VITASSAY

Calprotectin
-Turbidimetric Assay-

Rapid test for the quantitative detection of human calprotectin in human stool samples.

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only. Professional trained in turbidimetric techniques.

INTENDED USE

Vitassay Calprotectin –Turbidimetric Assay- is a rapid Turbidimetric assay for the quantitative detection of human calprotectin in human stool samples.

Simple, non-invasive and highly sensitivity assay for the presumptive diagnosis of human calprotectin, which might be useful for the diagnosis of inflammatory gastrointestinal disorders. This product is optimized for several automated analyzer. The results should be used to differentiate between IBD patients with inflammation from IBD patients without inflammation and from with irritable bowel syndrome.

INTRODUCTION

Calprotectin is a 36 KDa neutrophil cytosolic protein with antimicrobial properties. Increased concentration of this protein in stool samples is tightly associated to bowel inflammation. This protein remains stable in faeces for up to 7 days at room temperature, becoming it an ideal disease marker.

Calprotectin releasing is associated to leukocytes activation, giving increased levels in plasma, urine or stools as a consequence of disease in the relevant organ(s).

Calprotectin action mechanism is related to zinc-dependent enzymes inhibition, killing microbes and inducing apoptosis in normal and cancer cells. In presence of calcium, calprotectin is remarkably resistant to protelolytic degradation.

PRINCIPLE

Vitassay Calprotectin –Turbidimetric Assay- is a quantitative turbidimetric assay for the detection of human calprotectin in human solid stool samples.

Calprotectin Turbidimetric Assay is based on antigen-antibody agglutination reactions between the antigen contained in the sample and the antibodies anti-antigen coated on polystyrene latex particles.

Such agglutination is measured as an increase in absorbance proportional to the quantity of antigen contained in the sample.

The use of two external controls, Control 1 and Control 2, is used to verify that the test is working properly.

PRECAUTIONS

- For professional *in vitro* use only.
- A trained person in Turbidimetric technique and autoanalyzer use is required.
- Do not use after expiration date.
- Do not use the test if its primary containers are damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. Avoid contamination errors, follow proper work procedure.
- The reagents after use should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay Calprotectin-Turbidimetric Assay-**. Do not use any other commercial component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not use any other commercial kit component.
- If measure range is exceeding, use the sample diluent to dilute the sample and repeat the assay again.
- Read and follow up the instructions for use provided in the kit.
- Prepare and adjust the analyzer before starting measurements.

STORAGE AND STABILITY

Store as packaged in the original primary container, the reagents should be preserved at refrigerated temperature (2-8ºC/35.6-46.4ºF), the sample diluent could be preserved refrigerated or at room temperature (2-30ºC/35.6-86ºF).

The product is stable until the expiration date printed on the label, if they have been preserved under the recommended conditions.

Do not freeze and keep away from the sunlight.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ Reagent R1 (Enhancer buffer) ▪ Reagent R2 (Latex particles) ▪ Calibrator 0 ▪ Calibrator 1 ▪ Calibrator 2 ▪ Calibrator 3 ▪ Calibrator 4 ▪ Calibrator 5 ▪ Control 1 ▪ Control 2 ▪ Universal vials with diluent for the sample dilution. ▪ Instruction for use. ▪ Additional screw cap	▪ Specimen collection container. ▪ Disposable gloves and laboratory equipment. ▪ Vortex ▪ Automatic analyzer. ▪ Microcentrifugue (10000g).

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SPECIMEN COLLECTION

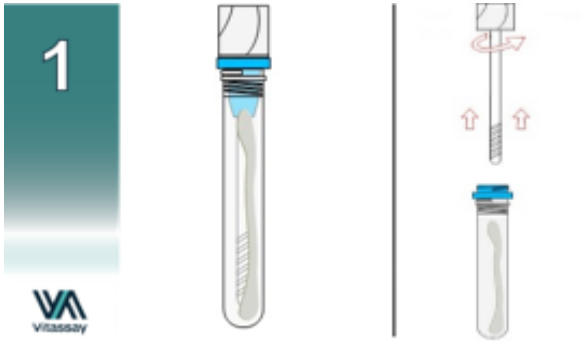
Collect sufficient quantity of feces: 1-2 g or mL for liquid samples. Stool should be collected in clean and dry containers.

