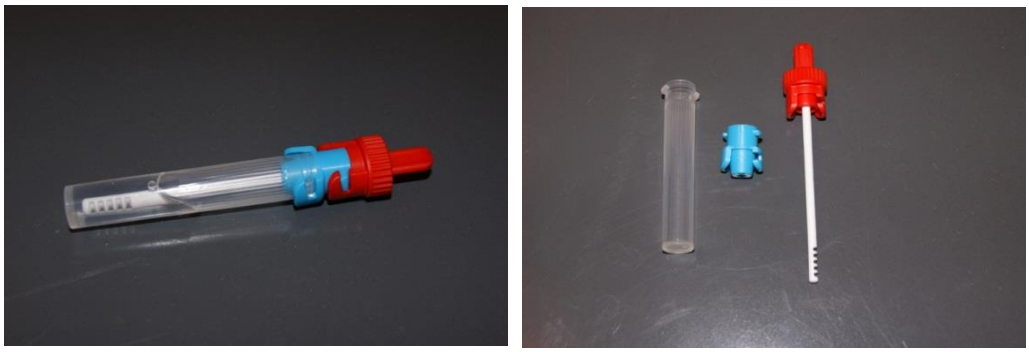
Collection conditions

Collect specimens using standard laboratory techniques; use only suitable procedures, tubes, or collection containers.

Since Calprotectin is very stable in stools, patients can collect small faecal samples at home.
Collect 1 – 5 g (approximately one teaspoonful), place it in a suitable clean container and deliver it to the laboratory as soon as possible but within four days. When put in a container approved for transport, it can be sent by ordinary mail, i.e. no refrigeration is needed. Exposure to temperatures above 25°C should be avoided.
Samples can also be stored frozen, at -20°C or lower, until delivery or mailing. Frozen samples must be thawed and equilibrated to room temperature before extraction and testing. Note that freezing faecal samples can in some cases result in increased Calprotectin levels, most likely due to release from granulocytes.
Note: Before commencing extraction, the stool sample should be homogenised well using for example a spatula, before the small amount for extraction is taken out.
For extraction we recommend the use of Calpro EasyExtract™ according to package insert. Other methods and devices, validated by the customer, can be used.

- Extraction using the Calpro EasyExtract™

Instructions for use: please read package insert for product No. CAL0510



(Calpro AS, Product No. CAL0510)

Sample type

Faecal samples

Storage and stability of specimens

- Before extraction :

Temperature	Stability
2 – 25°C	5 days
- 20 °C	2 years

This information comes from data originating from internal measurements.

- After extraction with Calpro EasyExtract™ :

Temperature	Stability
2 – 8°C	7 days
8 – 25°C	5 days

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This information comes from data originating from manufacturer of the device (see Calpro EasyExtract™ instruction for use). It is the responsibility of the laboratory to use all available references and/or its own studies to determine the stability criteria specific to its laboratory.

Reagents

Composition and concentrations/Storage

Active components:
Reagent R1: none
Reagent R2: Suspension of colloidal gold particles coated with monoclonal human calprotectin antibodies (mouse).

Other components:
Reagent R1: buffer, stabiliser, inorganic salt and preservative.
Reagent R2: buffer, inorganic salt and preservative.

Conservation temperature:
Reagent R1: 2 - 8 °C.
Reagent R2: 2 - 8 °C.

Preparation

Ready to use.

Storage and stability

Reagents are stable until the expiration date printed on the packaging (months passed), under the following recommended storage and handling conditions:

- Unopened vial stored at temperature indicated on packaging.
- Opened vial: closed immediately after use or placed on closed analyser intended for this purpose, not contaminated by handling and stored at the temperature indicated on the packaging.

Reagents are shipped at 2-8°C.

Note:

- Do not freeze the reagents.
- Antisera or nanoparticle-based reagents may sediment over time. It is advisable to gently homogenize them by turning them over before putting them on board the analyzer.

Other materials required

Usual laboratory equipment including an analytical system equipped with a photometric detector.

Calibration

Calibration

The calibration curve is performed and validated using the calibration kit and associated controls listed in the "Order references" section. The zero point of the calibration curve is performed with level 1 of the calibration kit.

Traceability

The method has been standardised with a benchmark method traceable to the CALPROLAB™ Calprotectin ELISA (ALP) as described in the associated calibrators data sheet (see the “Order references” section).
Calibrate the method when the reagent batch number changes or in case of change in performance (contact the manufacturer if the changes persist) or if quality control requires it.

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Quality control

For quality control, use the control materials listed in the section "Order references". It is also possible to use other suitable control materials.
It is recommended that quality control be performed at each calibration and that the routine be supported by the assay of controls to ensure the reliability of patient assays performed during the routine. The results should be within the defined confidence limits. Each laboratory should establish the procedure to be followed if the results are outside the defined limits. Comply with local legislation and guidelines on quality control**.**

Reference values

	Reference values
Normal value	5 – 50 mg/kg
Positive value	> 50 mg/kg
Median value in patients with symptomatic colorectal cancers	350 mg/kg
Active, symptomatic inflammatory bowel disease	200 – 40.000 mg/kg

International units: mg/kg
Conventional units: µg/g

This information is based on data from the scientific literature. Each laboratory should verify the validity of these values and establish its own reference values, if necessary, according to the population under examination.

