Instructions for Use

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English

OC-FCa Reagent

for OC-SENSOR PLEDIA REF V-PH11
for OC-SENSOR Ceres REF V-PH09

INTENDED USE

OC-FCa Reagent is *in vitro* diagnostic assay reagent intended for the quantitative measurement of calprotectin, inflammatory protein, in faeces. The OC-FCa Reagent aids in diagnostic and monitoring of inflammatory bowel diseases (IBD; e.g. ulcerative colitis, and Crohn's disease) in conjunction with other clinical findings. The test can be utilized in diagnostic aid and in monitoring for symptomatic patients. The test is noninvasive, using stool/faeces as test sample. The reagent is used on the dedicated automated analysers by qualified personnel in clinical laboratories and hospitals.

SUMMARY

Calprotectin is an inflammatory protein abundantly present in neutrophils.[1] The amount of calprotectin in faeces increases accompanied with intestinal mucosal inflammation.[2] Therefore measuring the amount of calprotectin in faeces can be used for disease monitoring of IBD patients and distinguishing inflammatory bowel disease (IBD; e.g. ulcerative colitis, Crohn's disease) from functional intestinal disorders (e.g. Irritable bowel syndrome).[3-5]
Currently, the methods used for detection of calprotectin include immunoassay latex agglutination, ELISA, FEIA, chemiluminescent immunoassay and immunochromatography.[6]
OC-FCa Reagent is an immunoassay test reagent used together with an automatic analyser for measurement of calprotectin in faeces. OC-FCa Reagent contains the latex particles coated with anti-human calprotectin antibodies for optical measurement of the latex agglutination reaction.

PRINCIPLE OF THE METHOD

The test method is based on a latex agglutination reaction. A latex reagent is prepared by coating polystyrene latex particles with anti-human calprotectin antibodies. When this reagent is mixed with the sample, the anti-human calprotectin antibodies on the latex particles react with the calprotectin in the sample, and the latex aggregate is formed in the latex agglutination reaction. The change in absorbance per unit time resulting from the latex agglutination reaction is proportional to the concentration of calprotectin in the sample. A dose response curve of the absorbance unit (OD) vs. concentration is generated using the results obtained from the calibrators. The concentration of calprotectin in the patient sample is determined from this curve.

MATERIALS PROVIDED

Product code	*Product name*	*Contents*	*Storage*	*Compatible analyser*
V-PH11	OC-FCa Reagent	2 x 8 mL 2 x 15 mL	2-10 °C	PLEDIA
V-PH09	OC-FCa Reagent	2 x 8 mL 2 x 15 mL	2-10 °C	Ceres

MATERIALS REQUIRED BUT NOT PROVIDED

Product code	*Product name*	*Contents*	*Storage*	*Compatible analyser*
V-PH12	OC-FCa Calibrator	6 x 1 mL	2-8 °C	PLEDIA, Ceres
V-PH13	OC-FCa Control LV1	2 x 5 mL	2-8 °C	PLEDIA, Ceres
V-PH14	OC-FCa Control LV2	2 x 5 mL	2-8 °C	PLEDIA, Ceres
V-PH15	OC-FCa Control LV3	2 x 5 mL	2-8 °C	PLEDIA, Ceres
V-PH19	OC-SENSOR Sample Diluent	3 x 45 mL	2-8 °C	PLEDIA
V-PH08		2 x 20 mL	2-8 °C	Ceres
V-PZ25	OC-Auto Sampling Bottle 3	100 bottles	1-30 °C	PLEDIA, Ceres
V-PZ26	OC-Auto Sampling Bottle 3 without barcode	100 bottles	1-30 °C	PLEDIA, Ceres

REQUIRED MATERIALS NOT PROVIDED BY THE MANUFACTURER

Prepare these materials before measurement

- **Wash solution: Sodium hypochlorite 0.15% (0.10%–0.30% is acceptable)**
- **Purified water for wash: Distilled or de-ionized water (1.0-10.0 MΩcm is acceptable)**
- **Sample cups**
- **Printer paper: Thermal printer paper which fits the analyser**

REAGENTS

Reagents are stable until the date printed on the label assuming the container remains unopened at a storage temperature of 2-10 °C.

