DyonMed S.A.
Point of Care Solutions

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ISO 9001:2008 (No. 010110003)| ISO 13485:2003 (No 01210005)
by TÜV Austria Hellas

DyonCalpro® (*Calprotectin 50,200*)

Cat.No: DNCT101dd (50, 200)

Rapid test for qualitative detection of Calprotectin in sample of human stool

SUMMARY

The **DyonCalpro® (Calprotectin)** is a rapid immunochromatographic test for the qualitative detection Calprotectin in stool samples

Calprotectin is a complex protein of molecular weight 36 kDa, complex proteins S100A8 and S100A9. It binds calcium and zinc; it has antibacterial and antifungal properties. It comes mainly from neutrophils and, to a lesser extent, monocytes and macrophages. Calprotectin is approximately 5% of the total protein and up to 60% of the cytoplasmic protein of human neutrophils. Increased presence of neutrophils in the intestinal mucosa and hence the intestinal content indicates inflammatory response of the intestinal wall. Because of its strong resistance to enzymatic cleavage, Calprotectin maintained almost intact in stools and therefore it can be measured reliably.

The concentration of Calprotectin is proportional to the presence of neutrophil polymorphonuclear leukocytes within the lumen of the intestine. However, since the presences of white blood cells are responsible for the activity of Inflammatory Bowel Disease (IBD) thus understand the value display high concentrations of Calprotectin in stools.

The levels of Calprotectin directly correlate with the presence of white blood cells in stool; that is proved with the correlation between Calprotectin and the labeled scan with In111 (Indium 111) white blood cells, the gold standard method for detection of bowel inflammation.

The using of stool's Calprotectin presents great utility in differentiating between irritable bowel syndrome and inflammatory bowel disease (Langhorst et al, 2008). Recent studies found that the diagnostic accuracy of using stool's Calprotectin for IBD shows sensitivity of 95% and specificity of 91% for the diagnosis of IBD compared with histological diagnosis (Gisbert & McNicholl, 2009).

Diagnosis, prognosis and monitoring of disease activity in patients with inflammatory bowel disease require clinical radiological and histological criteria, with the necessary support of specific blood markers of inflammation. Methods for detection of specific inflammatory markers in stools, like Calprotectin, have developed in recent years. The C-reactive protein (CRP) but more specifically the Calprotectin exhibit increased correlation with the activity and the extent of the disease in ulcerative colitis; about Crohn's disease, the stool sample's Calprotectin has highest correlation as an index of inflammatory lesion (Ricanek et al, 2011). Specifically, the stool's Calprotectin presents the greatest correlation with endoscopic disease activity index of Crohn (SES-CD) and the disease activity index (CDAI). So, Calprotectin is emerging as an indicator reliably distinguishes between inactive from mild, moderate and high disease activity, which demonstrates its great importance of the clinical monitoring (Schöpfer et al, 2010; Lewis, 2011).

Diagnostic access with the assistance of stool's Calprotectin expands in populations of asymptomatic first-degree relatives of patients with IBD and it has proved that they exhibit increased levels of Calprotectin, implying a more extensive presence of subclinical disease and aiding the rapid diagnosis (Gisbert & McNicholl, 2009).

The use of Calprotectin extends to cases of children with idiopathic inflammatory bowel disease and has proven its reliability and of course the fact that it is a non-invasive monitoring method of pediatric patients (Canani et al, 2008).

PRINCIPLE OF THE METHOD

DyonCalpro® (*H. pylori* stool Ag) is a qualitative immunoassay of the sandwich type colloidal gold for detecting stool's Calprotectin. The method utilizes a membrane coated with antibodies in order to detect stool's Calprotectin selectively and with high sensitivity. At the start of the test the sample is mixed with the complex of the chromophore and begins to move through the membrane. If the sample contains an antigen, it will interact with the pre-capped complex chromophore, so will appear in a color bar test zone (T). Both negative and positive samples should generate a colored line in the Control Zone (C), which are indicated proper execution of technique, sample volume, and mode of examination.

STORAGE AND STABILITY

Store the kit at 2-30 ° C. If the kit was stored in the refrigerator, it should be transferred to room temperature before proceeding with the examination. Under these conditions, the kit is stable until the expiration date printed on the package.

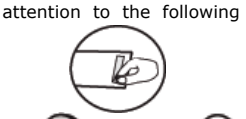
PACKAGE CONTENTS

Each kit contains 1 individually wrapped (or multiples thereof), as follows:

-Paper for Collection
-Collection vial containing a solution and bring Strain
-Instructions for sample collection
-Sealed plastic package with test cassette
-1 package

STOOL SAMPLE COLLECTION AND PREPARATION

Please ensure that patients should pay attention to the following instructions for collecting stool samples.



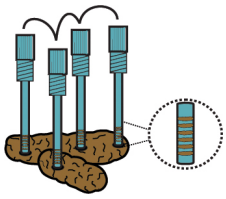
1. Remove the bond line from 2 edges of special collection paper.

2. Lift the toilet lid. Set the Collection Paper in the middle of the basin so that the Collection paper is relatively weak. Put the adherence film on the ring of the basin. Put down the cover.

3. Bowel on the Collection Paper.

Attention: (a) stool should not come into contact with water before the toilet sample collection (b) The Collection Paper should not come in contact with urine.

4. Keep the collection vial vertically so the blue cap is upwards. Unscrew the blue cap from the collection vial and extract the collection strain. DO NOT POUR LIQUID of the Collection Vial. Enter Collection Strain in the stool sample in 4 different sides. Collect little sample as to cover the tip of the Collection Strain . Do not overload the COLLECTION STRAIN (see picture). With a cotton swab remove from the swab off excess of sample.

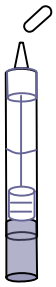


5. Holding the collection vial in a vertical position, tighten the gripping behind the collection vial and tighten the blue cap securely. The dissolved sample is now ready for examination by the doctor.

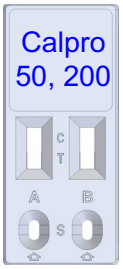
Note that (a) the collection vial contains a small transparent ring with holes, which functions as a "net" prohibiting excess stool enter the space where the solubilizing liquid. (Do not try to push stool through the transparent ring.) (B) Solubilized in the collection vial stool samples are stable up to 5 days when stored at room temperature.

TEST PROCEDURE-RESULTS INTERPRETATION

Before the examination, make sure that the collection vial and plastic packaging with the test cassette have come to room temperature. The examination cartridge must be opened only just before the test.



1) Shake the collection vial to be good solubilization of the sample.
2) Keep the collection vial so that the white cap is upwards (blue downwards).
3) Unscrew the white cap. Recognize the clear plastic edge Collection Vial. Keeping upright collection vial and having put a paper towel on the edge of the vial, push the transparent plastic tip to break.
4) Turn the collection vial and press the relative strengths that they drop 4 drops solubilized sample per round hole sample (position "S") examination cartridge.



4+4 drops

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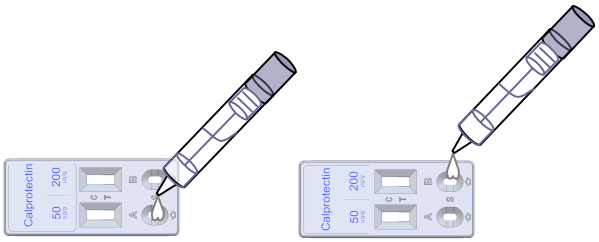


5) Immediately start the timer (not supplied)

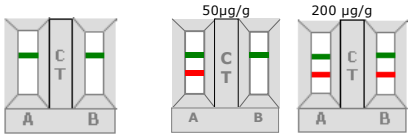
6) Observe the dissolved sample moves zones (T) and (C). Interpret the results in 10 minutes. Any results obtained after 10 minutes have not diagnostic significance.

Result Interpretation DyonCalpro® (50, 200)

Negative Results **DyonCalpro®**(50, 200) = 2 green lines (C)
The test is negative if both green lines appear in the Control Zone (C), while no line appears in test zone (T). The intensity of color in a Zone (C) could be stronger or achnoteri the intensity of the color in another zone (C). The interpretation of a negative result is guaranteed in 10 minutes.

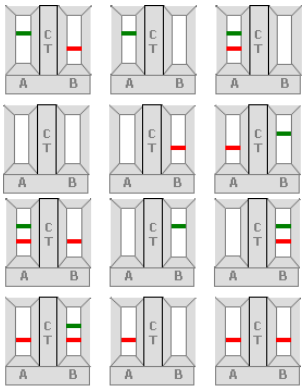
Positive Results **DyonCalpro®**(50, 200) = 3 or 4 color lines.
Provided that the two green lines of the control zone (C) are also visible at 10 minutes, then
(a) if a red line appears in the test zone (T) 50, then the test is positive for **DyonCalpro®** (50) which are indicated the presence of Calprotectin at concentrations of 50 mg/g in stool.
(b) if 2 red lines emerge in the test zone (T) 50 and 200, then the test is positive for **DyonCalpro®** (50, 200) which are indicated the presence of Calprotectin at concentration of 200 mg / g in stool.

Negative Positive

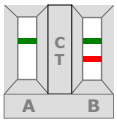


Invalid Results **DyonCalpro®**(50, 200)

(a) General cases for invalid results **DyonCalpro®**(50, 200): The test **DyonCalpro®** (50, 200) is mandatory invalid if most one of the two green lines in the Control Zone (C) appear in 10 minutes, regardless of what happens with the red lines of the test zone (T). Examples of such invalid test:



(b) A special case of invalid results DyonCalpro ® (50, 200) on raising error in concentration occurs when we see two green lines in zones (C) and one red line (T) 200 without indicator (C) 50 to be similarly positive. The test is invalid if the positive index in test zone (T) 50 of Calprotectin requires less concentration than that of the index (T) 200.



(c) Appearance of any brown lines in zones or in the background of the test is indicated excess sample during collection. The test is invalid and recommended dilution of the original stool sample.

PRECAUTIONS

Precautions regarding the collection, handling, storage and disposal of samples and used kit components. Handle all specimens as if they contain infectious agents. Take precautions against microbiological hazards throughout the process. Apply standard procedures for proper disposal of specimens. Operation is not affected by known environmental factors other than temperature.

RESTRICTIONS

1. Positive or negative test results **DyonCalpro®** should be considered with other symptoms, clinical findings or diagnostic results to a diagnosis.
2. The characteristics are valid only if the contents of the package are in good condition.
3. **DyonCalpro®** device is for single use only. In the event that a dissolved sample should be retested, use another device.
4. **DyonCalpro®** is exclusively for health care professionals.

QUALITY CONTROL

Internal Quality Control: The colored line appears in the Zone (C) is an internal control procedure. It confirms the correct specimen volume and the correct technical process. A clean background (background) is the internal negative control. If the test is working correctly the background in the Control Zone should not be difficult to interpret the result. External Quality Control: Each facility or health practitioner who uses the test should develop its own test instructions and process certification test.

FUNCTIONAL CHARACTERISTICS

1. The distinctive threshold of test (cutoff value) **DyonCalpro®** is 50 or 200 micrograms Calprotectin per gram of stool. This was demonstrated by the method of serial dilutions of a control solution containing or not stool negative in Calprotectin.

2. Performance. **DyonCalpro®** was tested for detection of Calprotectin in a study of 64 patients. **DyonCalpro®** was compared with a commercially available test. Initially all samples identified as positive or negative by the commercial test. Then the same samples were tested with **DyonCalpro®**. In these conditions, the yield of the device **DyonCalpro®** compared to a commercially available test was:

-Specificity & Sensitivity of device **DyonCalpro®** 92.3% and 94.4% respectively

-Positive Predictive Value of the device **DyonCalpro®** : 94.4%

-Negative Predictive Value of the device **DyonCalpro®** : 92.9%

-Accuracy of the device **DyonCalpro®** : 93.4%

3. Interference. **DyonCalpro®** assay is specific for Calprotectin and unaffected by transfection with transferrin and hemoglobin.

REFERENCES

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