Calprotectin-Lactoferrin (Feces)

Insert

REF C-90	20 test

A rapid and one step test for the qualitative detection of calprotectin and lactoferrin in human faeces. For professional *in vitro* diagnostic use only.

INTENDED USE

The Calprotectin-Lactoferrin Device test 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.

SYNTHESIS

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 lisozyme. 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 Calprotectin-Lactoferrin Device is a qualitative immunoassay for the detection of calprotectin and lactoferrin in feces samples. The membrane is pre-coated with monoclonal antibodies against calprotectin and lactoferrin on the test lines region. During testing, the sample reacts with the particles coated with anti-human calprotectin antibodies and 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 conjugate and generate colored line. A green colored band 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.

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.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (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.

MATERIALS PROVIDED	MATERIALS REQUIRED BUT NO PROVIDED
- Calprotectin-Lactoferrin Device	- Specimen collection container
- Instructions for use	- Disposable gloves
- Specimen collection vial with buffer	- Timer

SPECIMEN COLLECTION AND PREPARATION

Collect sufficient quantity of faeces (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 (2-4ºC/36-40ºF) for 7 days prior to testing. For longer storage the specimen must be kept frozen at –20ºC/-4ºF. In this case, the sample will be totally thawed, and brought to room temperature before testing.

PROCEDURES

To process the collected stool samples (see illustration 1):
1. Use a separate specimen collection vial for each sample. Unscrew the cap of the vial and introduce the stick three times into the fecal specimen to pick up the sample. Close the vial with the buffer and stool sample. This vial with the sample can be storage during 5 days.
2. Shake the vial in order to assure good sample dispersion. For liquid stool samples, aspirate the fecal specimen with a dropper and add approx. 10-20uL into the specimen collection vial with buffer.
Test Procedure (see illustration 2)
Allow the tests, stool samples and buffer to reach to room temperature (15-30°C/59-86°F) prior to testing. Do not open pouches until ready to perform the assay.
3. Remove the Calprotectin-Lactoferrin Device from its sealed pouch and use it as soon as possible. Place in a clean and flat surface.
4. Shake the specimen collection vial to assure good sample dispersion. Break off the tip of the vial.
5. Use a separate device for each sample. Dispense 4 drops or 100 µL into the specimen well (S). Start the timer.
6. Read the result at **10 minutes** after dispensing the sample.

Illustration 1

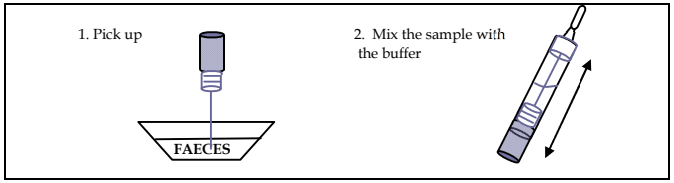
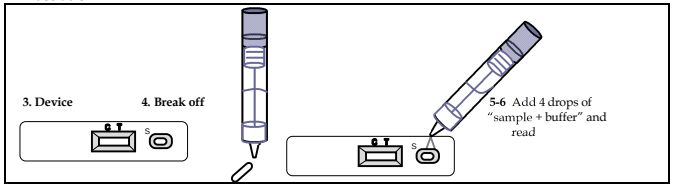


Illustration 2



INTERPRETATION OF RESULTS

Illustration 3

Calprotectin POSITIVE: Two lines appears across the central window, in the result line region (**red** test line marked with the letter T) and in the control line region (**green** control line marked with the letter C). A calprotectin positive result could be indicative of gastrointestinal inflammatory pathology is present.
Lactoferrin POSITIVE: In addition to the GREEN control band, a BLUE band (lactoferrin test line) also appears in the site marked with the letter T (result region). Interpretation: probably IBD (Inflammatory bowel disease).
Calprotectin (FCP) and Lactoferrin (FLF) POSITIVE: Three lines appears across the central window, in the result line region two lines (**red** test line and **blue** test line marked with the letter T) and in the control line region (**green** control line marked with the letter C). Calprotectin (CP) and Lactoferrin (LF) positive result could be indicative of intestinal inflammation (organic disease) is present in the patient.
NEGATIVE: Only one **green** band appears across the control line region marked with the letter C (control line). A negative results shows that neither active gastrointestinal inflammation is present.
INVALID: A total absence of the green control colored band regardless the appearance or not of the red or blue test line. 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 or blue colored band in the result line region (T) will vary depending on the concentration of calprotectin and lactoferrin in the specimen.

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.
- A clear background is an internal negative background control. If the test is working properly, the background in the result area should be clear and not interfere with the ability to read the result.

LIMITATIONS

1 An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
2 Some stool samples can decrease the intensity of the control line.
3 In the case of patients with active neutrophilic inflammatory bowel diseases such as Crohn's disease and Ulcerative Colitis, would be positive for fecal calprotectin. Calprotectin-Lactoferrin device could be used for patients with chronic diarrhea.
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 Calprotectin-Lactoferrin Device.

PERFORMANCE CHARACTERISTICS

Sensitivity
A sample containing calprotectin at concentration equal to or higher than 50µg/g faeces and/or lactoferrin at concentration equal to or higher than 10ug hLf/g feces produces positive results when using Calprotectin-Lactoferrin Device test.
Different calprotectin and 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 calprotectin with Calprotectin-Lactoferrin test showed >94% of sensitivity correlation compared to another commercial immunoassay (Calprest® Eurospital).
The detection of human lactoferrin with Calprotectin-Lactoferrin Device test showed >99% of sensitivity compared to another commercial immunoassay.
Specificity
The detection of human calprotectin with Calprotectin-Lactoferrin test showed 93% of specificity correlation compared to another commercial immunoassay (Calprest® Eurospital). And the detection of human lactoferrin with the Calprotectin-Lactoferrin Device test showed 99% of specificity compared to another commercial immunoassay IBD EZ VUE®TechLab®).
Calprotectin-Lactoferrin Device test is specific for human calprotectin and human lactoferrin, showing no cross-reaction with other calprotectins or bovine lactoferrin.

REFERENCES

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SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	Manufacturer	IVD	For *in vitro* diagnostic use only
EC REP	Authorized representative		Consult instructions for use
Σ n	Contains sufficient for <n> tests		Keep dry
REF	Catalogue Code		Temperature limitation
LOT	Lot Number		Use by
DIL	Sample diluent		

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