Calprotectin - Turbidimetric reagent Ref.:CPT-01B/CPT-02B
Determination of Calprotectin in stool samples
Only for *in vitro* use in clinical laboratory
Store at 2-8ºC

Calprotectin

CE

CLINICAL SIGNIFICANCE

Calprotectin is a neutrophil cytosolic protein with antimicrobial properties, which is present at increased concentration in stool samples during bowel inflammation. The stability of the protein to degradation keeps it stable in faeces 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 remarkably resistant to protelolytic degradation and so is stable in stools kept at room temperature for 7 days.

PRINCIPLE OF THE TEST

Calprotectin Turbilatex is a latex turbidimetric assay **only for the quantitative detection of Calprotectin in human solid stool samples**.

The intended use of the test is exclusively to differentiate IBD patients with inflammation from IBD patients without inflammation and from irritable bowel syndrome (IBS).
Calprotectin latex turbidimetric assay is based on agglutination reactions. These involve *in vitro* aggregation of microscopic latex particles. This aggregation consists in the specific reaction between antigen and antibodies, antigen contained in the sample and the antibodies anti-antigen coated on polystyrene latex particles**.** The sample is mixed with a suspension containing antibodies against the antigen bound to latex particles. If antigen is present in the sample it will react with the antibodies and form an aggregate. If no antigen is present in the sample the mixture will keep its appearance as a smooth suspension. Such turbidity is measured as an increase in absorbance at the determinate wave and is proportional to the quantity of antigen contained in the sample.

WARNING AND PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- If the result exceeds the measurement range, use the sample diluent to dilute the sample and repeat the assay again.
- Do not use after expiration date.
- Do not use the reagents if pack is damaged or opened.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, googles and mask. Do not eat, drink or smoke in the working area.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The solutions should be discarded in a proper container after testing following local regulations.

INSTRUMENTS:

Instrument applications are available upon request.

REAGENT PREPARATION AND STABILITY

Reagent 1 and 2 are ready for use.
Before use, mix reagent by gently inverting each bottle.
If stored and handled properly:

- Unopened components are stable until expiry date stated on the label.
- Once open, components are stable for 1 month at 2-8°C.

TYPE OF SPECIMENS

Collect sufficient quantity of human solid stool samples. These samples should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-8ºC) for 7 days prior to testing. Homogenize stool samples as thoroughly as possible prior to preparation.

To process the collected stool samples:

Use a centrifugation tube for each sample to be tested. Label centrifugation tube with name or number of patient.

1. Homogenize the sample. Add 20mg of sample into centrifugation tube.
2. Add 2mL of sample diluent.
3. Shake vigorously the tube in order to assure good sample dispersion (vortex) until sample is completely dissolved. Rest tubes on the bench for 10 minutes to get a proper calprotectin extraction.
4. Centrifuge for 15 minutes at 10000g or 10 minutes at 15000g.
5. Take the supernatant to automated analyser vial.

TEST PROCEDURE:

Allow reagents and stool samples to reach room temperature (15-30ºC) prior to testing.
R1 and R2 are ready to use.

Preparation of the calibration curve

For calibration use only the following materials:
CPT-CAL (Calprotectin Turbilatex Calibrator vials): liquid calibrator containing recombinant human calprotectin.
Calibrate the system every week is extremely recommended. Recalibrate the system when reagent lot is changed or when the controls are out of the assigned range given on the control label or certificate of analysis provided with the kit.
Calprotectin Turbilatex Calibrator is ready to use.

Prepare the tubes for calibration curve (5 points) as follow:

Vials	Blank	Cal. 1	Cal. 2	Cal. 3	Cal. 4	Cal. 5
Conc (µg/g)	0	50	100	250	750	1500

Positive results: higher or equal than the cut-off fixed by the clinical lab.

Positive results determine the abnormal presence of human Calprotectin (hCp) in stool samples.

QUALITY CONTROL

For quality control only use the following materials:

CPT-CT1 (Calprotectin Turbilatex Control 1) and **CPT-CT2** (Calprotectin Turbilatex Control 2): liquid controls at two different concentrations of recombinant human calprotectin. Concentration is indicated on the label of the vial. The use of control materials at two different concentrations is recommended in order to verify test precision.

Controls should be assayed every day before running patient faecal samples extract to validate the calibration curve.

If the obtained results are out of the tolerance range, the equipment, the reagents or the technique must be reviewed.

Allow Calprotectin Turbilatex C1 & C2 to reach room temperature (15-30ºC) prior to testing.

Calprotectin Turbilatex Control vials (Control 1 and Control 2) are ready to use.

EXPECTED VALUES

Recommended: 50 µg of hCp/g of stool for diagnostic procedures and 200 µg of hCp/g of stool for screening procedures.
Each laboratory should establish its own reference range. Results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Analytical sensitivity

Limit of Detection (LOD): **see annex information**.

Calprotectin concentration values low than 50 µg of hCp/g of stool are considered normal values and that is not indicative of an inflammation of gastrointestinal tract.

Calprotectin concentration values higher than 200 µg of hCp/g of stool are indicative of an inflammation of gastrointestinal tract.

Prozone effect

Studies have been made up to a concentration of 8000 µg of hCp/g of stool and no false negative results have been observed. Studies using higher concentrations have not been carried out.

Method Comparison

An evaluation was performed comparing our turbidimetric assay with other commercial immunoassays. The results were as follows:

Sensitivity	Specificity
94 %	> 99 %

The results showed a high sensitivity and specificity to detect human calprotectin (hCp) using Calprotectin Turbilatex.

Interferences and cross reactivity

An evaluation was performed to determine the cross reactivity: no cross reactivity was founded against other faecal markers occasionally present in faeces, such as bovine and pig haemoglobin, bovine and pig transferrin, bovine lactoferrin and human haemoglobin, transferrin and lactoferrin.

LIMITATIONS

1. Calprotectin Turbilatex should **only be used in human solid stool samples (not to be used for body fluid such as blood, serum, plasma, urine, cerebrospinal fluid, oral fluid, synovial fluid or empyema fluid**). The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper faecal specimens must be obtained.
2. Positive results determine the presence of hCp in faecal samples. A positive result should be followed up with additional diagnostic invasive procedures, a colonoscopy and a biopsy in order to confirm the diagnosis and to establish the inflammation extent.
3. If symptoms or situation still persist, calprotectin determination should be carried out invasive techniques. Negative results do not exclude IBD with inflammation, some diseases such as celiac sprue and microscopic colitis polyps that mainly involve mononuclear inflammation.
4. Stool samples from patients with non-steroidal anti-inflammatory drug (NSAID) treatment could show positive result.
5. Neonatal faecal calprotectin levels have been reported higher than those in normal children with a mean of 167µg/g (range 22-860 µg/g).

BIBLIOGRAPHY

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