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EN

Hemo One+, Calprotectin

REF R33300000059

R1 1x 18.5mL | **R2** 1x 2.7mL

Instructions For Use

100 Tests

Doc Control: IFU90053-B-00
Rev. B – 26/05/2022

CE IVD

Intended Use

Calprotectin is a latex turbidimetric assay for the quantitative detection of calprotectin in human solid stool samples and is intended for use on the I.S.E. S.r.l. **Hemo One** analyser.

Test results should exclusively be used to differentiate IBD patients with inflammation from IBD patients without inflammation and from irritable bowel syndrome (IBS).

For professional in vitro diagnostic use only.

Diagnostics Application

Calprotectin is a neutrophil cytosolic protein with antimicrobial properties, which is present at increased concentration in stool samples during bowel inflammation. 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.

Method

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.

Reagents Provided

R1	**Reagent 1**:	1 Vials x 18.5 mL	Ready to Use
R2	**Reagent 2**:	1 vials x 2.7 mL	Ready to Use

Stability and Storage

The reagents are stable until expiry date on the label when stored at 2-8°C. DO NOT FREEZE
After opening the reagents are stable for 1 month at 2-8°C.

Reagents required but not supplied.

The following materials are required to run test samples and must be ordered separately from your local authorised ISE S.r.l representative.

Reference	Description
R3330000061	Universal Stool Extraction Vials
R3330000065	Standard Set Calprotectin
R3330000069	Control Set Calprotectin

Sample collection and preparation

Samples should be collected utilising the Universal Stool Extraction Vials. Homogenize stool samples as thoroughly as possible prior to preparation.

- R3330000061 Universal Stool Extraction Vials

Consult the Universal Stool Extraction Vial instructions for use for correct stool sample extraction.

The samples can be stored in the refrigerator (2-8°C) for 7 days prior to testing.

ASSAY PROCEDURE

Application parameters

The Application parameters are included in the Hemo One software. In the event of a missing the Calprotectin protocol please contact your authorised ISE representative.

Any application not explicitly approved by I.S.E. S.r.l. cannot be guaranteed in terms of performance.

Hemo One Use

Consult the I.S.E. S.r.l. Hemo One user manual for instructions on the proper use of the analyser.

Material Preparation

- Ensure samples are collected correctly in accordance with the sample preparation procedure.
- Allow reagents and stool samples to reach room temperature (15-30°C) prior to testing.
- Allow any calibrator or control material to reach room temperature (15-30°C) prior to testing.

Calibration curve establishment

A calibration curve must be performed prior to running samples. The curve must be validated with controls.

Calibration Stability

The calibration is recommended to be run every 7 days on the Hemo One analyser.

Quality control

For quality control purposes only use the following materials:

- R3330000069 Control Set 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 across the measuring range. Control frequency should be run in accordance with the laboratory's quality management system.

If the obtained results are out of the tolerance range;

- Ensure all materials are not expired
- Ensure all materials have been stored and prepared correctly
- Perform a calibration

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In the event of controls results not meeting the defined tolerances please contact you local ISE authorised representative for support.

ANALYTICAL CHARACTERISTICS / PERFORMANCE

NORMAL VALUES

Calprotectin cut-off value:

Calprotectin concentration values lower 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.

< 50 hCp/g	not indicative of an inflammation
50 - 200 hCp/g	Inconclusive, repeat and follow up recommended
> 200 hCp/g	indicative of an inflammation

Method Comparison

An evaluation was performed comparing I.S.E. S.r.l. Calprotectin against Calprest®, Eurospital. The results were as follows:

	Sensitivity	Specificity
ISE Calprotectin vs Calpro	**94%**	**> 99%**

Linearity

Using the calibrator kit Calprotectin is linear in the calibration range of **0-1500 µg hCp/g**

Limit of detection (LOD):

Limit of detection (LOD): **15 µg hCp/g**
The lower limit of detection of Calprotectin was determined on 20 samples and 2 sample replicates as the mean value + 2 SD

Limit of quantification (LOQ):

Limit of quantification (LOQ): **20 µg hCp/g**
The lower limit of quantification is defined as the lowest actual amount of analysis that can be reliably detected; imprecision is < 20% as CV%

Precision

Precision was assessed with 3 different controls

Precision	Low	Medium	High
Number	20	20	20
Mean (ng/mL)	51.7	208.1	765.8
SD	2.6	9.2	27.2
CV (%)	5	4	4

Prozone effect

Using the reported parameters, no hook effect was observed up to 8000 µg hCp/g of stool. Samples with calprotectin concentration of 8000 µg hCp/g of stool give a typical positive result >1500 µg hCp/mL

Disposal of reagent

Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.

The product is in conformity with D.L: 8 September 2000, no. 332 "Actuation of the directive 98/79/EC regarding in vitro medical diagnostic devices".

Symbols on labels and packaging

IVD	In vitro diagnostic medical device
REF	Catalog Number
LOT	Lot or batch number
	Manufacturer
	Expiry date
	Temperature limitation
	Consult Instructions for use

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