Atlas Medical CE

Calprotectin Test Device

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

IVD *For In-Vitro Diagnostic and Professional Use Only*

2 30°C **Store at 2-30 °C**

INTENDED USE

Atlas Calprotectin Device 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.

SYNTHESIS

Calprotectin is a protein belonging to the S100 family and occurring in large amounts in neutrophil granulocytes, where it accounts for 5% of total proteins and 60% of cytoplasm proteins. A smaller amount of calprotectin has also been detected in monocytes and activated macrophages.

The structure of calprotectin consists of a light polypeptide chain and two heavy polypeptide chains, with a molecular weight of 36,5 kDa. Bacteriostatic and mycostatic properties of calprotectin are comparable to those of antibiotics. For this reason the abundance of calprotectin in neutrophil granulocytes and its antimicrobial activity suggest a substantial role in the defence of the organism. Calprotectin has been found in several human biological materials: serum, saliva, cerebrospinal fluid and urine. However, the assessment of faecal calprotectin is a widely used method for the detection of bowel inflammation severity. Calprotectin is an extremely stable protein, and it can be found unaltered in stool for longer than 7 days.

When inflammatory processes occur, calprotectin is released due to the degranulation of neutrophil granulocytes. In bowel inflammation, calprotectin may be detected in the stool. The faecal assay provides direct information about the inflammation site, whereas with serum or plasma, inflammation might be located anywhere.

PRINCIPLE

The Calprotectin test device is a qualitative lateral flow immunoassay for the detection of calprotectin in human feces samples. The membrane is pre-coated with monoclonal antibodies against calprotectin on the test line region. During testing, the sample reacts with the particle coated with anti-human calprotectin 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 a coloured line. A green colored line 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

Materials provided

- Test Devices.
- Extraction tube with buffer.
- Instructions for use.

Materials required but no provided

- Timer.
- Specimen collection container.
- Disposable gloves.

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 (2-8ºC/36-46.4ºF) for 7 days prior to testing. For longer storage (maximum 6 months), 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

PROCEDURE

To process the collected stool samples (see illustration 1)

1. Use a separate specimen collection vial for each sample.
2. Unscrew the cap of the vial and introduce the stick four times into the fecal specimen to pick up the sample.
3. Close the vial with the buffer and stool sample. This vial with the sample can be storage during 7 days (2-8ºC/36-46.4ºF).
4. Shake the vial in order to assure good sample dispersion. For liquid stool samples, aspirate the fecal specimen with a dropper and add approx. 15µL into the specimen collection vial with buffer.

Test Procedure (see illustration 2)

Allow the tests, stool samples and buffer to reach room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

1. Remove the Calprotectin test Device from its sealed pouch and use it as soon as possible.
2. Shake the specimen collection vial to assure good sample dispersion. Break off the cap of the vial.
3. Use a separate device for each sample. Dispense 4 drops into the specimen well (S). Start the timer.
4. Read the result at 10 minutes. Do not interpret the result after 10 minutes.

Illustration 1

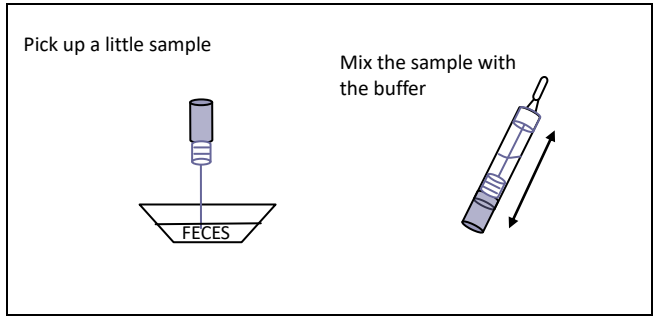
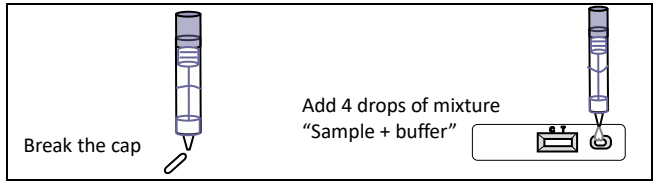


Illustration 2



INTERPRETATION OF RESULTS

Illustration 3

POSITIVE NEGATIVE INVALID INVALID

POSITIVE: Two lines appear across the central window, a **red** test line marked with the letter T and a **green** control line marked with letter C.
NEGATIVE: Only one **green** line appears across the control line region marked with the letter C (control line).
INVALID: Total absence of the green control coloured line 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. If the problem persists, discontinue using the test kit and contact your local distributor. See illustration 3.

NOTES ON THE INTERPRETATION OF RESULTS

The intensity of the red coloured test line in the result line region (T) will vary depending on the concentration of calprotectin 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.

LIMITATIONS

1. Calprotectin test device 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.
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 line.
4. 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 test device 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. Stool samples from patients with non-steroidal anti-inflammatory drug (NSAID) treatment could show positive result.
7. Neonatal fecal calprotectin levels have been reported higher than normal children with a median of 167µg/g.

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.

PERFORMANCE CHARACTERISTICS

CUT-OFF VALUE

Cut-off value of test is 500 ng/mL (50 µg hCp/g feces) for human calprotectin.

SENSITIVITY AND SPECIFICITY

The Sensitivity and specificity was performed using Calprotectin test device . The Calprotectin test device was evaluated compared with a commercial immunoassay (Calprest®, Eurospital).

Sensitivity >94% and specificity 93%.

CROSS-REACTIVITY

The cross-reactivity test was performed an evaluation to determine the cross reactivity of Calprotectin test device. There is not cross reactivity against other fecal markers occasionally present in feces.

- Bovine and pig hemoglobin
- Bovine and pig transferrin
- Bovine lactoferrin
- Human hemoglobin
- Human lactoferrin
- Human transferrin

REFERENCES

1. CUKOR G., and BLACKLOW N. R., "Human Viral Gastroenteritis", Microbiological Reviews, Vol. 48 No 2, June 1984, pp. 157-179
2. NEEL K. KRISHNA, B.A, "Identification of Structural Domains Involved in Astrovirus Capsid Biology", Viral Immunol. 2005; 18(1): 17–26.
3. BON, F. et al. "Prevalence of group A rotavirus, human calicivirus, astrovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France." J. Clin.

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PPI1420A01
Rev A (02.09.2019)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size.		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Do not re-use		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry