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CE

IVD

Lactoferrin Card

One step Lactoferrin Card test for the qualitative detection of Lactoferrin in faeces.

Instruction manual

INTENDED USE:

The Lactoferrin Card is a one step coloured chromatographic immunoassay for the qualitative determination of human lactoferrin in stool samples that may reflect intestinal inflammation in inflamatory bowel disease (IBD).

INTRODUCTION:

Lactoferrin is a glycoprotein component of neutrophil secondary granules, a primary component of the acute inflammatory response, and is released from fecal leukocytes. This protein is resistant to proteolysis in the feces and may serve as a marker of inflammation in the intestine. The major cause of fecal neutrophils in patients with chronic diarrhea is chronic inflammatory bowel disease of the colon (i.e., Crohn's Disease and ulcerative colitis). Non infections inflammatory diarrhoea may be seen in ulcerative colitis and Crohn's Disease.

Lactoferrin has been studied as a predictor of infection with invasive enteropathogens in children with diarrhoea. Bacterial inflammatory diarrhea may be caused by *Shigella, Salmonella, Campylobacter* and *Clostridium difficile*.

PRINCIPLE:

The Lactoferrin Card is a qualitative immunochromatographic assay for the determination of human lactoferrin in stool samples. The membrane is pre-coated with antibodies on the test band (result region), against human lactoferrin.
During testing, the sample is allowed to react with the coloured conjugate (anti-human lactoferrin antibodies-red microspheres) pre-dried on the test. The mixture then moves up ward on the membrane by capillary action. As the sample flows through the test membrane, the coloured particles migrate. In the case of a positive result the specific antibodies present on the membrane will capture the coloured conjugate. A coloured band will be visible, depend on the lactoferrin content of the sample. This band is used to interpret the result. The mixture continues to move across the membrane to the immobilized antibody placed in the control band region; this red coloured band always appears.
The presence of this red band serves as 1) verification that sufficient volume is added, 2) that proper flow is obtained and 3) as an internal control for the reagents.

MATERALS PROVIDED:

- Lactoferrin Card tests
- Instructions for use
- Specimen collection vial with buffer

MATERIALS REQUIRED BUT NO PROVIDED:

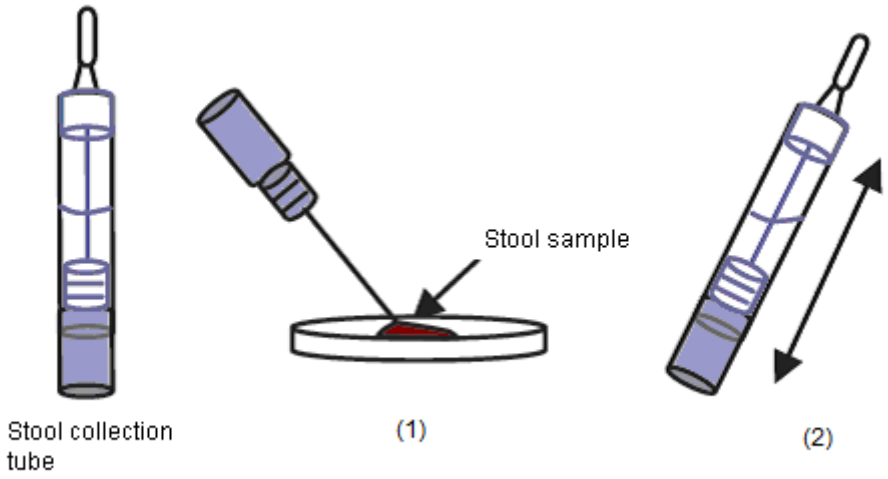
- Specimen collection container
- Disposable gloves
- Timer

SPECIMEN COLLECTION AND PREPARATION:

Stool samples should be collected in clean containers and the assay should be done right after collection. The samples can be stored at room temperature or in the refrigerator (2-4 ºC) for 5 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at –20ºC. In this case, the sample will be totally thawed, and brought to room temperature before testing.

Specimen preparation (see illustration):

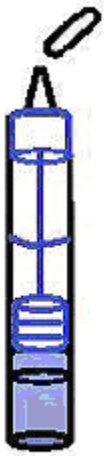
1. Unscrew the tap and use the stick by introducing four times into the fecal specimen to pick up a little sample and adding the sample (approx. 10 mg) into the stool collection tube (1).
2. Close the tube with the diluent and stool sample. Shake the tube in order to assure good sample dispersion (2).



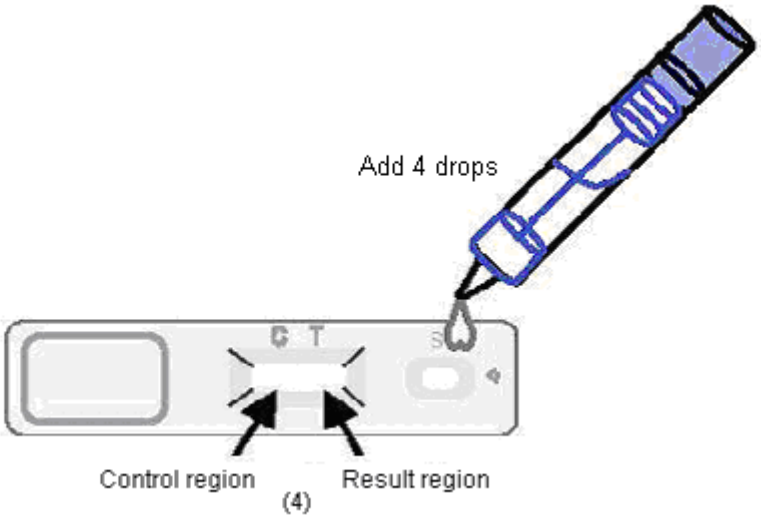
PROCEDURES:

Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open the pouch until ready to perform the assay. Only bring to room temperature the number of tests required to assay before opening it.

