VITASSAY

Calprotectin 50+200

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

IUE-7455005 Ed01 September 2023

CE IVD EN

Vitassay

EN

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Calprotectin 50+200 is a rapid immunochromatographic, one step assay for the simultaneous qualitative detection of human calprotectin in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay for the detection of human calprotectin. To determine inflammatory disease, to monitor treatment response in these patients, to predict risk of relapse and to monitor small bowel transplantation or graft rejection.

INTRODUCTION

Calprotectin is a protein that is released by neutrophils in the bowel in case of inflammation, as is the case in inflammatory bowel disease. In patients suspected for inflammatory bowel disease, a negative fecal calprotectin indicates a low chance of having bowel inflammation (high negative predictive value), whereas an elevated fecal calprotectin needs further investigation with endoscopy and histology. A recent meta-analysis calculated that calprotectin screening might reduce the need for endoscopic procedures in adults with suspected inflammatory bowel disease in up to 67%.

PRINCIPLE

Vitassay Calprotectin 50+200 is a qualitative immunochromatographic assay for the detection of human calprotectin in human stool samples.

Strip A: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against human calprotectin (≥ 50µg/g).

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against human calprotectin (≥ 200µg/g).

During the process, the sample reacts with the antibodies against calprotectin, forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is human calprotectin positive (concentration ≥ 50µg/g), antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in the strip A, and if the sample is human calprotectin positive (concentration ≥ 200µg/g), antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible in strip B. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears (for both strips).

The presence of these green lines (in the control zone (C)) indicates that sufficient volume is added; proper flow is obtained and serves as an internal control for the reagents.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Do not use the test if its pouch is damaged.
- Read the instructions for use carefully before using the test.
- Do not use the kit if the label sealing the outer carton is torn or if the bags are open or damaged on arrival.
- Do not use the tests if the desiccant material is missing or broken inside the aluminium pouch.
- Tests should be discarded in a proper biohazard container after testing.
- Specimens should be considered potentially hazardous and should be handled in the same manner as an infectious agent, following local/national regulations. A new test should be used for each sample to avoid contamination errors.
- Material exposed to the samples should also be considered potentially hazardous and should be handled in the same manner as an infectious agent, following local/national regulations.
- Do not reuse. This is a single-use device.
- Used material should be disposed of in an appropriate 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 50+200** Do not use any other commercial kit component or components from other batches.
- Follow Good Laboratory Practices. These practices should include, but are not limited to, personal protective equipment (PPE), such as lab coat, surgical or appropriate mask or face shield, disposable gloves and eye protection. Take the necessary precautions during sample collection, transport, storage, handling and disposal. Each sample must be correctly and unequivocally identified to ensure proper traceability of samples.
- In case of spillage, clean thoroughly with a suitable disinfectant.
- Do not eat, drink or smoke in the workplace.
- The presence of yellow lines in the result window (control line area and test line area), before using the test, is completely normal and does not imply a failure in the functionality of the test.
- The visual interpretation of the results is done by coloured lines, the interpretation of the results should be done by a professional user without problems of visualisation and colour interpretation.
- A certificate of analysis can be provided on request (not included).

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STORAGE AND STABILITY

The storage temperature of the kits should be 2-30ºC.

Do not freeze.

Under these conditions, they can be used until the expiry date indicated on the kit label.

All kit components are for single use only and must remain in their primary packaging until use. The test must remain in the sealed pouch until use.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ 25 tests/kit **Vitassay Calprotectin 50+200** ▪ Instructions for use. ▪ **25** vials with diluent for the sample dilution.	▪ PPE, such as disposable gloves ▪ Specimen collection container ▪ Timer ▪ Micropipette (in case of liquid stool)

SPECIMEN COLLECTION

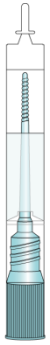
Collect sufficient quantity of feces: 1-2 g or 1-2 mL for liquid samples. Stool should be collected in clean and dry containers.

Samples can be stored in the refrigerator (2-8ºC) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC. Samples must be brought to room temperature before testing.