R-2 Latex Reagent (Suspension of latex particles coated with anti-human calprotectin mouse antibodies)
for OC-SENSOR PLEDIA ·····8 mL x 2 bottles; approx. 50 tests/bottle
for OC-SENSOR Ceres ·····8 mL x 2 bottles; approx. 115 tests/bottle
Stable for 4 weeks if stored back in a refrigerator after use with cap closed.
Stable for 4 weeks on-board with cap closed after use on PLEDIA and Ceres.
R-1 Buffer (50 mM N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES))
for OC-SENSOR PLEDIA ·····15 mL x 2 bottles; approx. 50 tests/bottle
for OC-SENSOR Ceres ·····15 mL x 2 bottles; approx. 115 tests/bottle
Stable for 4 weeks if stored back in a refrigerator after use with cap closed.
Stable for 4 weeks on-board with cap closed after use on PLEDIA and Ceres.

WARNINGS AND PRECAUTIONS

1. **For *in vitro* diagnostic use only.**
2. **Before performing measurement, be sure to read the instruction manual of the analyser.**
3. **Fully prepare and adjust the analyser before measurement.**
4. **Use OC-FCa Calibrator (REF V-PH12) to create calibration curve. Follow its instruction for use when to create a new calibration curve.**
5. **If the measurement results exceed the measurement range, use OC-SENSOR Sample Diluent (REF V-PH19, V-PH08) to dilute the sample and perform measurement again.**
6. **Store all reagents at 2-10 °C and avoid freezing.**
7. **On-board stability can vary depending on the conditions of measurement, such as tests/day and laboratory environment. It is advised to close the bottle of Latex Reagent with a cap when the measurement is not in process.**
8. **Do not use reagents that have passed their expiration date.**
9. **The test sample may contain pathogens. Handle with care. After use, all samples and other materials must be considered as medical waste and properly disposed of.**
   Example of treatment: Soak for 1 hour or longer in a sodium hypochlorite solution (available chlorine concentration 1000 ppm or greater). (Neutralize any substances that contain acids before soaking.) Alternatively, treat in an autoclave at 121 °C for 20 minutes. (Do not treat any items which sodium hypochlorite have adhered to in this way.)
10. **Dispose of used reagents and containers as medical waste in accordance with local regulations.**
11. **If the product is used in any way other than that specified here, the reliability of measurement results cannot be guaranteed. Be sure to follow the procedure.**
12. **A clinical diagnosis based on the measurement results must be a comprehensive judgment made by the attending physician, including factors such as clinical symptoms and other test results.**

SAMPLE COLLECTION

- **Use the following designated sampling device for collecting specimens.**
  OC-Auto Sampling Bottle 3 (REF V-PZ25, V-PZ26) (Sold separately)
- **Collect faecal samples by scraping the surface of the stools in different areas. Collect the amount necessary to cover the groove of the probe.**
- **Check that the test sample has become fully suspended in the buffer solution inside OC-Auto Sampling Bottle 3.**
- **Use the sample for measurement at least 1 hour after collection in OC-Auto Sampling Bottle 3.**
- **Calprotectin in the OC-Auto Sampling Bottle 3 is stable at room temperature for 3 days, at 2-10 °C for 14 days.[7]**
- **If a specimen is received in a specimen container, it should be stored at 2-8 °C and sampled with OC-Auto Sampling Bottle 3 within 3 days.[8]**

PREPARATION OF REAGENTS

OC-FCa Latex Reagent for OC-SENSOR PLEDIA
Ready to use.
Before use, bring reagent to room temperature (20-25 °C) and invert the bottle gently several times to assure uniform suspension.
Do not mix reagent with another bottle.

OC-FCa Buffer for OC-SENSOR PLEDIA
Ready to use.
Before use, bring the buffer to room temperature (20-25 °C).
Do not mix buffer from another bottle.
Do not use precipitated buffer, as precipitate may cause problems on analysers.

