

Application Note for the Gentian Calprotectin Immunoassay on the Cobas c501¹

For *in vitro* diagnostic use by laboratory professionals.

This document describes the instrument specific settings and performance of the product on the instrument above. For assay information, please refer to the IFU available on www.gentian.com.

Assay kit components

Products available	
Gentian GCAL [®] Calprotectin Reagent Kit	REF 1201
- R1 Assay Buffer (54 mL) - R2 Immunoparticles (9 mL)	
Gentian GCAL [®] Calprotectin Reagent Kit S	REF 1202
- R1 Assay Buffer (30 mL) - R2 Immunoparticles (5 mL)	
Gentian GCAL [®] Calprotectin Calibrator Kit (6 levels x 1 mL)	REF 1251
Gentian GCAL [®] Calprotectin Control Kit (2 levels x 1 mL)	REF 1219
Additional equipment but not provided	
Instrument-specific bottles	

All products are ready for use.

Reagent stability

The in-use stability of the Gentian GCAL[®] Calprotectin Reagent Kit was found to be at least 11 weeks in an on board study based on the CLSI guideline EP25 [1]. If the instrument remains unused for more than a week, please ensure the reagents are gently inverted every 7 days.

Calibration stability

The calibration curve stability of the Gentian GCAL[®] Calprotectin Calibrator Kit was found to be at least 11 weeks in a study based on the CLSI guideline EP25 [1].

Performance characteristics

All results refer to validation of the Gentian GCAL[®] Calprotectin Immunoassay on one instrument site with one lot of reagents, unless otherwise stated.

Measuring range

The measuring range of the Gentian GCAL[®] Calprotectin Immunoassay was found to be 0.42-21.59 mg/L in lithium heparin plasma and 0.42-21.29 mg/L in serum samples. The exact measuring range is specific to the calibrator lot, please refer to the analytical value sheet available on www.gentian.com.

Analytical sensitivity

The analytical sensitivity of the Gentian GCAL[®] Calprotectin Immunoassay was tested in a study based on the CLSI guideline EP17 [2]. The limit of quantification (LoQ) is defined as the lowest concentration of an analyte that can be reliably detected and at which the total error meets the requirements for accuracy. The LoQ of the Gentian Calprotectin Immunoassay was measured as 0.41 mg/L in lithium heparin plasma and 0.36 mg/L in serum samples.

Linearity

The linearity range of the Gentian GCAL[®] Calprotectin Immunoassay was found to be 0.42-21.59 mg/L in lithium heparin plasma and 0.42-21.29 mg/L in serum samples in a linearity study based on the CLSI guideline EP06 [3].

Security zone

No antigen excess effect in samples below 114 mg/L was observed for the Gentian GCAL[®] Calprotectin Immunoassay in a study based on the CLSI guideline EP34 [4]. Samples with a calprotectin concentration above the highest calibrator and up to 114 mg/L return a value above the highest calibrator and are flagged for rerun.

Precision

Precision of the Gentian GCAL[®] Calprotectin Immunoassay was tested in a 3-day precision study based on the CLSI guideline EP05 [5]. 3 lithium heparin plasma (P1-3) and 3 serum (S1-3) pools and 2 controls (CL, CH) were measured 3 times with 5 replicates (n=25).

Sample ID	Mean [mg/L]	Within run CV [%]	Between run CV [%]	Total CV [%]
P1	0.70	6.48	2.00	6.78
P2	4.94	1.05	1.12	1.54
P3	12.98	0.93	0.86	1.27
S1	0.64	9.30	8.80	12.80
S2	5.28	0.81	1.03	1.32
S3	13.52	1.09	1.01	1.49
CL	0.94	4.36	5.69	7.17
CH	9.39	1.52	1.17	1.91

Recovery

Recovery was analysed by spiking a low analyte sample with a high analyte sample according to Westgard [6]. The Gentian GCAL[®] Calprotectin Immunoassay had a recovery of 94-112%.

Analytical specificity and limitations

Interference was tested in a study based on the CLSI guideline EP07 [7]. As the antibodies in the Gentian GCAL[®] Calprotectin Immunoassay are of avian origin, there is no interference due to Rheumatoid Factor in the samples [8]. No clinically relevant difference was detected at the tested interferent concentrations.

Potential interferents	Concentration with no interference
Haemoglobin	8 g/L
Intralipid	5 g/L
Bilirubin	0.2 g/L

Instrument variation

Results obtained with the Gentian Calprotectin Immunoassay were compared using Passing-Bablok regression with results from the AU480 instrument (Beckman Coulter) in a study based on the CLSI guideline EP09 [9].

n	Range of samples [mg/L]	Term	Co-efficient	95% CI
75	0.42 – 19.35	Intercept	0.02	[-0.01, 0.04]
		Slope	0.99	[0.99, 1.00]
		R ²	1.00	



