GCAL®

Application Note for the Gentian Calprotectin Immunoassay on the Cobas c503[1]

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 • R1 Assay Buffer (54 mL) • R2 Immunoparticles (9 mL)	REF 1201
Gentian GCAL® Calprotectin Reagent Kit S • R1 Assay Buffer (30 mL) • R2 Immunoparticles (5 mL)	REF 1202
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 material required 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 12 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 4 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 0.5-19.1 mg/L. 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 found to be 0.45 mg/L in in lithium heparin plasma and 0.48 mg/L in serum.

Linearity

The linearity range of the Gentian GCAL® Calprotectin Immunoassay was found to be 0.4-19.1 mg/L in a linearity study based on the CLSI guideline EP06 [3].

Security zone

No antigen excess effect in samples below 93 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 93 mg/L return a value above the highest calibrator and are flagged for rerun with automatic dilution.

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 pools (P1-3) and 2 controls (CL, CH) were measured 5 times with 5 replicates (n=25).

Sample ID	Mean [mg/L]	Within run CV [%]	Between run CV [%]	Total CV [%]
P1	0.81	6.6	5.5	8.6
P2	5.52	1.4	0.8	1.6
P3	13.02	0.5	0.7	0.8
CL	0.95	4.0	4.5	6.0
CH	9.96	0.7	0.5	0.8

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 91-96 %.

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	2.5 g/L
Intralipid	10 g/L
Bilirubin	600 mg/L

Instrument variation

Results obtained with the Gentian GCAL® Calprotectin Immunoassay were compared using Passing-Bablok regression with results from the Cobas c501 instrument (Roche) in a study based on the CLSI guideline EP09 [9].

n	Range of samples [mg/L]	Term	Co-efficient	95% CI
46	0.12 - 21.8	Intercept	-0.07	[-0.09, -0.04]
		Slope	0.99	[0.98, 1.00]
		R^2	1.00	

[1]Registered trademark of Roche

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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

- Updated stirring speed.

Date of issue

2025-01-22

Instrument Settings for the Gentian GCAL® Calprotectin Immunoassay on the Cobas c503[1]

Application definition	*

CDC File Creator version	*
Workspace version	*
Data concept version	*

Analytical system, analytical unit	cobas pro, cobas c 503
Code	*
Version	1
Laboratory name	*

Analytical Parameters

Long Name	*
Unit 1	mg/L
Unit 2	none
Conversion Factor	1
Sample Type	Serum/Plasma

Rerun Setting

Result outside technical limit -> Below	Off	
Result outside technical limit -> Above	On	
Technical Limit	0.4	20

Assay

Assay	2 Point End			
Time	10			
Primary Wavelength	660			
Secondary Wavelength	None			
Points	1	2	3	4
	15	27	0	0

Reagent Volume

Reagent Type	Reagent	Dilution Volume	Mode	Mixing
R1	120	0	Without	8
R2	0	0	Water Push	1
R3	20	0	Without	8

Sample Volume

Sample Type	Sample	Diluted	Diluent	Mixing
Normal	2	0	0	4
Decreased	10	2	90	4
Increased	10	0	0	4

[1]Registered trademark of Roche

Diluent -> Type	Diluent
Diluent -> ACN	951
Diluent -> Dilution	10

Cell Wash

Wash Cells With	Basic and Acid
Higher Uncertainty	0
QC Interval Timeout	False
QC Interval Timeout -> Hours	1

Calibration

Changeover Settings

Lot Changeover	Full
Automatic masking if calibration failed	True
Reagent Pack Changeover	Cancel

Calibration Trigger

QC Violation -> Method	Cancel
Timeout -> Method	Cancel
Timeout -> Stability	99 Days

Limit Values

SD Limit	0	
Duplicate Limit	99 %	3.3 Abs
Sensitivity Start	0	
Sensitivity End	0	
Sensitivity Limit	-9.9	9.9
S1 Abs. Limit	-3.3	3.3

Calibration Method

Curve Type	Spline
Point	6
Weighting	0
K-factor	0

RCM Weighting

1	0
2	0
3	0
4	0
5	0
6	0

Calibrators
Standards

Standard ID	Calibrator Code	Calibrator Volume	Diluted C. Volume	Diluent Volume
S 1	*	2	0	0
S 2	*	2	0	0
S 3	*	2	0	0
S 4	*	2	0	0
S 5	*	2	0	0
S 6	*	2	0	0

Calibrator Diluent -> Type	Water
Calibrator Diluent -> Code	None
Calibrator Diluent -> Dilution Factor	0

Checks
Kinetics unstable check
Module 1

Enabled	True
Type	Entry module
Limit low	-99999.99
Limit high	99999.99
Check type	Outside
MP1	1
MP2	1
Inactive below	0
Priority	10
Action	Flag only
Calculation formula	-

Ranges
Linearity Limit

4-8 Point	0 %
9 Point	0 %
Min. Total Rate	0
Min. Diff. Rate	0

Reaction Limit

Check	Off
Abs. Limit	0
Method	Decrease

Application Correction Factor	A	B
	1	0

Sample Index Limits	L	H	I
	0	0	0

R.P. Settings

c packs

c pack	*
No. of Tests	80

Position	Reagent Type	Pipetting Volume	Filling Volume	Max Volume
B	R1	120 µL	14.4 mL	95.0 mL
C	R3	20 µL	4.4 mL	30.0 mL

c pack	None
No. of Tests	-

Position	Reagent Type	Pipetting Volume	Filling Volume	Max Volume
B	-	-	-	95.0 mL
C	-	-	-	30.0 mL

* User defined

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.