Samples can be stored in the refrigerator (2-8ºC/35.6-46.4ºF) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature (15-30ºC/59-86ºF) before testing. Homogenise stool samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

1. Label the vial with the patients´s identification.
2. Open the cap of the vial (anticlockwise) without removing the blue separator and use the stick to pick up sufficient quantity of sample (figure 1).
3. Use the stick to collect sufficient sample quantity. Introduce the stick into 4 different zones and rotate the stick in order to make the samples as homogenous as possible and make sure all grooves are filled with faecal material (figure 2).
4. Remove the stool excess by rotating the stick on the internal wall of the sterile container.
5. Insert the stick with the sample into the vial through the blue separator. Close the white cap by holding the blue separator to prevent it from opening.
6. Shake each vial (30-60 seconds) by Vortex in order to assure good sample dispersion (figure 3). Proceed to step 7 if the grooves are visually empty of any stool material, otherwise repeat the shaking up to 120 seconds and proceed to step 7 regardless some residual stool sample in the grooves. The sample dilution vial with diluted sample can be stored for 7 days in the refrigerator (2-8ºC) prior to testing and bring the samples to room temperature.
7. Centrifuge the vial for 10 minutes at 1000-3000 x g in order to remove the possible residuals of faecal material. Alternatively, let the vial sit upright for a minimum of 15 minutes.
8. Remove the white screw cap and the blue separator from vials by rotating the separator (clockwise). The faecal extract is now ready to be tested. Insert the opened device directly into the clinical chemistry analyser or transfer the supernatant to an adaptor cup (not provided) (figure 4).
9. Close the collection vial by the provided additional screw cap (figure 4).

Note: Do not use the sample vials directly in the analyzer.

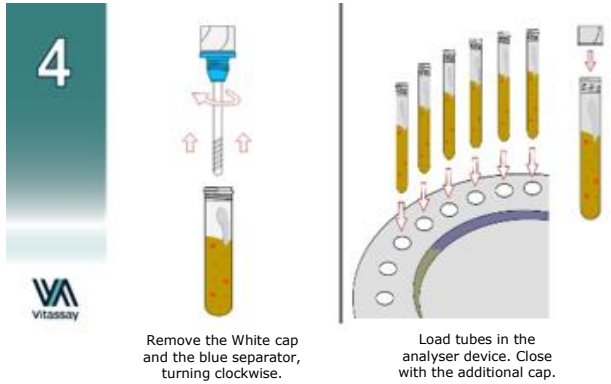


Sample dilution vial.

Pull out the shaped stick by turning the screw cap.

Introduce the stick into 4 different zones of the sample.

Shake the sample dilution vial with diluent+sample. Use a vortex. Centrifuge.



Remove the White cap and the blue separator, turning clockwise.

Load tubes in the analyser device. Close with the additional cap.

PROCEDURE

Reagent R1 y Reagent R2 are ready to use.

Calibration curve

For calibration only use Calprotectin Calibrator: Cal0, Cal1, Cal2, Cal3, Cal4 and Cal5. Contain human calprotectin at diferent concentrations indicated on the label of each of the vials.

	Reference	Calibrator 1	Calibrator 2	Calibrator 3	Calibrator 4	Calibrator 5
Conc.	0 µg/g	50 µg/g	100 µg/g	250 µg/g	750 µg/g	1500 µg/g
Vol.	1000µL	1000µL	1000µL	1000µL	1000µL	1000µL

Reagents are ready to use. The frequency in the realization of the calibration curve must be established by the end user in the function of the criteria established for the clinical laboratory.

Note: See section quality control.

Analytical procedure

Measure range: 20 – 8000 µg hCp/g.

Procedure	Steps	
R1 addition	250 µL	0 s
Sample addition	5 µL	10 s
R2 addition	30 µL	300 s
Blank measure	450 nm – 800nm	310 s
Mainly measure	450 nm – 800nm	610 s

*Dat obtained by Biolis i24 analyser (Tokio Boeki).

INTERPRETATION OF RESULTS

Positive results: higher or equal than the Cut-off fixed by the clinical laboratory.

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Recommended cut-off values: 50 µg de hCp/g of stool for diagnostics procedures and 200 µg de hCp/g of stool for screening procedures.

Positive results determine the abnormal presence of human Calprotectin (hCp) in stool samples.

Cut-off value of **Vitassay Calprotectin-Turbidimetric Assay**-:

Calprotectin concentration valueslower than 50 µg de hCp/g of stool are considerer normal values and that is not indicative of an inflammation of gastrointestinal tract.

