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 solid 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.
- 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 ▪ Reagent R2 ▪ Calibrator 0 ▪ Calibrator 1 ▪ Calibrator 2 ▪ Calibrator 3 ▪ Calibrator 4 ▪ Calibrator 5 ▪ Control 1 ▪ Control 2 ▪ Vials with diluent for the sample dilution. ▪ Instruction for use.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Automated analyzer. ▪ Vortex. ▪ Microtubes (analyser vial).

SPECIMEN COLLECTION

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

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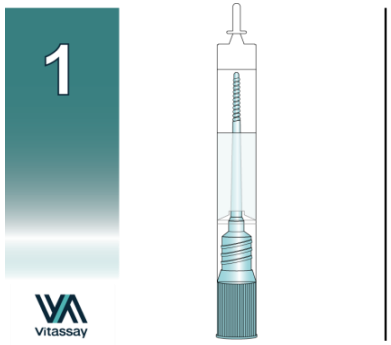
Samples can be stored in the refrigerator (2-8ºC/35.6-46.4ºF) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature before testing. Homogenise stool samples as thoroughly as possible prior to preparation.

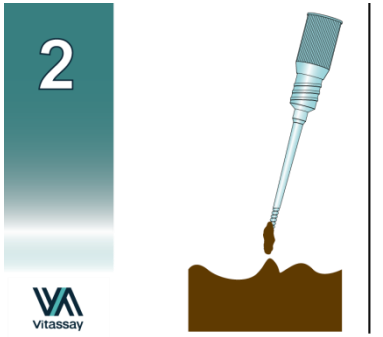
SPECIMEN PREPARATION

1. Label the vial with the patients´s identification.
2. Open the cap of the vial with diluent for sample dilution (figure1).
3. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample (figure 2), and add it into the vial with diluent for sample dilution. For liquid stool, take 20 µL of the sample using a micropipette and transfer it into the vial for the sample dilution.
4. Close the cap of the vial with the diluent and sample, shake the vial in order to assure good sample dispersion, using a vortex during 1 minute (figura 3). The sample dilution vial with dilute sample can be stored in a range of temperatures (2-8ºC / 35.6-46.4 ºF) for 7 days in the refrigerator prior the testing.
5. Let the vial stand for 10 minutes to get the best extraction.
6. Take the specimen collection vial with the sample diluted, cut the end of the cap and dispense 20 drops of the sample diluted (figure 4) into a analyser vial (microtube). This vial must be compatible with the analyzer.

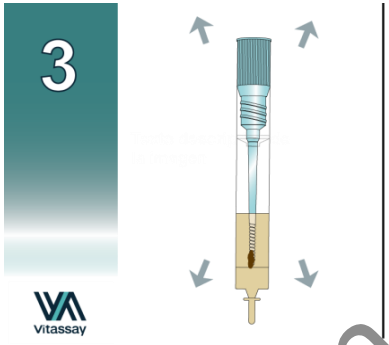
Note: Do not use the sample vials directly in the analyzer.



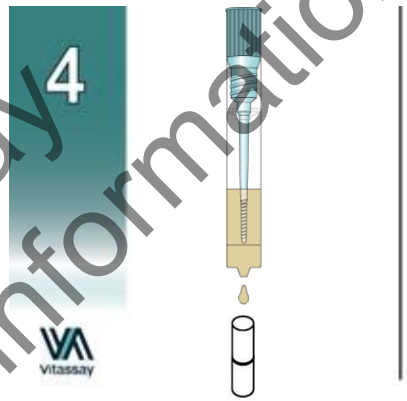
Vial for sample dilution.



Insert the stick in 4 different areas of the stool.



Put the sample into the vial, close the vial and shake for a good dispersion of the sample (vortex until the sample is fully resuspended máx.1 minute). Then, let the vial stand for 10 minutes to get the best extraction.



Dispense 20 drops into a analyser vial (microtube).

PROCEDURE

Allow reagents and solid stool to reach room temperature (15-30ºC/59-86ºF) prior the testing. 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 esblished 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

Cut-off must be fixed by the clinical laboratory.

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.

Values higher than the cut-off determine the abnormal presence of human calprotectin (hCp) in stool samples.

QUALITY CONTROL

Calprotectin C1 & C2 Controls are ready to use. Allow controls to reach room temperature (15-30ºC/59-86ºF) prior to testing.

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.

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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.

LIMITATIONS

- **Vitassay Calprotectin -Turbidimetric Assay** should be only used in human solid 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 considerer 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.

Cut-off value of **Vitassay Calprotectina-Turbidimetric Assay**-: 50-200 µg hCp/g.

Calprotectin concentration values greater than 200 µg hCp/g are indicators of inflammation of the gastrointestinal tract.

Prozone:

Lower concentrations of 8000 µg 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*	*Bovine Lactoferrin*	*None*
Pig Haemoglobin	*None*	*Human Haemoglobin*	*None*
Bovine Transferrin	*None*	*Human Transferrin*	*None*
Pig Transferrin	*None*	*Human Lactoferrin*	*None*

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1. Faecal calprotectin and faecal occult blood tests in the diagnosis of colorectal carcinoma and adenoma. Tibble J, Sigthorsson G, Foster R, Sherwood R, Fagerhol M, Bjarnason I. Gut. 2001 Sep;49(3):402-8.
2. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease.Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Gastroenterology. 2000 Jul;119(1):15-22.
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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	Σ n	Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number
	Keep out of the sunlight		

ADAPTED EQUIPMENT

- Biolis i24 (Tokio Boeki)
- BS200 (Mindray)
- Architect 4000/8000 (Abbott)
- Vitros 5600 (Ortho)
- ChemwellT (Awareness)
- A15 (Biosystems)
- Cobas c501 (Roche)

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