



Distribuito in ITALIA da  
**Li StarFish S.r.l.**  
Via Cavour, 35  
20063 Cernusco S/N (MI)  
telefono 02-92150794  
fax 02-92157285  
info@listarfish.it  
www.listarfish.it



## Rapid-VIDITEST

### Calprotectin-Lactoferrin

#### *One step calprotectin and lactoferrin Card Test*

#### Instruction manual

#### **INTENDED USE:**

The Rapid-VIDITEST Calprotectin-Lactoferrin Card is a rapid chromatographic immunoassay (non-invasive assay) for the qualitative detection of calprotectin and lactoferrin in human feces specimens, which might be useful for the diagnosis of inflammatory gastrointestinal disorders. Only for laboratory use.

#### **INTRODUCTION:**

**Calprotectin** is a calcium-containing protein that makes up 5% of the total protein and 60% of the cytosolic protein of neutrophil. It has bacteriostatic and fungistatic properties and is found in feces at levels six times higher than that in plasma. That fecal biomarker is useful to assess the activity of inflammatory bowel disease (IBD). IBD includes Crohn's Disease (CD) and Ulcerative Colitis (UC) and are associated with elevated neutrophils.

This fecal calprotectin assay is useful in differentiating organic (IBD) from functional gastrointestinal disease (IBS: Intestinal Bowel Syndrome). It is a simple, non-invasive biomarker that is especially useful in children, who may require general anesthesia for colonoscopy. And this fecal calprotectin detection can predict relapse.

**Lactoferrin** is a glycoprotein that is produced by neutrophils, mononuclear phagocytes and epithelial cells and is contained in the secretory fluids such as saliva and breast milk. Its function is to block bacterial growth by limiting the availability of iron and this effect is enhanced by the presence of specific secretory IgA antibodies directed against bacteria. Lf also has a bacteriocidal effect by causing direct damage to cell membranes in cooperation with lysozyme. When inflammation develops in the gastrointestinal tract, neutrophils and phagocytic cells migrate to the inflammatory focus and release the granules containing Lf. Lf is stable in faeces and is easily detected for immunochemical methods.

This marker is elevated in patients with inflammatory bowel disease. Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn disease (CD), represent a spectrum of diseases characterized by an idiopathic and chronic inflammation affecting the gastrointestinal (GI) tract. Pediatric and adult patients with IBD may present with a variety of clinical symptoms (including abdominal pain and diarrhea) that can be non-specific.

#### **PRINCIPLE:**

The Rapid-VIDITEST Calprotectin-Lactoferrin Card is a qualitative lateral flow immunoassay for the detection of human calprotectin and human lactoferrin in human feces samples. The membrane is pre-coated with monoclonal antibodies against human calprotectin and human lactoferrin on the test line regions. During testing, the sample reacts with the particle coated with anti-human calprotectin antibodies and/or with anti-human lactoferrin antibodies which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. In the case of a positive result the specific antibodies present on the membrane will react with the mixture conjugates and

generate one or two coloured lines. A green coloured line always appears in the control line and serves as verification that sufficient volume was added, that proper flow was obtained and as an internal control for the reagents.

#### **MATERIALS PROVIDED:**

- Rapid-VIDITEST Calprotectin-Lactoferrin Card tests
- Instruction for use
- Specimen collection vials with buffer

#### **MATERIALS REQUIRED BUT NO PROVIDED:**

- Specimen collection container
- Disposable gloves
- Timer

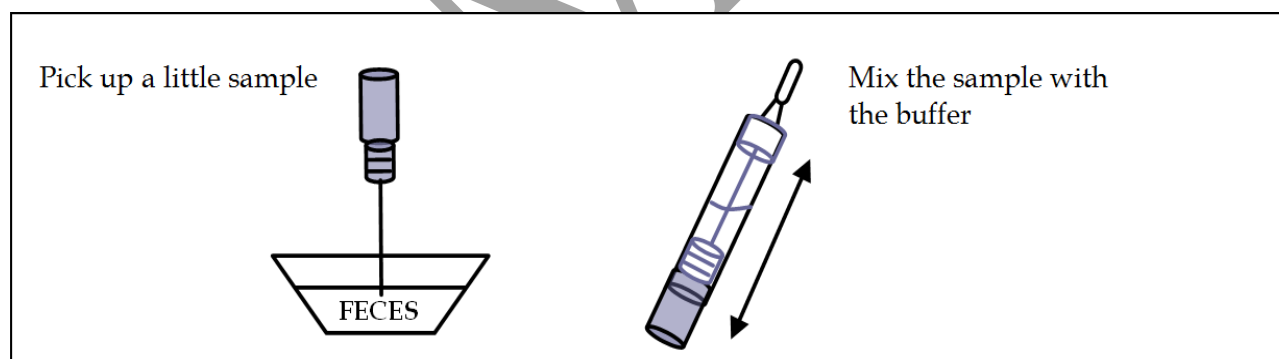
#### **SPECIMEN COLLECTION AND PREPARATION:**

Collect sufficient quantity of feces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The 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 month) the specimen must be kept frozen at -20°C/-4°F. In this case, the sample will be totally thawed, and brought to room temperature before testing.