Analytical performances

The analytical performances below were evaluated on a clinical biochemistry analyzer. The results obtained are representative of those expected from a photometric system. However, the results obtained in the laboratory may differ from these. Data sheets corresponding to a specific clinical biochemistry system are available on request.

Linearity

Low linearity was assessed according to Clinical and Laboratory Standards Institute (CLSI) protocol EP06-A, with dilution of high faecal extract in sample dilution buffer. The method has been demonstrated to be linear from 10,9 mg/kg, with an acceptance criterion of 20% of allowable nonlinearity.

High linearity was assessed according to Clinical and Laboratory Standards Institute (CLSI) protocol EP06-A, with dilution of high faecal extract in sample dilution buffer. The method has been demonstrated to be linear up to 1297,4 mg/kg, with an acceptance criterion of 20% of allowable nonlinearity.

Measurement range

10,9 mg/kg - 1297,4 mg/kg
The measurement range is bounded by the low and high linearity limits. Samples having a concentration lower than the lower limit must be concentrated. Samples having a concentration greater than the upper limit must be diluted.

Lower limits of measurement

Limit of Blank = 9,7 mg/kg
Limit of Detection = 16,9 mg/kg
Limit of Quantification = 21,4 mg/kg
The Limit of Blank was determined in accordance with the CLSI EP17-A2 requirements, based on 60 determinations of blank samples. The Limit of Blank is the 95th percentile of the standard normal distribution of the blank samples determination.
The Limit of Detection was determined in accordance with the CLSI EP17-A2 requirements and with a proportion of false positive (α) less than 5 % and false negative (β) less than 5 %, based on 120 determinations with 60 blank and 60 low level replicates.
The Limit of Quantitation was determined in accordance with the CLSI EP17-A2 requirements for the functional sensitivity determination, based on 80 determinations of 7 low levels during 20 days and with a %CV goal of 20 %.

Interferences (Analytical specificity)

No interference for:

- Prednisolon (0,05 mg/100mg)
- Imurel (0,25mg/100mg)
- Salazopyrin (1,95mg/100mg)
- Trimetoprim (0,3mg/100mg)
- Ciprofloxacin (1,17mg/100mg)
- Pentasa (3,1mg/100mg)
- Asacol (1,8mg/100mg)
- Ibux (2,5mg/100mg)
- Multivitamin (0,5mg/100mg)
- Blood Human Hemoglobin (3mg/100mg)
- Bacteria cultures at concentration of 10^8/100mg (*Citrobacter freundii, Escherichia coli, Klebsiella pneumonia, Salmonella enterica, Yeirsina enterolitica*)

All interference studies are performed at 4 calprotectin levels (~50mg/kg; ~90mg/kg; ~275mg/kg; ~1100mg/kg).

Precision

Precision was evaluated with 3 quality controls and 4 faecal extracts samples following the CLSI protocol EP05-A3. Within-run precision was determined using 2 runs per day with 2 replicates per run. Within-lab precision was determined using a single lot of reagent and at least 4 calibrations. These results are guidelines. Variables (e.g. instrument maintenance, environment, sample handling) can affect the reproducibility of test results.

	Number of days	Number of measures	Mean concentration	Within-run CV	Within-lab CV
Control 1	22	88	95,8 mg/kg	4,8 %	8,2 %
Control 2	22	88	475,8 mg/kg	3,5 %	7,3 %
Control 3	22	88	1320,3 mg/kg	6,4 %	7,3 %
Sample 1	22	88	66,9 mg/kg	3,1 %	11,5 %
Sample 2	22	88	221,4 mg/kg	2,4 %	9,9 %
Sample 3	22	88	553,6 mg/kg	3,0 %	6,5 %
Sample 4	22	88	1003,5 mg/kg	2,5 %	4,1 %

Limitations of the method

The results of this test should always be interpreted in relation to the patient's medical history, clinical signs and other findings.

Prozone

By limiting the linearity to the value of the upper limit of the measurement range, no excess antigen effect was observed for samples with a concentration up to 10 000 mg/kg.

Matrix effect

Results show no matrix effect. The inter-laboratory control samples and controls can yield different results from those obtained with other assay methods because there is no international standardized method. Each manufacturer will use internal method for calibrator value assignment. In this case, an analysis of the results according to specific target values of the method utilised may be necessary. If in doubt, contact the manufacturer.

Literature

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Symbols legend

The following symbols may appear on the packaging and the label:

LOT	*Batch code*	BUF	*Buffer*
	Use until	CAL	*Calibrator*
	Manufacturer	H	*High*
IVD	*In vitro diagnostic medical device*	M	*Moderate*
	Temperature (Storage at)	L	*Low*
REF	*Catalogue reference*	4 LEV	*4 levels*
	Read the usage instructions	5 LEV	*5 levels*
REAG	*Reagent*	6 LEV	*6 levels*
KIT	*Kit*	CONTROL	*Control*
CONT	*Content*	CE	*This product meets the requirements of European Directive 98/79 EC concerning in vitro diagnostic medical devices*
Ab	*Antibody or Antisera*		*Track version changes*

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