OC-FCa Latex Reagent for OC-SENSOR Ceres
Ready to use.
Before use, invert the bottle gently several times to assure uniform suspension.
Do not mix reagent with another bottle.

OC-FCa Buffer for OC-SENSOR Ceres
Ready to use.
Do not mix buffer from another bottle.
Do not use precipitated buffer, as precipitate may cause problems on analysers.
Note: OC-SENSOR Ceres is equipped with refrigerated reagent slots so that the reagents do not need to be brought to room temperature.

TEST PROCEDURE

Perform measurements according to the instruction manual of the analyser.

1) **Set the OC-FCa R-2 Latex Reagent and OC-FCa R-1 Buffer on the designated locations in the analyser.**
2) **Input the parameters into the analyser.**
3) **Check water, wash solution, and drain tank volumes.**
4) **Create calibration curve.**
5) **Set control materials, OC-FCa Controls as internal control and start measurement. Make sure the control values are within the acceptable range.**
6) **Set samples sampled with OC-Auto Sampling Bottle 3 and start measurement.**

RESULTS

The reaction of each sample is compared to the calibration curve that has been created previously. The concentration of calprotectin (µg/g faeces) is reported as a result.

LIMITATIONS

- **Patients taking NSAIDs (non-steroidal anti-inflammatory drug) regularly may raise faecal calprotectin levels.[9]**
- **Samples exceeding 50,000 µg/g of calprotectin concentration may result in decreased measurement value due to Hook effect.**
- **Patient with IBD fluctuate between active (inflammatory) and inactive (remission) stages of the disease. These stages should be considered when the result of the faecal calprotectin is interpreted.[10]**
- **Other intestinal diseases such as colorectal cancer and gastrointestinal infections can result in increase of calprotectin level. A clinical diagnosis based on the measurement results must be a comprehensive judgment made by the attending physician, including factors such as clinical symptoms and other test results.[10-12]**

INTERNAL QUALITY CONTROL

Each laboratory should establish a quality control program to monitor the performance of R-2 Latex Reagent. It is recommended to use the following materials for the quality control in your laboratory.
OC-FCa Controls: Liquid, ready-to use controls sold separatory
LV1 (REF V-PH13) LV2 (REF V-PH14) LV3 (REF V-PH15)

PERFORMANCE CHARACTERISTICS

1. Accuracy

When samples of known concentration (170, 250, 500, 1360 µg/g) were measured, the value obtained was within ±15% of the indicated value. Example of measurement resulted in 95-103%, 98-100%, 97-100%, and 96-100% for the samples 170, 250, 500, and 1360 µg/g, respectively.

2. Repeatability and Precision

When the same samples were measured in 2 replicates, twice a day, for 20 days (2x2x20), the coefficient of variation (CV) for the values obtained was 10% or less, using ANOVA analysis. Examples of measurement of controls and faecal samples (N=80 each) using OC-SENSOR PLEDIA and Ceres are shown below.

OC-SENSOR PLEDIA

Samples	Mean (µg/g)	Repeatability		Between-Run		Between-Day		Precision	
		SD	CV	SD	CV	SD	CV	SD	CV
LV1	252.7	2.8	1.1%	2.2	0.9%	2.9	1.2%	4.6	1.8%
LV2	496.0	4.1	0.8%	2.3	0.5%	4.4	0.9%	6.5	1.3%
S1	53.0	3.0	5.6%	1.5	2.8%	0.7	1.4%	3.4	6.4%
S2	284.3	2.6	0.9%	1.3	0.4%	2.7	0.9%	3.9	1.4%

OC-SENSOR Ceres

Samples	Mean (µg/g)	Repeatability		Between-Run		Between-Day		Precision	
		SD	CV	SD	CV	SD	CV	SD	CV
LV1	252.7	2.4	1.0%	1.1	0.4%	3.1	1.2%	4.1	1.6%
LV2	508.5	4.9	1.0%	0.3	0.0%	6.1	1.2%	7.8	1.5%
S1	49.7	1.7	3.4%	0.8	1.5%	1.2	2.5%	2.2	4.5%
S2	307.3	3.3	1.1%	0.9	0.3%	3.6	1.2%	4.8	1.6%

