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Electronic Instruction For Use: version

280_5

ORG 280 Calprotectin

INTENDED PURPOSE

Calprotectin for Alegria® is an ELISA-based test system for the quantitative measurement of calprotectin in human stool samples to be used in the assessment of intestinal inflammatory disorders. This product is intended for professional in vitro diagnostic use only.

Elevated faecal calprotectin levels may be found in patients with chronic inflammatory bowel disease (IBD), and other abdominal disorders, e.g. infectious gastro-enteritis, necrotising enterocolitis, cystic fibrosis, and colorectal carcinoma. Calprotectin in stool is a sensitive marker for IBD and is particularly useful for differentiating irritable bowel syndrome (IBS). In addition, measurement of the calprotectin level may contribute to the evaluation of disease activity.

SYMBOLS USED

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
24	Sufficient for ... determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
280_5	Electronic Instruction For Use: version

ALEGRIA TEST STRIPS	Alegria® Test Strips
WASH	Wash Buffer
SYSTEM FLUID	System Fluid
RTU	Ready to use
EXTRACT	Stool Extraction Medium

PRINCIPLE OF THE TEST

Calprotectin contained in a stool sample has to be released from the stool with extraction medium. Then, extracted Calprotectin can be analyzed with the Alegria® Calprotectin assay.

The Alegria® assay features barcoded 8-well-microstrips, called Alegria® Test Strips. Each strip is designed for a single determination of one patient sample. The Alegria® Test Strip holds a complete set of reagents. Included are enzyme conjugate, enzyme substrate, sample buffer and a test specific control. Furthermore each strip has two antibody-coated wells which serve as reaction wells for one control and one patient sample.

The determination is based on an indirect enzyme linked immune reaction with the following steps: Calprotectin present in control / positive samples binds to the surface of the reaction wells forming an antibody-antigen-complex. After incubation, a first washing step removes unbound and unspecifically bound molecules. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complex. After incubation, a second washing step removes unbound enzyme conjugate. Addition of enzyme substrate solution results in hydrolisation and colour development during incubation. The intensity of the blue colour correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 650 nm. The Alegria® Test Strip is based on the proprietary SMC®-Technology (Sensotronic Memorized Calibration): information about the assay, analysis and evaluation, and the lot-specific expiry date is contained on the barcode printed on each Alegria ® Test Strip.

The Alegria® Test Strip can be used with the diagnostic instrument Alegria® - a fully automated Random Access Analyser. By means of SMC®-Technology data encoded on the barcode are transferred from the Alegria® Test Strip to the instrument and the assay is automatically processed and evaluated. The instrument reads the date of expiry and rejects further processing if the Alegria® Test Strip is out of date.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- The control contains material of human origin. Since no test can guarantee the absence of infective agents in human material, we recommend handling Alegria® Test Strips as potentially infective material.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- System fluid contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, sample buffer, extraction medium and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate, control and sample buffer contain ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

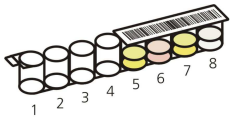
During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Remove contact lenses if this can be done easily. Get medical attention if necessary. Respiratory tract: take person to the fresh air. Swallowing: Rinse the mouth and spit the fluids out. Drink 1 - 2 glasses of water immediately. During spontaneous vomiting hold the head of the casualt low with the body in a prone position in order to avoid the penetration of vomit into the air tube.
- Personal precautions, protective equipment and emergency procedures:
  Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex.
  Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitiv. Store Alegria® strips in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.

CONTENTS OF THE KIT

Σ 24 ORG 280		Sufficient for 24 determinations
ALEGRIA TEST STRIPS	24	Alegria® Test Strips are modules of 8 wells each composed of:



Wells 1 + 2: empty and not coated (wells for the sample dilution)
Wells 3 + 4: coated with antibody (reaction wells)
Well 5: **Control**; yellow; containing Calprotectin, PBS, BSA, detergent, preservative sodium azide 0.09% and ProClin 300 0.05%.
Well 6: **Enzyme Conjugate**; light red; containing anti-Calprotectin antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%.
Well 7: **Sample Buffer**: yellow; containing PBS, BSA, detergent, preservative sodium azide 0.09% and ProClin 300 0.05%.
Well 8: **TMB Substrate**: clear; containing 3,3', 5,5'- Tetramethylbenzidin.
Anti-Calprotectin antibodies are coated onto microwells.

