

Application Note

Calprotectin Turbilatex, BS-200/200E, Mindray (AN-Cp-BS-200.EN rev. 2019.05.28)

For *in vitro* diagnostic device
ENGLISH



General Information

Intended use:

Calprotectin Turbilatex is a latex turbidimetric **assay only for the quantitative detection of calprotectin (hCp) in human stool samples** (not to be used for body fluid as blood, serum, plasma, urine, cerebrospinal fluid, oral fluid, synovial fluid or empyema fluid).

This assay is simple and widely applicable. 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.

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 S.L.:

Reagents	Quantity	Cat. reference
Turbidimetric reagents (R1 & R2) 200 Det/kit	R1: 2 vials, 2x27 mL R2: 1 vial, 1x8 mL	TL-022CP01 TL-022CP02
Auxiliary Reagents	Quantity	Cat. reference
Calibration kit	Calibrator: 6 vials, 6x1mL.	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, 2x1 mL/vial.	TL-022CP08 TL-022CP09
Sample diluent kit	4 vials, 4x125 mL/vial	TL-022CP03E
Sample dilutions vials	1x2 mL/vial 1x2 mL/vial	MST-0006MC MST-0008C

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 (2-8°C) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20°C. In this case, the sample will be totally thawed, and brought to room temperature (15-30°C) 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 (2-8°C) 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 BS-200 (Mindray) analyzer manual and instructions for use provided with the kit.

Loading of reagents:

Load reagents according to the BS-200 (Mindray) analyzer manual.

Calibration curve establishment:

A 6 point calibration curve can be established in BS-200 (Mindray) 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 labels and CoA.

QC controls:

Calprotectin Turbilatex controls C1 and C2 must be assayed each day before running patient faecal 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 faecal 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.

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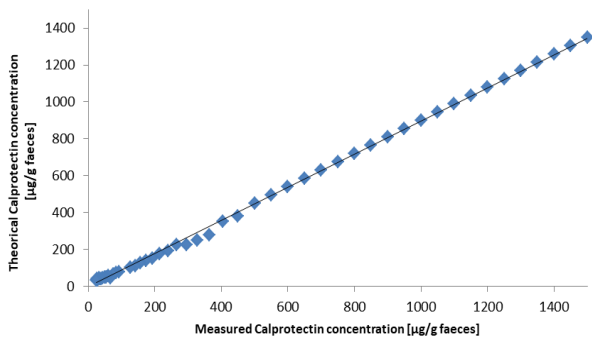


Performance characteristics

The following results have been obtained during the validation of Calprotectin Turbilatex on the BS-200 (Mindray) analyzer.

Linearity:

Calprotectin Turbilatex on BS-200 (Mindray) 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 41.4-8000 µg hCp/g of stool on the BS-200 (Mindray) analyzer. 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 prozone effect (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/g.

Detection limit

Limit of detection (LOD): 30 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 + 2xSD.

Limit of quantification (LOQ): 41.4 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 BS-200 (Mindray) analyzer.

Precision

Calprotectin Turbilatex was tested with three different controls levels.

	Low (50 µg/g)	Medium (100 µg/g)	High (500 µg/g)
N	20	20	20
Mean (µg/g)	46.8	93.8	484.1
SD (µg/g)	4.5	7.6	30.6

CV (%)

10

8

6

Method comparison

Results obtained with Calprotectin Turbilatex on the BS-200 (Mindray) analyzer were compared with a commercial immunoassay (Calprest®, Eurospital).

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

Shipping damage

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

NOTES

Please refer to the instruction for use for the detailed information about the test on the following:

Synthesis; Principle; Precautions; Reagents; Specimen collection and preparation; Interpretation of results and limitations.

Symbols key

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

Manufacturer

CERTEST BIOTEC S.L.

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

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The parameters optimized for BS-200 might be applied for BS-200E.

BS200, Mindray / 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	800 nm
Direction	Increase
Reagent blank lecture	1 cycle
Final lecture	20 cycle
Reaction time	10 min
Linear range	0-1500 µg/g
CALIBRATION	
Calibration Method	Linear
Calibration set	6 calibrators
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 (1500µg/g)
STEPS	
Addition R1	
Addition Sample	
Incubation	5 min
Addition R2	
Blank Lecture	
Incubation	5 min
Final lecture	



The screenshot shows the CerTest software interface. At the top, it displays 'Espera' (Waiting) mode, 'HOST' status, and the date/time '05/12/2017 14:40:29'. The main menu includes 'Reactivo', 'Calibrac', 'CC', 'Estado', 'Estadist', 'Parámetr', 'Config', and 'Mantenim'. The 'Test' configuration screen is active, showing parameters for 'CP Turbilatex':

Parámetros	Referenc	Calibración	CC
Test	CP Turbilatex	R1	200
Nº	2	R2	30
Nomb comp		Volum muestra	5
Nº estándar		Blanc R1	
Tipo reac	Pto fin	Blan react mez	
Onda prim	450nm	Interv lineali	
Onda secu	800nm	Límite lineali	
Dirección	Aumentar	Límit sustrato	
Tiem reac	0 - 20	Factor	
Tiem incu	14	Compensar	Pend 1 Interse 0
Unidad	ug/g	Comprobación pro:	q1 q2 q3 q4
Precisión	0.1	CP	Abs

Buttons at the bottom include 'Añadir', 'Borrar', 'OK', 'Cancel', and 'Configurar'.

The screenshot shows the 'Curva calibrac' (Calibration Curve) window. It displays the test name 'CP Turbilatex', the date/time '04/04/2018 15:58:5', and the rule 'Logit-Log 5P'. The calibration equation is shown as:

$$C = C + C[1], R = R_0 + K \frac{1}{1 + \exp[-(a + b \ln C + cC)]}$$

The parameters are:

Blan reac	9184.89813	R0	-50.11668	A	-8.89351
K	9693.93536	C	-0.00018	D	
B	1.31306				

The graph shows 'Respuest' (Response) on the y-axis (ranging from -100 to 7050) versus 'Concentración' (Concentration) on the x-axis (ranging from 0.0 to 0.1500). The data points are plotted as green dots, and a smooth orange curve is fitted to them. Buttons at the bottom include 'Intervalo', 'Actualiz', 'Imprimir', 'Anterior', 'Sigu', and 'Cerrar'.