#### **PROCEDURES:**

##### **To process the collected stool samples:**

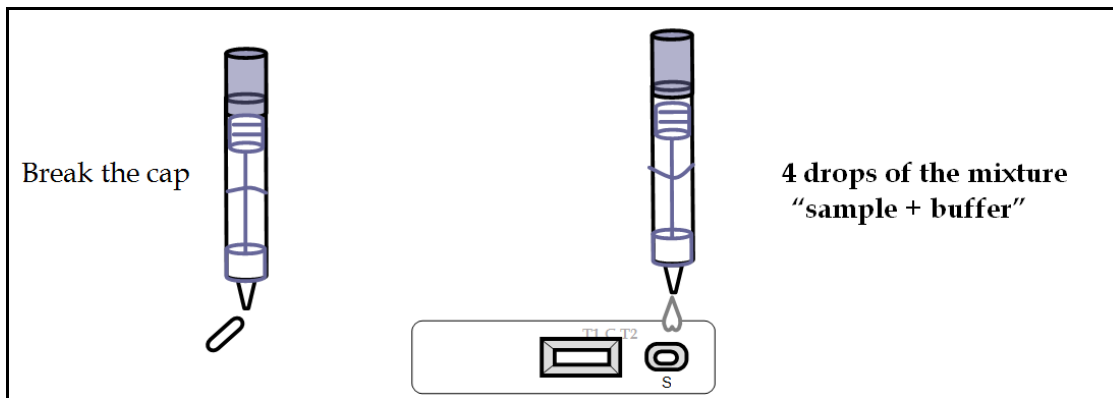
Use a separate specimen collection vial for each sample. Unscrew the cap of the vial and introduce the stick four times in different parts of the fecal specimen to pick up the sample. Close the vial with the buffer and stool sample. Shake the vial in order to assure good sample dispersion. For liquid stool samples, aspirate the fecal specimen with a dropper and add 15 µL into the specimen collection vial with buffer.



#### **Test procedure:**

**Allow the tests, stool samples and buffer to reach room temperature (15-30°C/59-86°F) prior to testing. Do not open pouches until ready to perform the assay.**

1. Remove the Rapid-VIDITEST Calprotectin-Lactoferrin Card device from its sealed pouch and use it as soon as possible.
2. Shake the specimen collection vial to assure good sample dispersion. Break off the cap of the vial.
3. Use a separate device for each sample. Dispense exactly 4 drops into the specimen well (S). Start the timer.
4. Read the result at **10 minutes** after dispensing the sample.



## INTERPRETATION OF RESULTS:



### **POSITIVE:**

**Calprotectin positive:** Two lines appear across the central window, a **red** test line marked with the letter T1 and, in the control line region, a **green** control line marked with the letter C.

**Lactoferrin positive:** Two lines appear across the central window, a **red** test line marked with the letter T2 and, in the control line region, a **green** control line marked with the letter C.

**Calprotectin-Lactoferrin positive:** Three lines appear across the central window, the two **red** test lines (T1 and T2) and, in the control line region, a **green** control line marked with the letter C.

**NEGATIVE:** Only one **green** line appears across the control line region marked with the letter C (control line).

**INVALID:** Total absence of the green control coloured line regardless the appearance or not of the red test lines. Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the reagents are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit and contact your local distributor.

### **NOTES ON THE INTERPRETATION OF RESULTS:**

The intensity of the red coloured test lines in the result line regions (T1 and T2) will vary depending on the concentration of calprotectin and/or lactoferrin in the specimen. However, neither the quantitative value, nor the rate of increase in calprotectin and/or lactoferrin can be determined by this qualitative test.

### **QUALITY CONTROL:**

Internal procedural controls are included in the test:

- A green line appearing in the control line region (C). It confirms sufficient specimen volume and correct procedural technique.

### **LIMITATIONS:**

1. Calprotectin-Lactoferrin will only indicate the presence of human calprotectin or/and lactoferrin in the specimen (qualitative detection) and should be used for the detection of calprotectin and lactoferrin in feces specimens only. Neither the quantitative value nor the rate of increase in calprotectin or lactoferrin concentration can be determined by this test.
2. An excess of sample could cause wrong results (brown lines appear). Dilute the sample with the buffer and repeat the test.

