I. Intended Use

The Biomerica Calprotectin test is a rapid chromatographic immunoassay for the qualitative detection of Calprotectin in human stool samples which might be useful for the diagnosis of inflammatory gastrointestinal disorders. The *In Vitro* test is intended **for professional use only.**

II. Summary and Explanation

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 is associated with elevated neutrophils. The calprotectin assay is useful in differentiating organic (IBD) from functional gastrointestinal disease (IBS; Irritable Bowel Syndrome). It is a simple, non-invasive biomarker that is especially useful in children, who may require general anesthesia for colonoscopy. Also, fecal calprotectin detection can predict relapse.

III. Principles of the Procedure

The Biomerica Calprotectin test is a qualitative immunoassay for the detection of calprotectin in human stool samples. The membrane is pre-coated with antibodies against calprotectin on the test line region. During testing, the sample reacts with the particle coated with anti-human calprotectin antibodies which was 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 colored lines. 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.

IV. Materials Provided

1. Test device in foil pouch
2. Stool collection vial with buffer
3. Instructions for use

V. Materials Required but Not Provided

Timer and Gloves
Dropper or Pipette (for liquid specimens)

VI. Warning and Precautions

Safety Precautions: Human stool should be handled as if capable of transmitting infectious agents. It is recommended that these specimens be handled using established good laboratory working practices. The test should be discarded in a proper biohazard container after testing.

For *in vitro* diagnostic use: Do not use the kit beyond the expiration date printed on the outside of the foil pouch. Discard all used test devices into a proper biohazard container. The test should remain in the sealed pouch until use. Do not use the test if pouch is damaged. The test must be carried out within 2 hours of opening the sealed pouch.

VII. Kit Storage and Stability

The Calprotectin tests can be stored refrigerated or at room temperature (2-30°C). The test is usable until the expiration date stamped on the foil pouch. Do not freeze.

VIII. Quality Control

Although the Calprotectin test contains an internal quality control (green color band in the Control region) good laboratory practice recommends the daily use of an outside control to ensure proper kit performance. Quality control samples should be tested according to the quality control requirements established by your laboratory.

IX. Specimen Collection And Preparation

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

X. Test Procedure

To process the collected stool samples (see illustration 1):
Use a separate specimen collection vial for each sample. Unscrew the cap of the vial and introduce the stick three times into the stool sample to pick up a small amount of 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 faecal specimen with a dropper and add 10-20 uL into the specimen collection vial with buffer.

Test Procedure (see illustration 2). Allow the tests, stool samples and buffer to reach to room temperature (15-30ºC) prior to testing. Do not open pouches until ready to perform the assay.

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

Illustration 1

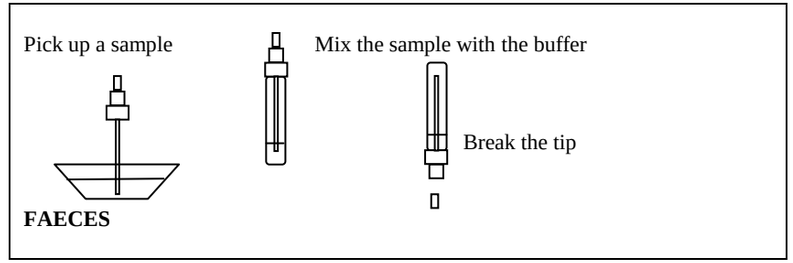
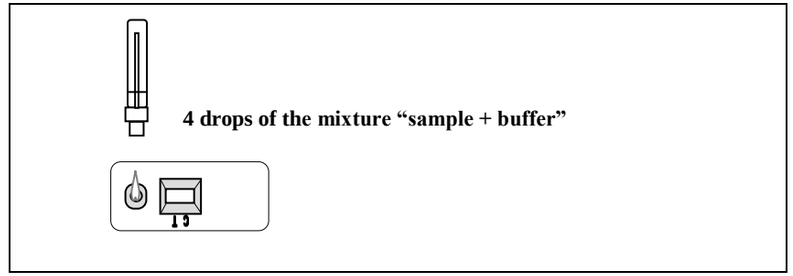
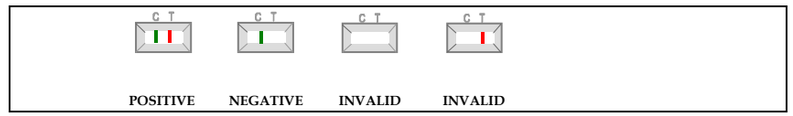


Illustration 2



XI. Interpretation of Results



POSITIVE: Two lines appear across the central window, in the Test region (a pink test line in the region marked with the letter T) and in the Control region (a green control line in the region marked with the letter C). A calprotectin positive result could be indicative that gastrointestinal inflammatory pathology is present.

NEGATIVE: Only one band appears in the Control region (a green control line in the region marked with the letter C).

INVALID: A total absence of the control line regardless of the appearance of the test line. Note: Insufficient specimen volume, incorrect procedural techniques or deterioration of the test materials 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 supplier.

The intensity of the pink colored band in the test line region (T) will vary depending on the concentration of calprotectin in the sample.

Internal procedural controls are included in the test:
- A green line appearing in the control region (C) confirms sufficient specimen volume and correct procedural technique.

XII. Limitations of the Test

1. The Calprotectin device will only indicate the presence of calprotectin in the sample (qualitative detection) and should be used for the detection of calprotectin human stool samples only. Neither the quantitative value nor the rate of increase in calprotectin concentration can be determined by this test.
2. An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
3. Some stool samples can decrease the intensity of the control line.
4. Patients with active neutrophilic inflammatory bowel diseases such as Crohn's Disease and Ulcerative Colitis would be positive for fecal calprotectin. The Calprotectin test could be used for patients with chronic diarrhea.
5 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.
6. Neonatal fecal calprotectin levels have been reported higher than normal children with a median of 167µg/g.
7. Higher levels of calprotectin in the stool are associated with an increased risk of relapse in patients with inflammatory bowel disease (IBD).
8. Some studies have established a cut-off value equal to or greater than 50µg hFCP/g faeces for the detection of adult patients with GI inflammatory problems.

XIII. Performance Characteristics

Sensitivity
A sample containing calprotectin at concentration equal to or greater than 50µg/g faeces produces positive results when using the Biomerica Calprotectin 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 the Calprotectin test showed >94% of sensitivity correlation compared to another commercial immunoassay (Calprest® Eurospital).

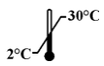
Specificity
The detection of human calprotectin with the Biomerica Calprotectin test showed 93% of specificity correlation compared to another commercial immunoassay (Calprest® Eurospital). The Calprotectin test is specific for human Calprotectin, showing no cross-reaction with other calprotectins.

XIV. 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. Hanal, 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.

XV. Ordering information

ORDERING: Send purchase order to:
Telephone: 949-645-2111
Fax: 949-553-1231
E-mail: bmra@biomerica.com
www.biomerica.com



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INTERNATIONAL VERSION

EZ-CALPROTECTIN

One-Step Calprotectin Test

January 2014

Cat.# 1012, 1012-15

Immunoassay kit for the detection of Calprotectin in human stool specimen

FOR *IN VITRO* DIAGNOSTIC USE ONLY

FOR PROFESSIONAL USE ONLY

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