VITASSAY

# Lactoferrin

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

IUE-7355046 Ed01 Septiembre 2023

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Vitassay

EN

**For professional *in vitro* diagnostic use only.**

## INTENDED USE

**Vitassay Lactoferrin** is a rapid, immunochromatographic, one step assay for the qualitative detection of human lactoferrin (hLf) in human stool samples.

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

## INTRODUCTION

Inflammatory bowel disease (IBD), i.e., Crohn's disease (CD) and ulcerative colitis (UC), are chronic, nonspecific, and relapsing inflammatory conditions affecting varying layers of the gastrointestinal tract with a poor prognosis. In routine clinical practice, early and accurate diagnosis of IBD is essential for optimal treatment and the avoidance of surgery.

Lactoferrin (LF) is an iron binding glycoprotein secreted by most mucosal membranes and a major component of secondary granules of polymorpho-nuclear neutrophils, a component of the inflammatory response.

An increase in fecal lactoferrin levels occurs during intestinal inflammation due to mucosal infiltration and degranulation of neutrophils.

Elevated LF has been used as a marker of active IBD and for monitoring patients for response to treatment. LF levels were significantly higher in patients with inactive IBD than in patients with IBS (irritable bowel syndrome), making it a valuable investigative tool.

Fecal lactoferrin has high positive and negative predictive values for the diagnosis of small bowel Crohn's disease. This highlights how fecal lactoferrin could be used to aid in diagnosing small bowel Crohn's disease. Taking this approach may avoid some of the delays in diagnosis which can occur in patients with small bowel Crohn's disease.

## PRINCIPLE

**Vitassay Lactoferrin** is a qualitative immunochromatographic assay for the detection of human lactoferrin in human stool samples.

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

During the process, the sample reacts with the antibodies against lactoferrin, 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 Lactoferrin**. 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.



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- 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.

## MATERIALS

| MATERIAL PROVIDED | MATERIAL REQUIRED BUT NOT PROVIDED |
|---|---|
| ▪ 25 tests/kit **Vitassay Lactoferrin**<br>▪ Instructions for use.<br>▪ 25 vials with diluent for the sample dilution. | • PPE, such as disposable gloves<br>▪ Specimen collection container<br>▪ Timer<br>▪ Micropipette (in case of liquid stool) |

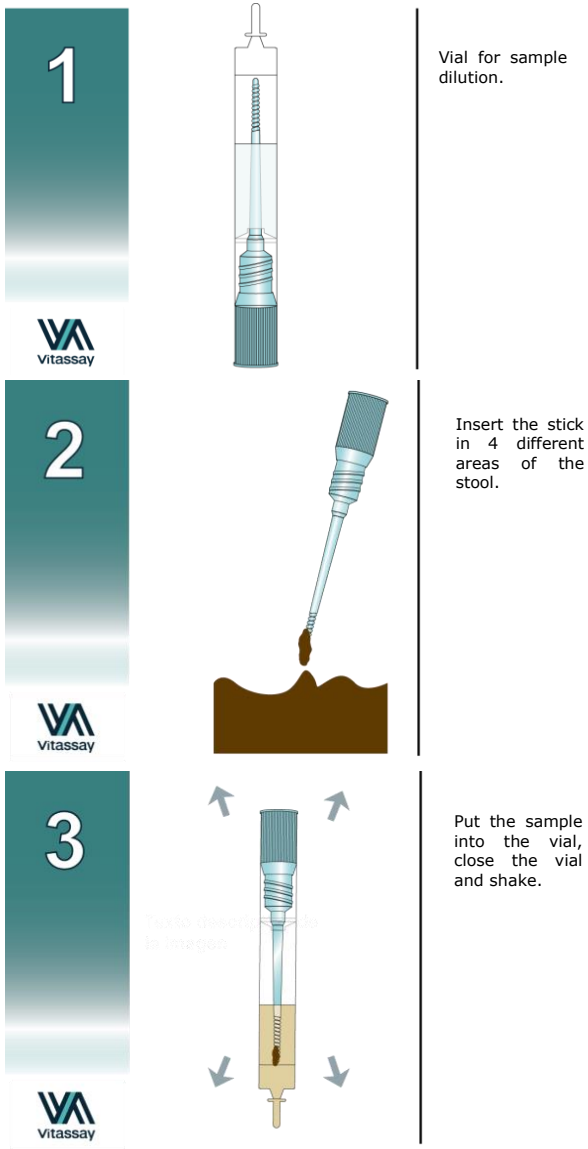
## SPECIMEN COLLECTION

Collect sufficient quantity of faeces: 1-2g 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 15µL 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.

