CALPROTECTIN CARD

In Vitro **diagnostic (IVD)**

Immunochromatographic card test for the qualitative detection of calprotectin in human faeces

I. INTRODUCTION AND INTENDED USE

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.
The Calprotectin Card test is a rapid chromatographic immunoassay (non-invasive assay) for the qualitative detection of calprotectin in human feces specimens, which might be useful for the diagnosis of inflammatory gastrointestinal disorders.

II. PRINCIPLE OF THE TEST

The Calprotectin Card is a qualitative immunoassay for the detection of calprotectin in feces samples. The membrane is pre-coated with monoclonal antibodies against calprotectin on the test lines region. During testing, the sample reacts with the coloured particles coated with anti-human calprotectin antibodies which were pre-dried on the gold pad. 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.

III. REAGENTS AND MATERIALS

1. Calprotectin card (20 devices).
2. Extraction buffer (1,0 mLx20 bottles)
3. Instruction for use (1 item)
REQUIRED MATERIALS (NOT SUPPLIED)
Specimen collection container
Disposable gloves and container
Timer
Plastic dropper.

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- Calprotectin Card is for in vitro diagnosis only.
- Avoid touching the nitrocellulose with your fingers.
- Wear gloves when handling the samples.
- Disposable gloves, swabs, test tubes, and sensitized strips in accordance with GLP.
- Never use reagents from another lot.
- Discard the dilution buffer if it is contaminated with bacteria or mould.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.

V. STORAGE

The test must remain in the sealed pouch until use and in a dry environment. The kit must not be frozen. 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.

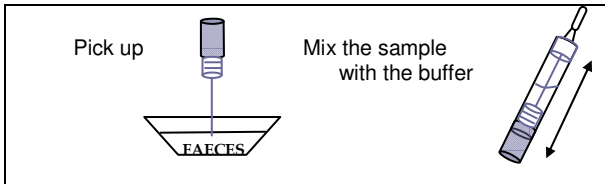
VI. SPECIMEN COLLECTION

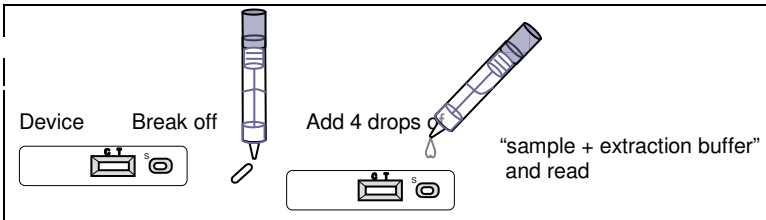
Collect sufficient quantity of faeces (1-2 g or mL for liquid sample). Stool samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-4ºC/36-40ºF) for 7 days prior to testing. For longer storage the specimen must be kept frozen at –20ºC/-4ºF. In this case, the sample will be totally thawed, and brought to room temperature before testing.

VII. 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 three times into the fecal specimen to pick up the sample (about 150 mg). Close the vial with the buffer and stool sample. This vial with the sample can be storage during 5 days.
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
Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the Calprotectin device from its sealed pouch and use it as soon as possible. Place in a clean and flat surface.
2. Shake the specimen collection vial to assure good sample dispersion. Break off the tip of the vial.
3. Use a separate device for each sample. Dispense 4 drops or 100 µL into the specimen well (S). Start the timer.
4. Read the result at 10 minutes after dispensing the sample.



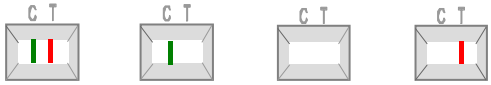


VIII. INTERPRETING THE RESULTS

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.

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 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.



Positive Negative Invalid

IX. INTERNAL QUALITY CONTROL

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

X. PERFORMANCES

A. 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.

B. Sensitivity-Specificity (correlation)-

A sample containing calprotectin at concentration equal to or higher than 50µg/g faeces produces positive results when using Calprotectin Card test.

Different calprotectin 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 Card test showed >94% of sensitivity correlation compared to another commercial immunoassay (Calprest® Eurospital).

The detection of human calprotectin with Calprotectin Card test showed 93% of specificity correlation compared to another commercial immunoassay (Calprest® Eurospital).

C. Interference and cross-reactivity

The Calprotectin Card test is specific for human Calprotectin, showing no cross-reaction with other calprotectins.

XI. LIMITS OF THE TEST

- Calprotectin Card will only indicate the presence of calprotectin in the specimen (qualitative detection) and should be used for the detection of calprotectin in feces specimens only. Neither the quantitative value nor the rate of increase in calprotectin concentration can be determined by this test.
- An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
- Some stool samples can decrease the intensity of the control line.
- 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 Card could be used for patients with chronic diarrhea.
- Positive 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.
- Neonatal fecal calprotectin levels have been reported higher than normal children with a median of 167µg/g.

XII. BIBLIOGRAPHY

- 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.
- 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.
- 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.

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (20 tests) **REF. VT81610**

Calprotectin card	20 devices
Extraction buffer	20 bottles x 1.0ml
Instruction for use	1 item

EDMA Code 12 01 90 10 CE IVD

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