1. Proceed to shake the stool collection tube in order to assure good sample dispersion. Cut the end of the top (3).
2. Remove the Lactoferrin Card device from its sealed bag just before using.
3. Use a separate stool collection tube and device for each sample or control. Dispense 4 drops or 100 µl into the circular window marked with an arrow (4).
4. Read the result at **10 minutes** (the coloured bands appear). Do not read the test result after more than 10 minutes.

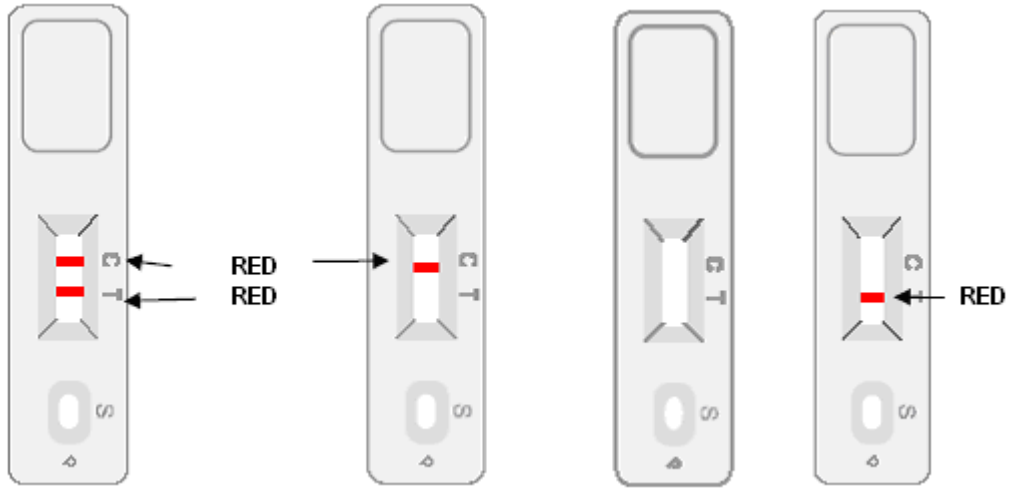


(3)



(4)

INTERPRETATION OF RESULTS:



POSITIVE ***NEGATIVE*** ***INVALID***

POSITIVE: In addition to the RED control band, a RED band (lactoferrin test line) also appears in the site marked with the letter T (result region). That probably would mean an inflammatory bowel disease (IBD).

NEGATIVE: Only one RED band appears across the central window in the site marked with the letter C (control line). That would mean neither active inflammatory bowel disease nor irritable bowel syndrome (IBS).

INVALID: A total absence of the control coloured band (RED) regardless the appearance or not of the result line (RED). Note: Insufficient specimen volume, incorrect procedural echniques 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 band in the result line region (T) will vary depending on the concentration of human lactoferrin in the specimen. However, neither the quantitative value, nor the rate of increase in lactoferrin can be determined by this qualitative test.

QUALITY CONTROL:

Internal procedural controls are included in the test. A red line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

LIMITATIONS:

1. The test must be carried out within 2 hours of opening the sealed bag.
2. An excess of stool sample could result in wrong results (brown bands appear or absence of the control coloured band).
3. Stool from paients with active inflammatory bowel diseases that usually involve significant neutrophilic inflammation of the intestine, such as Crohn's disease and ulcerative colitis, would be positive for fecal lactoferrin. Lactoferrin Card could be sensitive for this diagnosis in patients with chronic diarrhea.
4. Positive 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.
5. Negatiave results do not exclude inflammation, some diseases such as celiac sprue and microscopic colitis polyps that involve mainly monocuclear inflammation.
6. 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.

PERFORMANCE CHARACTERISTICS:

Sensitivity

A sample containing lactoferrin at concentration equal to or higher than 50 ng/ml produces positive results when using Lactoferrin Card.

Different lactoferrin dilutions were tested directly in the extraction buffer or spiked in a negative stool sample in accordance with the kit instructions to determinate the detection limit of the test.

The detection of human lactoferrin with Lactoferrin Card test showed >99% of sensitivity compared to another commercial immunoassay.

Specificity

The detection of human lactoferrin with Lactoferrin Card test showed 92% of specificity compared to another commercial immunoassay.

The Lactoferrin Card test is specific for human lactoferrin, showing no cross-reaction with bovine lactoferrin.

STORAGE AND STABILITY:

Store as packaged in the sealed pouch at 2-30ºC (36-86ºF). 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.
- All the specimens should be considered potentially hazardous and handled as if they were infectious agents.
- The test should be discarded in a proper biohazard container after testing.

REFERENCES:

1. Angriman I. et al.: Enzymes in feces: Useful markers of chronic inflammatory bowel disease. *Clinica Chimica Acta* 381, Feb. 2007, p. 63-68.
2. Guerrant R. et al.: Measurement of Fecal Lactoferrin as a Marker of Fecal Leukocytes. *Journal of Clinical Microbiology*, Vol. 30 No. 5; May 1992, p. 1238-1242.
3. Langhorst, M.d. et al.: Noninvasive Markers in the Assessment of Intestinal Inflammation in Inflammatory Bowel Diseases: Performance of Fecal Lactoferrin, Calprotectin and PMN-Elastase, CRP, and Clinical Indices. Am. J. Gastroenterol. 2008; Vol. 103, p. 162-169.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS:

IVD	*In vitro* diagnostic device	LOT	Batch code
	Use by		

Last Revision: January 2011