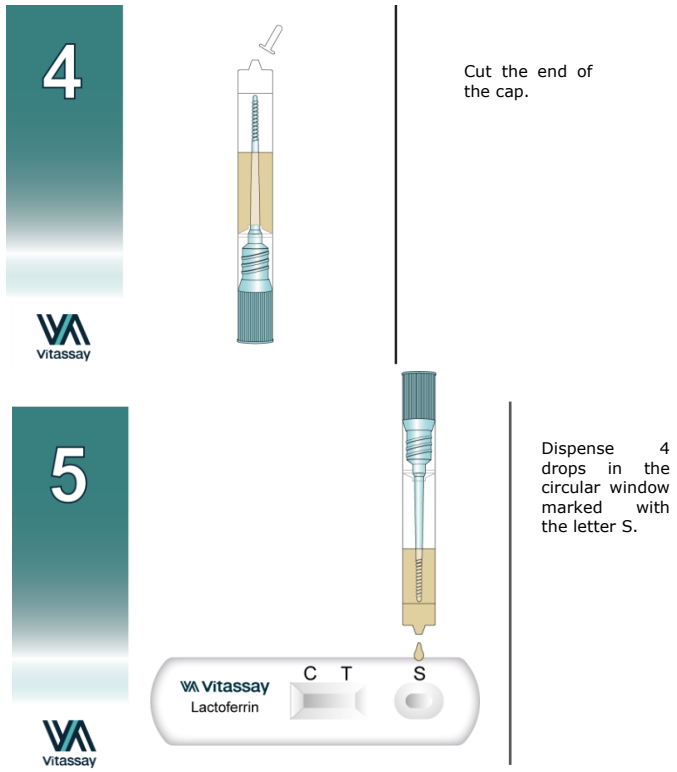



## PROCEDURE

Allow the test, stool sample, controls (if applicable) 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 vigorously to obtain a good sample dilution.
2. Remove the **Vitassay 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 (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.



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## INTERPRETATION OF THE RESULTS

| | | |
|---|---|---|
|  | **NEGATIVE**<br>Only one green line in the control zone **(C)**. | There is no human lactoferrin presence which could mean absence of gastrointestinal inflammation. |
|  | **POSITIVE**<br>In addition to the green line (**control line C**), a red line appears (**test line T**). | There is human lactoferrin presence which could mean there is inflamatory intestinal disease. |
| **Any other results** | | Invalid result, we recommend repeating the assay using the sample with another test.<br>**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 lactoferrin in the specimen.

## QUALITY CONTROL

Internal procedural control is included in **Vitassay Lactoferrin**. 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 lactoferrin.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay Lactoferrin** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of human lactoferrin in fecal samples; nevertheless, it can be due to several causes besides inflammatory bowel disease (IBD). A positive result should be followed up with additional diagnostic procedures, endoscopy and histology on biopsy specimens, for detecting and quantifying bowel inflammation.
- Negative results should not be considered as conclusive; it is possible that the concentration of human lactoferrin is lower than the cut-off value. Negative results do not exclude inflammation, some diseases such as celiac sprue and microscopic colitis polyps that mainly involve mononuclear inflammation.
- Breast- fed children could show positive results due to lactoferrin is a component of breast milk, therefore the test should not be used to evaluate neonates receiving breast milk.
- 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 lactoferrin. **Vitassay 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.

## EXPECTED VALUES

Diarrheal illnesses are extremely common throughout the world, causing 2 to 16 or more illnesses per person per year in developed and developing countries and often posing diagnostic and therapeutic questions for physicians. The causes of diarrhea include a wide variety of etiologic agents, many of which have been recognized only in the past two decades.

In order to decrease the misdiagnosis rate, the LF could be used in conjunction with other parameters (blood inflammatory markers) to determine the subset of patients who have active disease or who may require a step up in therapy.

Elevated fecal lactoferrin levels have been reported in IBD with a sensitivity of 78%, and specificity of 90% in identifying inflammation in adults with chronic UC and CD. In addition, fecal lactoferrin showed good correlation to disease activity (endoscopic and histopathologic) and was 100% specific in ruling out IBS.

## PERFORMANCE CHARACTERISTICS

### Cut-off value

Cut-off value of **Vitassay Lactoferrin** is 100ng/mL (10µg hLf/g feces) for human lactoferrin.