Code on barcode: ***Calprotectin*** on printout: ***Calpro***

WASH	1x 20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x concentrate.
SYSTEM FLUID	1x 2.5 ml	System Fluid, contains acid; 1000 x concentrate.
EXTRACT	1x 20 ml	**Stool Extraction Medium**; containing TBS, stabilizing protein, extraction reagent, preservative sodium azide 0.09%. Ready to use. (**F5126**)
	1	Certificate of Analysis

MATERIALS REQUIRED

- Vortex mixer, rocking shaker, benchtop centrifuge, optional: precision scales
- Pipettes for 10 µl
- Measuring cylinder for 1000 ml and 2500 ml, microtubes
- Distilled or deionized water

AUXILIARY IVD REAGENTS

- F5126 EXTRACT Stool Extraction Medium. This kit component is also available separately.

FURTHER ACCESSORIES

- ORG 282 Stool Extraction Tubes; containing 100 tubes.
- SSCO Stool Sample Collector

These auxilliaries are available separately.

STORAGE AND STABILITY

- Store test kit at 2-8 °C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store Alegria® Test Strips sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 15 months from day of production.
  Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and System Fluid are stable for at least 30 days when stored at 2-8 °C.
  Once transferred to the reagent container we recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- All materials must be at room temperature (20-28 °C) prior to use.
- To avoid carryover or contamination, change the pipette tip between samples.

SPECIMEN COLLECTION, PREPARATION, STORAGE AND HANDLING

Collecting stool samples

Stool Sample Collector (SSCO) may be used.
- Avoid contamination with toilet water containing disinfectants.
- Keep the collected stool sample no longer than 5 days at 2-8 °C. Alternatively, store at -20 °C.

Preparing stool sample for Extraction

Stool sample and EXTRACT have to be at room temperature.

For the Alegria® Calprotectin assay the stool sample has to be diluted **1:50** in the EXTRACT provided.
Inhomogeneous samples should be homogenised prior to sampling, e.g. by using an inoculation loop.
For liquid stool samples we recommend to weigh out sample.

Extraction of Calprotectin using Stool Extraction Tube (ORG 282)

Prepare Stool Extraction Tube (ORG 282)
- Fill **750 µl** EXTRACT into the transparent tube to gain a **1:50** dilution.
  Use Stool extraction Tubes according to Instruction for Use ORG 282
- Extraction
  Vortex 30 sec at 1800 rpm to remove stool sample from the notches of the spatula.
  Further homogenize for 15 min at top speed on a rocking shaker.

Open the tube at the blue cone insert and transfer the homogenate to a microtube.
Centrifuge for 2 min at 3000xg. Transfer clear fluid to another microtube and test for Calprotectin **immediately**.

Extraction of Calprotectin using other tubes
- Weigh out empty tube.
  Pick stool sample and transfer to tube. Weigh out and determine net weight of sample.
- Fill EXTRACT into the tube: 49-times net weight of sample needed to gain a 1:50 dilution

stool sample	+	EXTRACT	stool sample	+	EXTRACT
15 mg	+	0.75 ml	60 mg	+	2.9 ml
20 mg	+	1.0 ml	70 mg	+	3.4 ml
30 mg	+	1.5 ml	80 mg	+	3.9 ml
40 mg	+	2.0 ml	90 mg	+	4.4 ml
50 mg	+	2.5 ml	100 mg	+	4.9 ml

Close tube. Vortex 30 sec at 1800 rpm. Further homogenize for 15 min at top speed on a rocking shaker.
Open tube. Transfer the homogenate to a microtube. Centrifuge for 2 min at 3000xg.
Transfer clear fluid to another microtube and test for Calprotectin **immediately**.

Such an extracted stool sample may be stored at 2-8 °C for up to 5 days or at -20 °C for up to 4 months. The extracted stool sample is not stable at room temperature!

PREPARATION OF REAGENTS

WASH

Dilute the content of the Wash Buffer concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Transfer the diluted Wash Buffer into the instrument reagent container. If only one Alegria run is to be performed on one day we recommend transferring only 500 ml diluted Wash Buffer.

SYSTEM FLUID

Dilute the content of the System Fluid concentrate (1000x) with distilled or deionized water to a final volume of 2500 ml prior to use. Transfer the diluted System Fluid into the instrument reagent container.

ALEGRIA TEST STRIPS

Take the required number of Alegria® Test Strips out of the clip bag and let them reach room temperature (20-28 °C). Do not remove foil covering the empty wells until you are ready to start the assay.

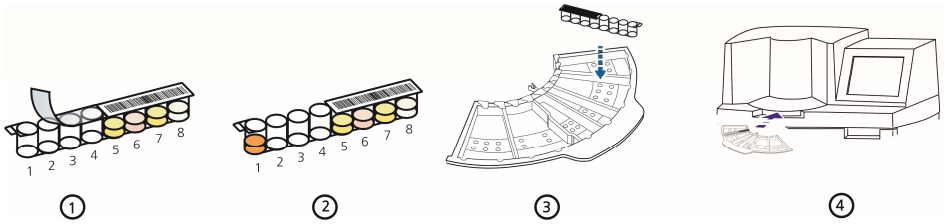
EXTRACT

Ready to use

TEST PROCEDURE

Alegria® Test Strips with SMC® technology are used with the diagnostic instrument Alegria®.
Detailed information about operating the instrument can be taken from the Instrument User Manual.