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References

1. CLSI. Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline. CLSI document EP25-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
2. CLSI. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012
3. CLSI. Evaluation of Linearity of Quantitative Measurement Procedures. 2nd ed. CLSI guideline EP06. Clinical and Laboratory Standards Institute; 2020
4. CLSI. Establishing and verifying an extended measuring interval through specimen dilution and spiking. 1st ed. CLSI guideline EP34. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
5. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical Laboratory Standards Institute; 2014
6. Westgard JO. Basic Method Validation, 3rd Edition. 2008; ISBN13: 9781886958258
7. CLSI. Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical Laboratory Standards Institute; 2018.
8. Larsson A, et al. Poultry Science 1993;72:1807-12
9. CLSI. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 3rd ed. CLSI guideline EP09c. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

Modification from the Previous Version

- Added additional reagent stability information

Date of issue

2025-08-25

Instrument Settings for the Gentian Calprotectin Immunoassay on the Cobas c501¹

Analyze		Calib.		Range		Other													
Assay/Time/Point		2 point End ▼	10 ▼	30	55	0	0												
Wavelength (2nd / Pri.)		Cancel ▼	660 ▼																
Sample Volume		<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td>Norm.</td> <td>3</td> <td>0.0</td> <td>0</td> </tr> <tr> <td>Dec.</td> <td>15</td> <td>3.0</td> <td>135</td> </tr> <tr> <td>Inc.</td> <td>10</td> <td>0.0</td> <td>0</td> </tr> </table>						Norm.	3	0.0	0	Dec.	15	3.0	135	Inc.	10	0.0	0
Norm.	3	0.0	0																
Dec.	15	3.0	135																
Inc.	10	0.0	0																
Dilution		☐ Water ☒ Diluent 951 10 ▼																	
Cassette Configuration		Code 0777773 Expiration days 99 <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td colspan="2">Reagent Volume</td> </tr> <tr> <td>R1</td> <td>180 0 Inactive ▼</td> </tr> <tr> <td>R2</td> <td>0 0</td> </tr> <tr> <td>R3</td> <td>30 0</td> </tr> </table>						Reagent Volume		R1	180 0 Inactive ▼	R2	0 0	R3	30 0				
Reagent Volume																			
R1	180 0 Inactive ▼																		
R2	0 0																		
R3	30 0																		
Linearity Limit		0	% 0	% 0	0														
Prozone Limit		0	0	0	0	0	Inside ▼ 0 0												
Abs. Limit		0	Decrease ▼		Version No.00-01														
Cell Detergent		Detergent 1 ▼		Stirring Level		8													
Stirring Setting		M1		M2		M3													
UP		Stirring ▼	LOW	Stirring ▼	Stirring ▼	Stirring ▼	Stirring ▼												
							SAVE												

Cassette Type A

Bottle

a	R1 ▼	110	25.6
b	R1 ▼	90	18.0
c	R3 ▼	200	9.0

Cancel
OK

Cassette Type A

Bottle

a	R1 ▼	85	25.0
b	R1 ▼	20	5.0
c	R3 ▼	105	5.0

Cancel
OK

Settings for Gentian GCAL® Calprotectin Reagent Kit: REF 1201.

Settings for Gentian GCAL® Calprotectin Reagent Kit: REF 1202.

Analyze	Calib.	Range	Other
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Calibration Type Spline

Point 6

Span 4

Weight 0

Update Type None 0 0

SD Limit 999

Duplicate Limit 99 % 32000 Abs.

Sensitivity Limit -99999 99999

S1 Abs. Limit -32000 32000

☐ Auto Masking

Auto Calibration

☒ Timeout

Cassette Cancel

0 Day

Changeover

Cassette Cancel

☐ QC Violation

Method Blank

Rule 1s

Control1 None

Control2 None

Control3 None

Save

Analyze	Calib.	Range	Other
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Application Code *

Unit mg/L

Reporter name GCAL

Data mode Active

☐ Automatic Rerun

Technical Limit -99999 99999

Repeat Limit -99999 99999

☐ Control Interval Time 0

☐ Automatic QC On Board Stability 1

☐ Qualitative

(1) 0

(2) 0

(3) 0

(4) 0

(5) 0

(6) 0

Expected Values

Male

99	Year	-99999	99999
100	Year	-99999	99999
		-99999	99999

Female

99	Year	-99999	99999
100	Year	-99999	99999
		-99999	99999

Default

Sex

☒ Male ☐ Female

Range

☒ Range 1 ☐ Range 2 ☐ Range 3

Save

Analyze	Calib.	Range	Other			
	(1)	(2)	(3)	(4)	(5)	(6)
Calibrator Code	10*	11*	12*	13*	14*	15*
Concentration	**	**	**	**	**	**
Rack No.-Pos.	*	*	*	*	*	*
Sample Volume	3	3	3	3	3	3
Diluted S. Volume	0	0	0	0	0	0
Diluent Volume	0	0	0	0	0	0
Save						

Disclaimer: The specific settings above is what used to validate the application on the specific instrument. For any instrument specific settings, please refer to the instrument manual. Please be aware that illustrations or settings might be affected in case of an instrument software update.

* User defined

** Lot specific. See analytical value sheet available on www.gentian.com