General Information

Intended use:

Calprotectin Turbilatex is a latex turbidimetric assay for the quantitative detection of calprotectin (hCp) in human stool samples.

This assay is simple and widely applicable. Test results aid in a presumptive diagnosis of IBD patient with inflammation and from irritable bowel syndrome (IBS).

For professional *in vitro* diagnostic use only.

Calprotectin Turbilatex can be performed on every open chemistry analyser. Please follow the subsequent instructions in order to assure performance characteristics as describes in the instructions for use. This instruction has been validated by CerTest BIOTEC S.L. Laboratories.

Additionally, please read the "Instructions for use" for instructions on operating and programming user defined test.

Reagents:

Materials provided by CerTest BIOTEC:

Reagents	Quantity	Code
Turbidimetric reagents (R1 & R2) 200 Det/kit	R1: 2 vials, 2x27 mL R2: 1 vial, 1x8 mL	TL-022CP01 TL-022CP02
Auxiliary Reagents		
Calibration kit	Calibrator: 6 vials, 6x1 mL.	TL-022CP70, TL-022CP71 TL-022CP72 TL-022CP73 TL-022CP74 TL-022CP75
Controls kit	Control C1, 2 vials, 2x1 mL/vial. Control C2, 2 vials, 2x 1 mL/vial.	TL-022CP08 TL-022CP09
Sample dilutions vials	1x2 mL/vial 1x2.4 mL/vial	MST-0018MU MST-0019U

Preparation of reagents:

R1 and R2 are ready to use.

Calibrators are ready to use.

Controls are ready to use

Storage and stability

Kit components must be stored at temperature indicated on the label. Do not freeze.

Reagents are stable up to the expiration date printed on the label, always considering that reagent containers must be properly closed to avoid any contamination, must be kept away from the sunlight and conserved at temperature indicated on the label of each reagent.

Specimen:

Collect enough quantity of human 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. For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC. In this case, the sample will be totally thawed, and brought to room temperature (15-30ºC) before testing. Freezing and thawing cycles are not recommended. Homogenise stool samples as thoroughly as possible prior to preparation.

The sample dilution vial with diluted sample can be stored for 7 days in the refrigerator (2-8ºC) prior to testing.

Use Calprotectin Turbilatex stool collection tubes for sample collections described the instructions for use.

Assay procedure

Application parameter set up:

Specific analyzers settings for Calprotectin Turbilatex must be programmed onto the analyzer, see below. For instructions, consult the Vitros 5600 (Ortho Clinical Diagnostics) analyzer manual and instructions for use provided with the kit.

Loading of reagents:

Load reagents according to the Vitros 5600 (Ortho Clinical Diagnostics) analyzer manual.

Calibration curve establishment:

A 6 point calibration curve can be established in Vitros 5600 (Ortho Clinical Diagnostics) analyzer. For instructions consult analyzer manual.

Calibration stability:

Calibrate the system at least once a month is extremely recommended. Recalibrate the system when reagent lot is change or when the controls are out of the assigned range given in the control label and CoA.

QC controls:

Calprotectin Turbilatex controls C1 and C2 must be assayed each day before running patient fecal sample extract to validate the calibration curve. The controls have assigned value ranges indicated on the label and certificate of analysis supplied. The control measurements must be within the indicated value range to obtain valid results for patient fecal extract. If the control values are out of range, follow next procedures: 1) Repeat QC control measurement, 2) Repeat calibration measurement.

Results:

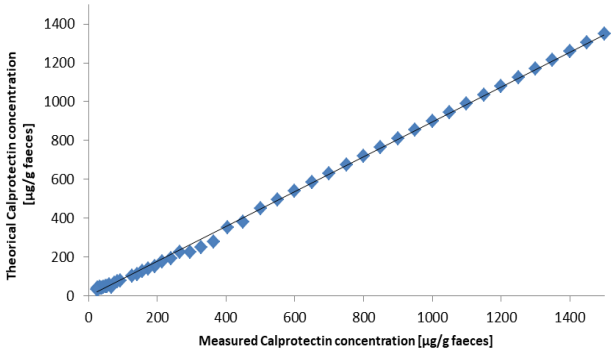
The results are evaluated automatically by the analyzer and presented in µg hCp/g of stool.

