

VITASSAY

Calprotectin

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

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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 feces 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.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors. Single use device.

- Tests 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**. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30°C/36-86°F).

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
<ul style="list-style-type: none"> • 25 tests/kit Vitassay Calprotectin • Instructions for use. • 25 vials with diluent for the sample dilution. 	<ul style="list-style-type: none"> • Specimen collection container. • Disposable gloves. • Timer.

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 (2-8°C/36-46.4°F) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20°C (-4°F). 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.

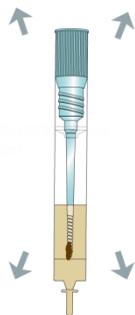
3. Close the vial with the diluent and stool sample. Shake the vial in order to assure good sample dispersion (figure 3). The vial for the sample dilution with diluted sample can be stored for 7 days in the refrigerator (2-8°C / 35.6-46.4°F) prior to testing.



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.

PROCEDURE

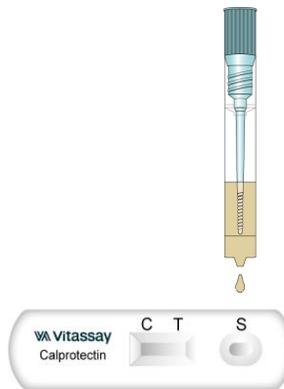
Allow the test, stool sample, controls and diluent to reach room temperature (15-30°C / 59-86°F) prior to testing. Do not open pouches until the performance of the assay.

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.



Cut the end of the cap.



Dispense 4 drops in the circular window marked with the letter S.

INTERPRETATION OF THE RESULTS

		NEGATIVE	There is no human calprotectin presence which might mean neither active gastrointestinal inflammation, nor risk of relapse (Ulcerative Colitis relapse or Crohn's Disease).
		Only one green line in the control zone (C).	
		POSITIVE	There is human calprotectin present which might mean active gastrointestinal inflammation or risk of relapse in clinical remission.
		In addition to the green line (control line C), a red line appears (test line T).	
Any other results			Invalid result, we recommend repeating the assay using the sample with another test. Note: Wrong procedural techniques or deterioration of the reagents are the main reasons of control line failure. If the symptoms or situation still 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

- **Vitassay Calprotectin** must be carried out within 2 hours of opening the sealed bag.
- 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.

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 feces) 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).

Results were as follows:

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

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

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 fecal markers that are occasionally present in feces:

Bovine and pig haemoglobin	Human haemoglobin
Bovine and pig transferrin	Human transferrin
Bovine lactoferrin	Human lactoferrin

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		Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number