Calprotectin concentration values between 50 and 200 µg hCp/g of stool are considered as an abnormal presence being indicative of mild inflammation of gastrointestinal tract, therefore monitoring and follow-up of the patient is recommended.

Calprotectin concentration values higher than 200 µg hCp/g are indicative of a severe inflammation of the gastrointestinal tract.

QUALITY CONTROL

Calprotectin C1 & C2 Controls are ready to use.

Calprotectin Control 1: is liquid control at a certain concentration of recombinant human calprotectin (lower than Control 2). Concentration is indicated on the label of the vial.

Calprotectin Control 2: is liquid control at a certain concentration of recombinant human calprotectin (higher than Control 1). Concentration is indicated on the label of the vial.

The use of controls at two different concentrations is recommended to verify test precision.

If the obtain results are out of the tolerance range, the equipment, the reagents or the technique must be reviewed. If the problems persists, stop using the reagents and contact your distribuitor.

NOTE: The labels on the corresponding tubes for both Control 1 and Control 2 indicate the range of values for the kit controls. For additional details, please request and refer to the certificate of analysis (not included).

LIMITATIONS

- **Vitassay Calprotectin -Turbidimetric Assay** should be only used in human stool samples.
- The quality of **Vitassay Calprotectin -Turbidimetric Assay-** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of human calprotectin in fecal samples; nevertheless, it can be due to several causes (inflammatory bowel disease, colorectal cancer and some other enteropathies). A positive result should be followed up with additional diagnostic invasive procedures, a colonoscopy and a biopsy in order to confirm the diagnosis and to establish the inflammation extent.
- If the test result is negative and the clinical symptoms or situation continue, it is recommended to perform another screening method. Negative results do not exclude inflammation; some diseases such as celiac sprue and microscopic colitis polyps that mainly involve mononuclear inflammation.
- Patients following non-steroidal anti-inflammatory drug treatment (NSAID) could show positive results.
- Neonatal fecal calprotectin levels have been reported higher than those in normal children with a mean of 167 µg/g (range 22-860 µg/g).
- All results must be interpreted along with the rest of the clinical information and other results obtained in the laboratory by a specialist doctor.

EXPECTED VALUES

Some studies established equal or higher 50 µg/g faeces are not considered indicative of intestinal inflammation. Values between 50-200 µg/g can be representative of diseases related to intestinal inflammation. Patients who are in this range should be reevaluated and may be susceptible to invasive clinical analysis. The exception to this range are infants whose calprotectin values are higher than in healthy adults. Values greater than 200 µg/g are indicative of an active inflammatory process, these patients will require additional clinical analyzes.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity:

Detection limit: 7 µg hCp/g.

Prozone:

Lower concentrations of 25 mg of hCp/g of stool do not show prozone effect and no false negative results have been observed.

Within-Run Precision

	Low (20 µg/g)	Media (80 µg/g)	High (250 µg/g)
N	20	20	20
Media (µg/g)	22.1	84.3	258
DS (µg/g)	3.6	11.9	17.7
CV (%)	16	14	6

*Data obtained by the analyzer Biolis i24 (Tokio Boeki)

Cross reactivity:

No cross reactivity was detected against other fecal markers that are occasionally present in feces:

Bovine Haemoglobin	*None*	*Human Haemoglobin*	*None*
Pig Haemoglobin	*None*	*Human Transferrin*	*None*
Bovine Transferrin	*None*	*Human Lactoferrin*	*None*
Bovine Lactoferrin	*None*		

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4. Fecal calprotectin: a quantitative marker of colonic inflammation in children with inflammatory bowel disease. Fagerberg UL, Lööf L, Lindholm J, Hansson LO, Finkel Y. J Pediatr Gastroenterol Nutr. 2007 Oct;45(4):414-20.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

IVD	*in vitro* diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
LOT	Batch code		Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number
	Keep out of the sunlight		

ADAPTED EQUIPMENT

- A15 (Biosystems)
- Advia 1800 (Siemens)
- Advia 2400(Siemens)
- Alinity (Abbot)
- Architect c1000/c4000/c8000 (Abbot)
- Atellica (Siemens)
- Au680 (Beckman Coulter)
- Biolis 24i/Biolis 50i (Tokio Boeki)
- BS200E/240 (Mindray)
- Chemwell-T (Awareness)
- Cobas c111 (Roche)

- Cobas c501 (Roche)
- Cobas c702 (Roche)
- Respons 910 (DiaSys)
- Vitros 5600 (Ortho)
- TC220 (Tecom)
- XL-180 & XL-200 (Erba)