SPECIMEN PREPARATION

1. Take out the cap of the vial with diluent for the sample dilution (figure 1).
2. 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 the sample dilution. For liquid stool, take 15 µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.
3. Close the tube with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dilution (figure 3). The stool collection vial with diluted sample can be stored for 7 days in the refrigerator (2-8ºC) prior to testing.

1

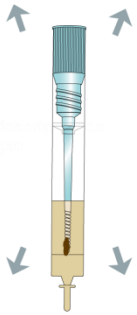


Vial for sample dilution.

2

Put the sample into the vial, close the cap and shake.

3



Put the sample into the vial, close the cap and shake.

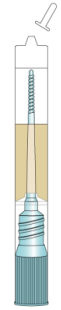
PROCEDURE

Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample to obtain a good sample dilution.
2. Remove the **Vitassay Calprotectin 50+200** from its sealed bag just before using it.
3. Take the vial containing the diluted sample, cut the end of the cap (figure 4) and dispense 4 drops in the circular window marked with the letter A-Calprotectin 50 (figure 5), and 4 drops, using the same vial in the circular window marked with the letter B-Calprotectin 200 (figure 6).
4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

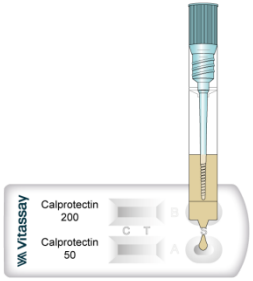
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.

4



Cut the end of the cap.

5



Dispense 4 drops in the circular window marked with the letter S for the strip A – Calprotectin 50.

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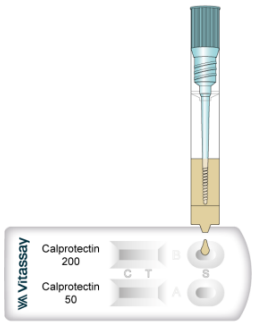
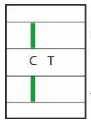
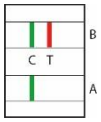
6



Dispense 4 drops in the circular window marked with the letter S for the strip B – Calprotectin 200.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A Calprotectin 50 (≥ 50µg/g)	Strip B Calprotectin 200 (≥ 200µg/g)	INTERPRETATION
	**Negative**	**Negative**	Calprotectin marker is not present in patient sample (<50µg/g), which might mean neither active gastrointestinal inflammation, nor risk of relapse (CD or UC relapse), nor graft or transplantation rejection.
	GREEN	GREEN	
	**Positive**	**Positive**	High quantity of calprotectin marker (≥200µg/g) is present in patient sample, which might mean active gastrointestinal inflammation, risk of relapse in clinical remisión, graft rejection or transplantation rejction.
	GREEN-RED	GREEN-RED	
	**Negative**	**Positive**	Invalid result. If B is positive, A has to be also positive.
	GREEN	GREEN-RED	
Any other result			Invalid result, we recommend repeating the assay using the sample with another test. **Note**: Wrong procedural techniques, insufficient sample volume or deterioration of the reagents are mostly the main reasons for control line failure. If the symptoms or situation persist, discontinue using the test kit and contact your local distributor.

Notes: The intensity of the red colored test line in the result line zone (T) will vary depending on the concentration of antigens in the specimen.

QUALITY CONTROL

Internal procedural control is included in **Vitassay Calprotectin 50+200**. Green line appearing in the results window is an internal control, which confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS

- An excess of stool sample could cause wrong results (brown bands appear). Dilute the sample with the diluent and repeat the test.
- The intensity of test line may vary depending on the concentration of human calprotectin.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay Calprotectin 50+200** 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.
- Negative results should not be considered as conclusive; it is possible that the concentration of human calprotectin is lower than the cut-off value. If symptoms or situation still persist calprotectin detection should be carried out using invasive techniques. 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).
- Active inflammatory bowel disease (such as Crohn's disease and ulcerative colitis) usually involves significant neutrophilic intestine inflammation. Stool from patients suffering from those illnesses might obtain positive results for fecal calprotectin.
- **Vitassay Calprotectin 50+200** might be sensitive for the diagnosis in patients with chronic diarrhea.
- **Bloody stool** samples and/or **mucuos stool** samples can cause non-specific reactions in the test. Such positive samples should be followed up with other diagnostic techniques to confirm the result

EXPECTED VALUES

Several studies have shown that about 90 per cent of colorectal cancer patients have increased calprotectin levels. This means that more cancer will be found by the calprotectin test at the expense of some extra endoscopies with negative results.