Performance characteristics

The following results have been obtained during the validation of Calprotectin Turbilatex on the Vitros 5600 (Ortho Clinical Diagnostics) analyzer.

Linearity:

Calprotectin Turbilatex on Vitros 5600 (Ortho Clinical Diagnostics) analyzer using calibrator kit is linear in the calibration range of 0-1500 µg hCp/g of stool.



Measuring range:

Calprotectin Turbilatex assay measuring range is 20-8000 µg hCp/g of stool on the Vitros 5600 (Ortho Clinical Diagnostics). Samples higher concentrated than 1500 µg hCp/g of stool must be diluted for proper quantification by the user, using additional sample buffer.

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.

Detection limit

Limit of detection (LOD): 7 µg hCp/g of stool (*). The lower limit of detection of Calprotectin Turbilatex was determined on 20 samples and 2 sample replicates as the mean value + 2·SD.

Limit of quantification (LOQ): 20 µg hCp/g of stool (*). The lower limit of quantification is defined as the lowest actual amount of analysis that can be reliably detected; imprecision is < 20% as CV% on the Vitros 5600 (Ortho Clinical Diagnostics) analyzer.

(*) Data obtained by the analyser Biolis 24i (Tokyo Boeki)

Precision

Calprotectin Turbilatex was tested with three different controls levels.

	Low (50 µg/g)	Medium (200 µg/g)	High (750 µg/g)
N	20	20	20
Mean (µg/g)	49.2	212.6	784.2
SD (µg/g)	1.4	7.7	23.1
CV (%)	3	4	3

Method comparison

Results obtained with Calprotectin Turbilatex on the analyser Biolis 24i (Tokyo Boeki) were compared with a commercial immunoassay (Calprest®, Eurospital).

	Sensitivity	Specificity
Calprotectin Turbilatex vs Calprest®	**94%**	**>99%**

Shipping damage

Please notify your distributor, it this product was received damaged.

Symbols key

IVD	For in *vitro* diagnostic use only		Keep dry
	Consult instructions for use		Temperature limitation
REF	Catalogue number	LOT	Lot number
	Use by		Manufacturer
Σ n	Contains sufficient for <n> test	DIL	Sample diluent
	Keep out of the sunlight		

Manufacturer

CERTEST BIOTEC

Pol. Industrial Río Gállego II,Calle J, Nº 1, 50840,
San Mateo de Gállego, Zaragoza (SPAIN)
www.certest.es

NOTES

Please refer to the instruction for use for the detailed information about the test on the following:
Synthesis; Principle; Precautions; Reagents; Specimen collection; Interpretation of results.

Vitros 5600 (Ortho Clinical Diagnostics) / Application parameters

ASSAY PARAMETERS	
Std. No	6
R1	200 µL
Sample	5 µL
R2	30 µL
Others	NA
Reaction mode	Endpoint
Primary wavelength	450 nm
Secondary wavelength	None
Direction	Increase
Reagent blank lecture	After R2 addition
Final lecture	294 sec after 1st lecture
Reaction time	10 min
Linear range	0-1500 µg/g
CALIBRATION	
Calibration Method	Linear
Calibration set	5 calibrators + Blank
Blank	Calibrator 1 (0 µg/g)
Calibrator 1	Calibrator 2 (50 µg/g)
Calibrator 2	Calibrator 3 (100 µg/g)
Calibrator 3	Calibrator 4 (250 µg/g)
Calibrator 4	Calibrator 5 (750 µg/g)
Calibrator 5	Calibrator 6 (1500 µg/g)
STEPS	
Addition R1	
Addition Sample	
Incubation	
Addition R2	
Blank Lecture	After R2 addition
Incubation	
Final lecture	294 sec after 1st lecture