### Clinical sensitivity and specificity

An evaluation, with fecal samples, was performed comparing the results obtained by **Vitassay Lactoferrin** and another commercial inmunochromatographic (IBD EZ VUE®, TechLab®).

Results were as follows:

| | | IBD EZ VUE® | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| **Vitassay Lactoferrin** | **Positive** | 20 | 0 | 20 |
| | **Negative** | 0 | 44 | 44 |
| | **Total** | 20 | 44 | 64 |

Table 1. Results obtained by immunochromatographic test **Vitassay Lactoferrin** compared to a commercial immunochromatographic test (IBD EZ VUE®, TechLab®).

| Vitassay Lactoferrin vs IBD EZ VUE® | | | |
|---|---|---|---|
| **Sensitivity** | **Specificity** | **PPV** | **NPV** |
| >99% | >99% | >99% | 99% |

Table 2. Sensitivity, specificity, positive predictive values and negative predictive values of the **Vitassay Lactoferrin** compared to a commercial immunochromatographic test (IBD EZ VUE®, TechLab®).

In addition, Vitassay Lactoferrin was evaluated with a total of 90 stool samples by purchasing the results obtained with a commercial for the detection of Lactoferrin.

The results are shown below:

| | | ELISA test: Human Lactoferrin ELISA kit | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| **Vitassay Lactoferrin** | **Positive** | 27 | 13 | 40 |
| | **Negative** | 3 | 47 | 50 |
| | **Total** | 30 | 60 | 90 |

Table 3. Results obtained by immunochromatographic test **Vitassay Lactoferrin** compared to a commercial ELISA test.

| Vitassay H. pylori vs ELISA test: Human Lactoferrin ELISA kit | | |
|---|---|---|
| | **Mean Value** | **95% confidence interval** |
| **Sensitivity** | 90.0% | 73.5 – 97.9% |
| **Specificity** | 78.3% | 65.8 – 87.9% |
| **PPV** | 67.5% | 50.9 - 81.4% |
| **NPV** | 94.0% | 83.5 –98.7% |

Tabla 4. Sensitivity, specificity, positive predictive values and negative predictive values of the **Vitassay Lactoferrin** compared to a commercial ELISA test.

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

### Cross-reactivity

No cross-reactivity was detected against the following microorganisms and other faecal markers occasionally present in feces:

| | | | |
|---|---|---|---|
| Adenovirus | *Entamoeba histolytica* | *Campylobacter coli* | *Shigella boydii* |
| Astrovirus | *Escherichia coli O111* | *Legionella pneumophila* | *Shigella dysenteriae* |



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|---|---|---|---|
| Human calprotectin | *Escherichia coli O26* | *Listeria monocytogenes* | *Shigella flexneri* |
| *Campylobacter jejuni* | *Escherichia coli O157* | Norovirus GI | *Shigella sonnei* |
| *Clostridium difficile* GDH antigen | *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 haemoblogib | *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. YAN WANG, FENGYAN PEI, XINGJUAN WANG, ZHIYU SUN, CHENGJIN HU, HENGLI DOU. "Diagnostic accuracy of fecal lactoferrin for inflammatory bowel disease: a meta-analysis". International Journal of Clinical and Experimental Pathology. 2015. Vol. 8, No 10, p. 12319-12332.

2. R. SIDHU, P. WILSON, A.WRIGHT, C.W.H.YAU, F.A.D'CRUZ, L.FOYE, S.MORLEY, A. J. LOBO, M. E. MCALINDON & D. S. SANDERS." Faecal lactoferrin – a novel test to differentiate between the irritable and inflamed bowel?". Alimentary Pharmacology and Therapeutics. 2010. 31, 1365–1370. 2010.

3. TAMARA PANG, STEVEN T. LEACH, TAMARA KATZ, ANDREW S. DAY AND CHEE Y. OOI. "Fecal biomarkers of intestinal health and disease in children". Frontiers in Pediatrics. Pediatric Gastroenterology, Hepatology and Nutrition. 28 January 2014.

4. RICHARD L. GUERRANT, VALTER ARAUJO, ELIZABETH SOARES, KAREN KOTLOFF, ALDO A. M. LIMA, WILLIAM H. COOPER AND AMELIA GAIL LEE. "Measurement of Fecal Lactoferrin as a Marker of Fecal Leukocytes". Journal of Clinical Microbiology, May 1992, Vol. 30, No. 5, p. 1238-1242.


## 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-7355046 Ed00 September 2016 | Original version. | 09/2016 |
| IUE-7355046 Ed01 September 2023 | The format has been updated. New evaluation has been included. The 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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