A rapid and one step test for the qualitative detection of calprotectin in human feces.

For professional in vitro diagnostic use only.

INTENDED USE

The Calprotectin 50+200 MonlabTest® is a rapid chromatographic immunoaassay (non-invasive assay) for the qualitative detection of human fecal calprotectin (hFCP) in human feces specimens to the following cut-off values: A: 50µg/g feces and B: 200µg/g feces.

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.

Calprotectin is a neutrophil cytosolic protein with antimicrobial properties, which is present at increased concentration in stool samples during bowel inflammation (intestinal inflammation). The stability of the protein to degradation keeps it stable in feces for up to 7 days at room temperature, making it an ideal analyte.

Calprotectin is released by activation of leukocytes, giving increased levels in plasma, cerebral spinal fluid, synovial fluid, urine or stools as a consequence of disease in the relevant organ(s).

Calprotectin inhibits zinc-dependent enzyme systems, as a result kills microbes and induces apoptosis in normal and cancer cells. In the presence of calcium, calprotectin is a remarkably resistant to protelolytic degradation.

This is a non-invasive marker of intestinal inflammation (for example in Oclusive Colitis (UC) and Crohn’s Disease (CD)).

PRINCIPLE

The Calprotectin 50+200 MonlabTest® is a qualitative lateral flow immunoassay for detection of human calprotectin in human feces specimens. The membrane of the Test A is pre-coated with monoclonal antibodies against human calprotectin and the membrane of the Test B is pre-coated with monoclonal antibodies against human calprotectin on the test lines region. During testing, the sample reacts with the red colored particles coated with anti-human calprotectin antibodies in the Test A and/or with anti-human calprotectin antibodies in the Test B which were pre-dried on the test strips. The mixture moves upward on the membrane by capillary action. In the case of a positive result in the Test A the specific antibodies present on the membrane will react with the mixture conjugate and generate one red colored line. In the case of a positive result in the Test B the specific antibodies present on the membrane will react with the mixture conjugate and generate one red colored line. The mixture continues to move across the membrane to the immobilized antibody places in the control line regions. A green colored line always appears in the control lines 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 (2-30°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.

MATERIALS PROVIDED

- 20 Tests
- Instructions for use
- 20 Specimen collection vial with buffer

MATERIALS REQUIRED BUT NOT 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 months), 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 (see illustration 1):

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   Use a separate specimen collection vial for each sample. Unscrew the cap of the vial and introduce the stick four 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 throughout 7 days (2-8°C/36-46.4°F).

2. Shake the vial in order to assure good sample dispersion. For liquid stool samples, aspirate the fecal specimen with a dropper and add 15µL into the specimen collection vial with buffer.

3. Use a separate device for each sample. Dispense 4 drops into the specimen well (S) of the Test A and dispense 4 drops into the specimen well (S) of the Test B using the same vial. Start the timer.

4. Read the result at 10 minutes after dispensing the sample.

Illustration 1

Illustration 2

Test A Calprotectin (50µg/g) procedure

Add 4 drops of “sample + buffer”

Test B Calprotectin (200µg/g) procedure

Add 4 drops of “sample + buffer”
**INTERPRETATION OF RESULTS**

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strip A</td>
<td>Calprotectin marker negative, (&lt;50µg/g) is not present in patient sample</td>
<td>Read as negative</td>
</tr>
<tr>
<td>Strip B</td>
<td>Calprotectin marker positive, (&gt;50µg/g and &lt;200µg/g)</td>
<td>Read as positive</td>
</tr>
</tbody>
</table>

**NOTES ON THE INTERPRETATION OF RESULTS**

- The intensity of the red coloured test lines in the test lines (T) in the results windows will vary depending on the concentration of human calprotectin present in the specimen.

**QUALITY CONTROL**

Internal procedural controls are included in the test:
- Green lines appearing in the control line regions (C). It confirms sufficient specimen volume and correct procedural technique.

**LIMITATIONS**

1. Calprotectin 50+200 MonlabTest® will only indicate the presence of calprotectin in different concentrations in the specimen (qualitative detection) and should be used for the detection of calprotectin in feces specimens only.
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 lines.
4. Stool from patients with non-steroidal anti-inflammatory drug (NSAID) treatment might show positive result.
5. Neonatal fecal calprotectin levels have been reported higher than those in normal children with a mean of 167µg/g.
6. Patients’ stool suffering from active inflammatory bowel disease, usually involve significant neutrophilic inflammation of the intestine, such as Crohn’s disease and ulcerative colitis, would be positive for fecal calprotectin. Calprotectin 50+200 MonlabTest® could be sensitive for this diagnosis in patients with chronic diarrhea.
7. Positive results confirm the presence of human calprotectin in fecal samples. Nevertheless, it can also be due to several causes such as Inflammatory Bowel Disease, colorectal cancer and some other enteropathies. A positive result should be followed up with additional diagnostic invasive procedures, colonoscopy and biopsy in order to confirm the diagnosis and establish the inflammation extent.
8. Negative results do not exclude inflammation. Some diseases such as celiac sprue and microscopic colitis polyps may mainly involve mononuclear inflammation.

**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 hCp/g feces as cut-off value to allow detect adult patients with GI inflammatory problems. The clinical course of inflammatory bowel disease is characterized by a succession of relapses and remissions. Some studies established equal or higher 200µg hCp/g feces as cut-off value to allow predict clinical relapse of disease activity in patients with Ulcerative Colitis and in Crohn’s Disease and detect some neonatal patients (higher levels than normal children) with gastrointestinal inflammatory pathology that will require to diagnosis additional diagnostic invasive procedures. Fecal calprotectin level evolution in individuals showed that fecal calprotectin level can predict rejection days before histopathological diagnosis.

**REFERENCES**

- VIEIRA, A. et al., “Inflammatory bowel disease activity assessed by Calprotectin 50+200 MonlabTest® to a commercial available assay: Calprotectin®Eurospital. Calprotectin 50+200 MonlabTest® was highly specific >93% and also highly sensitive >94% to compared with the results of that membrane assay.
- Bovine and pig hemoglobin
- Bovine and pig transferrin
- Bovine lactoferrin

**SYMBOLS FOR IVD COMPONENTS AND REAGENTS**

- Manufacturer
- IVD
- Don’t re-use
- Contains sufficient for <n> tests
- Catalogue Code
- Lot Number
- For in vitro diagnostic use only
- Consult instructions for use
- Keep dry
- Temperature limitation
- Use by

**PERFORMANCE CHARACTERISTICS**

**Cut-off value**
Cut-off value test is 500ng/mL (50µg hCp/g feces) for human calprotectin in strip A and 2000ng/mL (200µg hCp/g feces) for human calprotectin in strip B.

**Sensitivity and Specificity**
An evaluation was conducted comparing the results obtained using the Calprotectin 50+200 MonlabTest® and compared to the results of that membrane assay.

**Cross-Reactivity**
It was performed an evaluation to determine the cross reactivity of Calprotectin 50+200 MonlabTest®. There is not cross reactivity with common gastrointestinal pathogens occasionally present in feces.

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**Manufacturer**
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**MonlabTest® Langhorst, M.D. et al.**

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