3. Measurement range

Up to 2720 µg/g (faeces)

Examples of measurement using OC-SENSOR PLEDIA result showed LOD of 9 µg/g, LOQ of 23 µg/g; OC-SENSOR Ceres showed LOD of 5 µg/g, and LOQ of 22 µg/g. The values can vary due to faecal samples and whether/how calprotectin is spiked.

4. Cut-off value

Results for the OC-FCa Reagent greater than 50 µg/g (faeces) should be considered positive results. It is recommended for each laboratory to establish the cut-off value suitable for purposes in screening programs or diagnostic use.[6]

5. Acceptable range between-reagent batches

R-2 Latex Reagent

When control samples with known concentration (250 and 500 µg/g) were measured, the value obtained was within 100±15% of the indicated value.

6. Interference

Following substances were added to sample and the test results showed no interference or cross-reactivity.

- Toilet cleaners

0.01% Poly(oxyethylene)alkylether

- Co-existing substances

20 mg/dL Bilirubin (free and conjugated)
1500 FTU Chyle (Formazin turbidity)
125 mg/dL Haemoglobin
3.5 g/dL Protein
4.0 g/dL Glucose
100 mg/dL Barium sulphate

7. Sample Stability

Performance testing with the sample collection device demonstrated that samples stored at 2 °C for 31 days had 88±23.4%, at 25 °C for 3 days had 80±33.4%, and at 30 °C for 3 days had 66±42.4% of calprotectin recovery (in-house data, recovery rate shown as mean±2SD).

8. Comparison between analysers

When OC-SENSOR analysers, Ceres and PLEDIA are compared, there were no significant differences in measurement. Example of measurement of 168 samples resulted with the slope of 1.03 and r (correlation coefficient) of 0.9996 in regression analysis.

9. Traceability and Standard Material

No international reference material or reference measurement procedures are available for calprotectin. The calibrators and controls are traceable to the internal reference material.

10. Clinical Performance

Meta-analysis data is summarized from articles using various immunochemical faecal calprotectin tests as general information. Data from this product is not included.

Diagnostic sensitivity and specificity for IBD with different cut-offs[12]

	50 µg/g	100 µg/g	250 µg/g
Sensitivity	0.92	0.84	0.80
Specificity	0.60	0.66	0.82
PLR	2.33	2.95	4.17
NLR	0.13	0.23	0.22
N (patients)	693	559	763

PLR: positive likelihood ratio
NLR: negative likelihood ratio

Diagnostic sensitivity and specificity for different disorders[12]

	IBD	UC	CD
Sensitivity	0.85	0.88	0.81
Specificity	0.81	0.82	0.81
N (patients)	1471	744	727

(at the cut-offs with the highest diagnostic accuracy)

Differentiation of IBD and IBS at different cut-offs[6]

	50 µg/g	100 µg/g
Sensitivity	0.96	0.92
Specificity	0.77	0.86

Studies demonstrated that the reference intervals to be 10-33 µg/g (N=128),[13] and 3-35 µg/g (N=82)[14] in the normal population, 59–276 µg/g (N = 82) in patients with ulcerative colitis, and 110–353 (N=49) in patients with Crohn's disease,[14] respectively as 95% confidence intervals.

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NOTICE

In case of occurrence of any serious incident that has occurred in relation to the device shall be reported to the authorised representative, the manufacturer, and the competent authority of the Member State in which the user and/or the patient is established.

EXPLANATION OF SYMBOLS

LOT	Batch code		Manufacturer		Consult instructions for use
	Use by date	IVD	*In vitro* diagnostic medical device		Biological risks
REF	Catalog number		Temperature limitation	Σ	Contains sufficient for <n> tests

IVD CE 0123

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