The diagnostic accuracy of fecal calprotectin would decrease the numbers of endoscopies needed up to 3-fold in adults and 35% in children and, therefore, significantly reduces costs.

PERFORMANCE CHARACTERISTICS

Cut-off value

Cut-off value **Vitassay Calprotectin 50+200**:

- Strip A: 500ng/mL (50µg hCp/g feces)
- Strip B: 2000ng/mL (200µg hCp/g feces)

Clinical sensitivity and specificity

An evaluation, with fecal samples, was performed comparing the results obtained by **Vitassay Calprotectin 50+200** and another commercial immunoassay (Calprest®, Eurospital).
Results were as follows:

		Calprest®		
		Positive	Negative	Total
Vitassay Calprotectin 50+200	Positive	34	2	36
	Negative	2	26	28
	Total	36	28	64

Table 1. Results of **Vitassay Calprotectin 50 + 200** compared to a commercial ELISA test (Calprest) for calprotectin detection.

Vitassay Calprotectin 50+200 vs Calprest®			
Sensitivity	Specificity	PPV	NPV
>94%	93%	>94%	93%

Table 2. Sensitivity, Specifity, PPV and NPV of **Vitassay Calprotectin 50 + 200** compared to a commercial ELISA test (Calprest) for calprotectin detection.

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The results showed that **Vitassay Calprotectin 50+200** has a high sensitivity and specificity to detect human calprotectin.

Cross reactivity

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

Adenovirus	*Entamoeba histolytica*	Human Lactoferrin	*Shigella boydii*
Astrovirus	*Escherichia coli O111*	*Legionella pneumoniae*	*Shigella dysenteriae*
Campylobacter coli	*Escherichia coli O26*	*Listeria monocytogenes*	*Shigella flexneri*
Campylobacter jejuni	*Escherichia coli O157*	Norovirus GI	*Shigella sonnei*
Clostridium difficile antigen GDH	*Giardia*	Norovirus GII	*Streptococcus pneumococcal*
Clostridium perfringens Toxin A	*Helicobacter pylori*	Rotavirus	*Streptococcus pyogenes*
Clostridium perfringens Toxin B	Bovine Haemoglobin	*Salmonella enteritidis*	Bovine Transferrin
Clostridium perfringens	Porcine Haemoglobin	*Salmonella paratyphi A*	Human Transferrin
Cryptosporidium	Human Haemoglobin	*Salmonella typhi*	*Yersinia Enterocolitica O:3*
Entamoeba dispar	Bovine Lactoferrin	*Salmonella typhimurium*	*Yersinia Enterocolitica O:9*

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2. ERLING AADLAND; MAGNE K. FAGERHOL. "Faecal calprotectin: a marker of inflammation throughout the intestinal tract". European Journal of Gastroenterology & Hepatology 2002, 14: 823-825.

3. JOSÉ MANUEL BENITEZ; VALLE GARCÍA-SÁNCHEZ. "Faecal calprotectin: Management in inflammatory bowel disease". World Journal of Gastrointestinal Pathophysiology. 2015 November 15; 6(4): 203-209.

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
CE	CE Marking		

Changes control		
Nº version	**Changes**	**Date**
IUE-7455005 Ed00 October 2016	Original version	10/2016
IUE-7455005 Ed01 September 2023	The format has been updated. Limitations section has been updated and new cross-reactions have been added. Grammatical and editorial changes have been made to Precautions, Limitations, Sample Collection, Storage and Stability. Materials required, but not included updated with minor changes. A transcription error in the interpretation section has been corrected.	21/09/2023

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