

VITASSAY

Calprotectin+Lactoferrin

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

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EN

For professional *in vitro* diagnostic use only.

INTENDED USE

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

Simple, non-invasive and highly sensitivity immunoassay for the detection of human calprotectin and human lactoferrin in feces. To determine intestinal inflammatory activity, to monitor treatment response in these patients and to predict risk of relapse.

INTRODUCTION

Inflammatory bowel diseases (IBD), ulcerative colitis (UC), Crohn's disease (CD), and unclassified IBD (IBDU) are lifelong chronic inflammatory conditions that may break out even in very young children. Both in pediatric and adult patients, the mainstay in the diagnostics of IBD are endoscopy with biopsies. Endoscopy is also needed to detect and quantify intestinal inflammation during the disease course. Assessment of mucosal inflammatory activity is important, because targeting mucosal healing in IBD treatment instead of only controlling symptoms is becoming a new therapy goal. However, endoscopic procedure is invasive, requires a skilled operator and bowel preparation, and is unpleasant especially for young patients. Thus, the need for surrogate markers of intestinal inflammation that may substitute the use of endoscopy is strong.

Fecal markers fulfill all the criteria of being noninvasive, simple, inexpensive, sensitive, and specific markers to detect gastrointestinal inflammation. Calprotectin is a calcium-binding protein, representing up to 60% of the cytosolic proteins in neutrophils, its concentration is directly proportional to the concentration of neutrophils in the colonic/rectal mucosa. It is resistant to bacterial degradation in the gut and is stable in the stool for up to 1 week at room temperature. Lactoferrin is an iron-binding glycoprotein and a major component of the secondary granules of polymorph nuclear neutrophils. During intestinal inflammation, leucocytes invade the mucosa, which may lead to an increase in the excretion of lactoferrin into the stool. Lactoferrin can be measured by using simple and inexpensive techniques since it is stable in the stool for up to 4 days.

PRINCIPLE

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

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

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against human lactoferrin.

During the process, the sample reacts with the antibodies against calprotectin (strip A) and/or lactoferrin (strip B), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is human calprotectin positive, 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 lactoferrin positive, 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.
- 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.
- Do not reuse. This is a single-use device.
- 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.
- 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+Lactoferrin**. Do not use any other commercial kit component.
- 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 2-30°C.

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
<ul style="list-style-type: none"> ▪ 25 tests/kit ▪ Vitassay Calprotectin+Lactoferrin ▪ Instructions for use. ▪ 25 vials with diluent for the sample dilution. 	<ul style="list-style-type: none"> ▪ 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 (2-8°C) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20°C. 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.

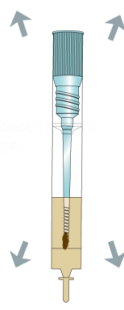
3. Close the tube with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dilution (figure 3). The stool collection vial with diluted sample can be stored for 7 days in the refrigerator (2-8°C) prior to testing.



Vial for sample dilution.



Insert the stick in 4 different areas of the stool.



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

PROCEDURE

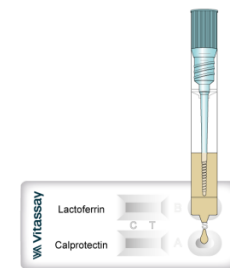
Allow the test, stool sample, controls and diluent to reach room temperature (15-30°C) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample to obtain a good sample dilution.
2. Remove the **Vitassay Calprotectin+Lactoferrin** 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 for the strip A-Calprotectin (figure 5), and 4 drops, using the same vial in the circular window marked with the letter S for the strip B-Lactoferrin (figure 6).
4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

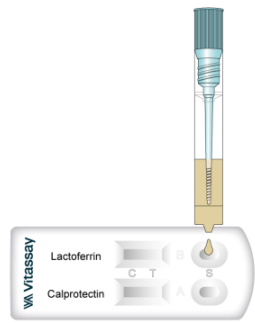
If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.



Cut the end of the cap.



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



Dispense 4 drops in the circular window marked with the letter B for the strip B - Lactoferrin.

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+Lactoferrin**. 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+Lactoferrin** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of human calprotectin and/or human lactoferrin 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 and/or lactoferrin 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+Lactoferrin** 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
- This test is a screening tool. The final diagnosis must be confirmed by a physician after a thorough evaluation of the clinical history and additional confirmatory tests.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A Calprotectin	Strip B Lactoferrin	INTERPRETATION
	Negative GREEN	Negative GREEN	There is no human calprotectin and/or human lactoferrin presence which might mean neither active gastrointestinal inflammation, nor risk of relapse (Crohn's Disease or Ulcerative Colitis).
	Positive GREEN-RED	Positive GREEN-RED	There is human calprotectin and human lactoferrin presence which might mean an inflammatory intestinal disease (caused by ulcerative colitis, Crohn's Disease, some type of carcinomas or an enteropathy caused by <i>Shigella</i> , <i>Salmonella</i> , <i>Campylobacter</i> or <i>Clostridium difficile</i>) or risk of relapse in clinical remission.
	Positive GREEN-RED	Negative GREEN	There is human calprotectin presence which might mean active gastrointestinal inflammation or risk of relapse in clinical remission.
	Negative GREEN	Positive GREEN-RED	There is human lactoferrin presence which might mean inflammatory intestinal disease.