3. Some stool samples can decrease the intensity of the control line.
4. Positive calprotectin results confirm the presence of calprotectin in fecal samples; nevertheless, it can be due to several causes, inflammatory bowel disease, colorectal cancer and some enteropathies). Positive results should be followed up with additional diagnostic procedures by a physician to determine the exact cause of inflammation.
5. Neonatal fecal calprotectin levels have been reported higher than normal children with a median of 167µg/g.
6. Positive lactoferrin results confirm the presence of human lactoferrin in fecal samples; nevertheless, it can be also due to several causes besides IBD. A positive result should be followed up with additional diagnostic procedures. Endoscopy and histology on biopsy specimens are the methods for detecting and quantifying bowel inflammation.
7. Lactoferrin is a component of breast milk; the test will be positive in breast fed children and should not be used to evaluate neonates receiving breast milk.

#### **EXPECTED VALUES:**

Higher levels of calprotectin in the stool are associated with an increased risk of relapse in patients with inflammatory bowel disease (IBD). Some studies established equal or higher 50µg hFCP/g faeces as cut-off value to allow detect adult patients with GI inflammatory problems. A sample containing lactoferrin at concentration equal to or higher than 10ug hLf/g feces produces positive results using Rapid-VIDITEST Calprotectin-Lactoferrin Card test.

#### **PERFORMANCE CHARACTERISTICS:**

##### **Cut-off value**

Cut-off value of Calprotectin-Lactoferrin test is 500ng/mL (50µg hCp/g feces) for human calprotectin and 100ng/mL (10µg hLf/g feces) for human lactoferrin.

##### **Sensitivity and Specificity**

It was performed an evaluation using Rapid-VIDITEST Calprotectin-Lactoferrin with specimens obtained from patients. Rapid-VIDITEST Calprotectin-Lactoferrin test was evaluated compared with others commercial immunoassays (Calpres®Eurospital and IBD EZ VUE®, TechLab®).

Sensitivity: >94% and specificity 93% compared with Calprest®Eurospital (calprotectin test line).

Sensitivity: >99% and specificity >99% compared with IBD EZ VUE® TechLab® (lactoferrin test line).

##### **Cross-Reactivity and interferences**

It was performed an evaluation to determine the cross reactivity and interferences of Calprotectin-Lactoferrin Device. There is not cross reactivity against other fecal markers occasionally present in feces.

- |                              |                     |
|------------------------------|---------------------|
| - Bovine and pig hemoglobin  | - Human haemoglobin |
| - Bovine lactoferrin         | - Human transferrin |
| - Bovine and pig transferrin |                     |

#### **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 through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. Do not freeze.

#### **PRECAUTIONS:**











- For professional *in vitro* diagnostic use only.
- Do not use after expiration date.
- The test should remain in the sealed pouch until use.
- Do not use the test if pouch is damaged.

- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The test should be discarded in a proper biohazard container after testing.
- The test must be carried out within 2 hours of opening the sealed bag.

#### REFERENCES:

1. VIEIRA, A. et al., "Inflammatory bowel disease activity assessed by fecal calprotectin and lactoferrin: correlation with laboratory parameters, clinical, endoscopic and histological indexes", BMC Research Notes 2009, 2:221.
2. HANAI, H. et al. «"Relationship Between Fecal Calprotectin, Intestinal Inflammation, and Peripheral Blood Neutrophils in Patients with Active Ulcerative Colitis" Digestive Diseases and Sciences, Sept. 2004, Vol 49, No 9, pp 1438-1443.
3. BONNIN TOMAS, A, et al. "Calprotectina fecal como marcador diferencia entre patología gastrointestinal orgánica y funcional". Rev. Esp. de Enf. Dig. 2007, Vol 99, No 12, pp. 689-693.
4. Amemoto K. et al. Clinical evaluation of fecal lactoferrin and  $\alpha$ -1-antitrypsin in pediatric gastrointestinal infections, Pathophysiology. Vol 3; 1996, p. 87-90.
5. Walker, T.R. et al. Fecal Lactoferrin is a Sensitive and Specific Marker of Disease Activity in Children and Young Adults with Inflammatory Bowel Disease. J. Pediatr Gastroenterol. Nutr. April 2007; Vol 44, No 4, p. 414-422.

#### SYMBOLS FOR IVD COMPONENTS AND REAGENTS:

	In vitro diagnostic use only		Batch code
	Use by		Manufacturer
	Consult instruction for use		Contains sufficient for <n> tests
	Keep dry		Catalogue Code
	Temperature limitation		Buffer (sample diluent)

Last Revision: 05/2015



Distribuito in ITALIA da  
**Li StarFish S.r.l.**  
 Via Cavour, 35  
 20063 Cernusco S/N (MI)  
 telefono 02-92150794  
 fax 02-92157285  
 info@listarfish.it  
 www.listarfish.it