(1) Remove the foil from the empty wells 1 to 4 of the Alegria® Test Strip.
Do not remove foil with printed barcode, covering wells 5 to 8.
(2) Pipette 10 µl extracted stool sample at the bottom of well 1.
(3) Insert the strip into the SysTray.
(4) Place loaded SysTrays into the correct position in the Alegria® instrument and start run. All further steps will be done automatically. The test run is completed when the instrument starts printing the results.



Calibration

The assay system is calibrated using a Calprotectin reference preparation purified from human neutrophils.

CALCULATION OF RESULTS

By means of SMC® Technology (Sensotronic Memorized Calibration), all test data are transferred to the system through individual barcodes on the Alegria® Test Strip. Calculation and interpretation of results will be performed automatically.

PERFORMANCE CHARACTERISTICS

Measuring range

The calculation range of this Alegria® assay is 0 - 1000 µg/g

Expected values

In a normal range study with samples from healthy donors the following ranges have been established with this Alegria® assay: Cut-off 50 µg/g

Interpretation of results

Normal range	< 50 µg/g
Slightly elevated values	50 - 200 µg/g
Significantly elevated values	> 200 µg/g

Calprotectin level in the normal range: gastrointestinal inflammation nearly ruled out; further invasive diagnostic measures are generally not required.
Calprotectin level slightly elevated: low level of inflammatory activity in the gastrointestinal tract (e.g. IBD in remission); repeated test and further diagnostic measures are recommended.
Calprotectin level significantly elevated: active organic disease of the gastrointestinal tract; further intensive diagnostic tests and treatment by a specialised gastroenterologist are urgently advised.

Linearity

Samples containing Calprotectin were serially diluted in sample buffer to demonstrate the dynamic range of the assay. Activity for each dilution was calculated by means of SMC® Technology.

Sample	Dilution	Observed	Expected	O/E
		[µg/g]	[µg/g]	[%]
1	1:100	746.8	746.8	100
	1:200	366.7	373.4	98
	1:400	186.7	186.7	100
	1:800	89.5	93.4	96
2	1:100	625.5	626.5	100
	1:200	325.0	313.3	104
	1:400	151.1	156.6	97
	1:800	65.2	78.3	83
3	1:100	112.9	112.9	100
	1:200	59.5	56.5	105
	1:400	26.5	28.2	94
	1:800	14.6	14.1	103

Sensitivity

Lowest detectable concentration: 5.2 µg/g

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 20 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 3 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean µg/g	% CV
1	49.5	5.5
2	218.2	6.1
3	847.9	9.9

Inter-Assay		
Sample	Mean µg/g	% CV
1	47.8	8.7
2	107.1	6.5
3	335.8	7.1

Interfering substances

The following substances were tested per 15 mg of stool sample and were found to be non-interfering: Vancomycin hydrochlorid 0.201 mg, Ciprofloxacin 0.15 mg, Prevazid 0.006 mg, Azathioprine 0.021 mg, Prednisone 0.003 mg, Pentasa 0.399 mg, Vitamin A 2.4 UI, Vitamin C 0.015 mg, Vitamin E 0.03 mg, Vitamin D3 0.33 UI, Hemoglobin 1.749 mg. The following microorganisms were tested at 1.1 x 10^7 CFU per 15 mg of stool sample and were found to be non-interfering: *E. coli, Klebsiella pneumoniae, Salmonella enterica, Shigella flexneri, Yersinia enterocolitica* .

Study results

Study population	n	n pos	%
Inflammatory bowel disease	186	182	97.8
Abdominal disorders	144	10	6.9
Healthy donors	18	0	0.0

		Diagnostic result		
		Pos	Neg	
ORG 280	Pos	182	10	
	Neg	4	152	
		186	162	348

Sensitivity:	97.8 %
Specificity:	93.8 %
Overall agreement	96.0 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.
The above pathological and normal reference ranges in patient stool samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.
Non-steroidal anti-inflammatory drugs (NSAIDs) induce enteropathy in up to 20-65 % of patients receiving these drugs. NSAID can lead to elevated concentrations of fecal calprotectin. In these cases reevaluation of fecal calprotectin after stopping NSAIDs should be considered.

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Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 280_IFU_EN_QM131061_2016-08-01_4* Reason for revision: *Introduction electronic IFU on homepage*