EXPECTED VALUES

The annual incidence of pediatric inflammatory bowel diseases is rising, being around 5.0 to 7.0/100,000. Studies have determined that the cut-off value for human calprotectin should be g hCp/g faeces and human lactoferrin 10µg hLf/g faeces, to allow detection of adult patients with inflammatory gastrointestinal pathology who will require additional invasive diagnostic procedures.

PERFORMANCE CHARACTERISTICS

Cut-off value
 Cut-off value **Vitassay Calprotectin +Lactoferrin**:

- Strip A: 500ng/mL (50µg hCp/g feces)
- Strip B: 100ng/mL (10µg hLf/g feces)

Clinical sensitivity and specificity
 An evaluation, with fecal samples, was performed comparing the results obtained by **Vitassay Calprotectin + Lactoferrin** and others commercial immunoassays (Calprest®, Eurospital, and IBD EZ VUE®, TechLab®).

Results were as follows:

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

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

Vitassay Calprotectin + Lactoferrin (Calprotectin) vs Calprest®			
Sensibility	Specificity	PPV	NPV
>94%	93%	>94%	93%

Table 2. Sensitivity, Specificity, positive predictive value and negative predicted value of **Vitassay Calprotectin + Lactoferrin** (Calprotectin) compared to a commercial ELISA test for calprotectin detection.

Vitassay Calprotectin + Lactoferrin		IBD EZ VUE®		
		Positivo	Negativo	Total
		Positivo	20	0
Negativo	0	44	44	
Total		20	44	64

Table 3. Results obtained by immunochromatographic test **Vitassay Calprotectin + Lactoferrin** (Lactoferrin) compared to a commercial immunoassay.

Vitassay Calprotectin + Lactoferrin (Lactoferrin) vs IBD EZ VUE®			
Sensibilidad	Especificidad	VPP	VPN
>99%	>99%	>99%	>99%

Table 4. Sensitivity, specificity, positive predictive values and negative predictive values of the **Vitassay Calprotectin + Lactoferrin** (Lactoferrin) compared to a commercial immunoassay.

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

Cross reactivity

No cross reactivity was detected against other fecal markers and microorganisms occasionally present in faeces:

Strip A (Calprotectin):

Adenovirus	<i>Entamoeba histolytica</i>	Human Lactoferrin	<i>Shigella boydii</i>
Astrovirus	<i>Escherichia coli</i> O111	<i>Legionella pneumoniae</i>	<i>Shigella dysenteriae</i>
<i>Campylobacter coli</i>	<i>Escherichia coli</i> O26	<i>Listeria monocytogenes</i>	<i>Shigella flexneri</i>
<i>Campylobacter jejuni</i>	<i>Escherichia coli</i> O157	Norovirus GI	<i>Shigella sonnei</i>
<i>Clostridium difficile</i> antigen GDH	<i>Giardia</i>	Norovirus GII	<i>Streptococcus pneumococcal</i>

<i>Clostridium perfringens</i> Toxin A	<i>Helicobacter pylori</i>	Rotavirus	<i>Streptococcus pyogenes</i>
<i>Clostridium perfringens</i> Toxin B	Bovine Haemoglobin	<i>Salmonella enteritidis</i>	Bovine Transferrin
<i>Clostridium perfringens</i>	Porcine Haemoglobin	<i>Salmonella paratyphi</i> A	Human Transferrin
<i>Cryptosporidium</i>	Human Haemoglobin	<i>Salmonella typhi</i>	<i>Yersinia Enterocolitica</i> O:3
<i>Entamoeba dispar</i>	Bovine Lactoferrin	<i>Salmonella typhimurium</i>	<i>Yersinia Enterocolitica</i> O:9












Strip B (Lactoferrin):

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

REFERENCES

- KAIJA-LEENA KOLHO; TAINA SIPPONEN; ELSA VALTONEN; ERKKI SAVILAHTI. "Fecal calprotectin, MMP-9, and human beta-defensin-2 levels in pediatric inflammatory bowel disease". Int J Colorectal Dis (2014) 29:43-50.
- TAKAYUKI YAMAMOTO; MANABU SHIRAKI; TAKUYA BAMBA; SATORU UMEGAE; KOICHI MATSUMOTO. "Fecal calprotectin and lactoferrin as predictors of relapse in patients with quiescent ulcerative colitis during maintenance therapy". Int J Colorectal Dis (2014) 29:485-491.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	in vitro diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
	Batch code		Contains sufficient for <n> test
	Sample diluent		Catalogue number
	CE Marking		

Changes control		
Nº version	Changes	Date
IUE-7455006 Ed00 July 2023	Original version	07/2016
IUE-7455006 Ed01 September 2023	The format has been updated. The limitations section has been updated and new cross-reactions have been added. Transcription error has been corrected in interpretation section. Grammatical and editorial changes have been made to Precautions, Limitations, Sample Collection, Storage and Stability. Required, but not included material updated with minor changes.	09/2023



