VITASSAY

Calprotectin

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

IUE-7355004 Ed01 September 2023

CE IVD EN

Vitassay

EN

For professional *in vitro* diagnostic use only.

INTENDED USE

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

Simple, non-invasive and highly sensitivity immunoassay for the presumptive diagnosis of human calprotectin, which might be useful for the diagnosis of inflammatory gastrointestinal disorders.

INTRODUCTION

The protein calprotectin constitutes approximately 60% of the soluble cytoplasmic proteins in neutrophilic granulocytes and has been found in increased concentrations in faeces from symptomatic patients with colorectal cancer (CRC), inflammatory bowel disease, and certain infections. The increased concentrations in patients with CRC is probably not related to intestinal bleeding but may reflect release from surrounding leucocytes.

PRINCIPLE

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

The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against calprotectin.

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 positive, antibodies present on the membrane (test line) capture the conjugate complex and a red line will be visible. Although the sample is positive or negative, the mixture continues to move across the membranes and the green control line always appears.

The presence of this green line (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**. 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).

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.

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MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ 25 tests/kit **Vitassay Calprotectin** ▪ 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

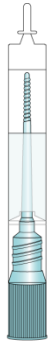
Collect sufficient quantity of faeces: 1-2 g or mL for liquid samples. Stool should be collected in clean and dry containers.

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

1

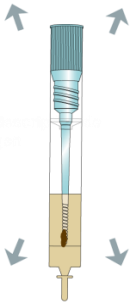


Vial for sample dilution.

2

Insert the stick in 4 different areas of the stool.

3



Put the sample into the vial, close the vial 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.

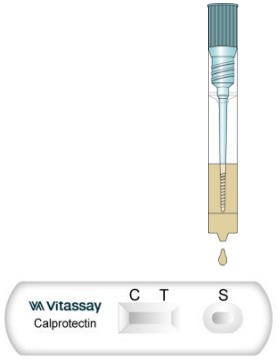
1. Shake the vial with the sample to obtain a good sample dilution.
2. Remove the **Vitassay Calprotectin** from its sealed bag just before using it.
3. Take the vial containing the diluted sample, cut the end of the cap (figure 4) and dispense 4 drops in the circular window marked with the letter S (figure 5).
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 circular 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.

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INTERPRETATION OF THE RESULTS

	**NEGATIVE** Only one green line in the control zone **(C)**.	There is no human calprotectin presence which might mean neither active gastrointestinal inflammation, nor risk of relapse (Ulcerative Colitis relapse or Crohn's Disease.
	**POSITIVE** In addition to the green line (**control line C**), a red line appears (**test line T**).	There is human calprotectin present which might mean active gastrointestinal inflammation or risk of relapse in clinical remission.
Any other results		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 human calprotectin in the specimen.

QUALITY CONTROL

Internal procedural control is included in **Vitassay Calprotectin**. Green line appearing in the 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** 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** might be sensitive for the diagnosis in patients with chronic diarrhea.
- **Bloody stool** samples and/or **mucous 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.

PERFORMANCE CHARACTERISTICS

Cut-off value

Cut-off value of **Vitassay Calprotectin** is 500ng/mL (50µg/ hCp/g faeces) for human calprotectin.

Clinical sensitivity and specificity

An evaluation, with fecal samples, was performed comparing the results obtained by **Vitassay Calprotectin** and another commercial immunoassay (Calprest®, Eurospital) based on the ELISA method.

Results were as follows:

		Calprest®		
		Positive	**Negative**	**Total**
Vitassay Calprotectin	**Positive**	34	2	36
	Negative	2	26	28
	Total	36	28	64

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

Vitassay Calprotectin vs Calprest®			
Sensitivity	**Specificity**	**PPV**	**NPV**
>94%	93%	>94%	93%

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

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

Cross reactivity

No cross reactivity was detected against other microorganisms or substances occasionally present in faeces:

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 difficile Toxin A	*Helicobacter pylori*	Rotavirus	*Streptococcus pyogenes*
Clostridium difficile 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*

REFERENCES

1. O KRONBORG; M UGSTAD; P FUGLERUD, B JOHNE, J HARDCASTLE; J H SCHOLEFIELD; K VELLACOTT; V MOSHAKIS; J R REYNOLDS. "Faecal calprotectin levels in a high risk population for colorectal neoplasia" Gut 2000, 46: 795-800.

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.

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-7355004 Ed00 January 2017	Original Version	01/2017
IUE-7355004 Ed01 September 2023	The format has been updated. A transcription error in the interpretation section has been corrected. New cross-reactivities has been added. The limitations section has been updated. Grammatical and editorial changes have been made to the Precautions, Limitations, Sample Collection, Storage and Stability sections. Required but not included material updated with minor changes.	21/09/2023

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