GCAL®

Application Note for the Gentian Calprotectin Immunoassay on the AU680[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

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 4 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 1 week 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 0.48-19.16 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 GCAL® Calprotectin Immunoassay was found to be 0.35 mg/L for lithium heparin plasma and serum.

Linearity

The linearity range of the Gentian GCAL® Calprotectin Immunoassay was found to be 0.44-22.02 mg/L for lithium heparin plasma and 0.41-20.98 mg/L for serum in a linearity study based on the CLSI guideline EP06 [3].

Security zone

No antigen excess effect in samples below 90 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 90 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, P2 and P3) and 3 serum (S1, S2 and S3) pools 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	0.90	3.50	1.27	3.72
P2	6.01	0.67	0.97	1.18
P3	13.64	0.71	1.87	2.00
S1	1.02	3.57	0.00	3.57
S2	5.84	0.61	0.73	0.95
S3	14.27	0.86	1.53	1.76
CL	1.01	3.23	3.00	4.41
CH	9.98	0.81	0.57	0.99

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 95-97 %.

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	0.6 g/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	Coefficient	95% CI
50	0.50-21.07	Intercept	-0.12	[-0.19, -0.05]
		Slope	1.13	[1.12, 1.15]
		R^2	1.00	

[1] Registered trademark of Beckman Coulter

Gentian AS CE 0123

Bjornasveien 5
N-1596 Moss
Norway
TEL: +47 99 33 99 05
www.gentian.com

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

- Harmonised analytical measuring range across Beckman Coulter instruments

Date of issue

2024-11-26

[1] Registered trademark of Beckman Coulter

Instrument Settings for the Gentian GCAL® Calprotectin Immunoassay on the AU680[1] (serum/plasma)

Reagent ID: 254

Specific Test Parameters

General | LIH | ISE | Range

Test Name: CAL1G ▽ < > Type: Serum*** ▽ Operation: Yes ▽

Sample Volume		4.0 µL	Dilution	0 µL	OD Limit			
Pre-Dilution Rate		1			Min. OD		Max. OD	
Reagents Volume:	R1(R1-1)	180 µL	Dilution	0 µL	Reagent OD limit:			
					First Low	-2.0000	High	2.5000
					Last Low	-2.0000	High	2.5000
	R2 Volume	30 µL	Dilution	0 µL				
					Dynamic Range Low	0.48	High	19.16
Common Reagent	Type	None	Name		Correlation Factor A	1	B	0
Wavelength:	Pri.	660 nm	Sec.	None nm	Factor for Maker A	1	B	0
Method:		END ▽						
Reaction slope:		+ ▽			Onboard Stability	28 Days	0	Hour
Measuring Point 1:	First	9	Last	15	LIH Influence Check	No ▽		
Measuring Point 2:	First		Last		Lipemia	▽		
Linearity:		%			Icterus	▽		
No Lag Time:		▽			Hemolysis	▽		

Specific Test Parameters

General | | ISE | Range

Test Name: CAL1G ▽ < > Type: Serum*** ▽

Value/Flag: * ▽ Level L: * Level H: *

Specific Ranges:

	Sex	From Year	From Month	To Year	To Month	Low	High
1.	* ▽	*	*	*	*	*	*
2.	* ▽	*	*	*	*	*	*
3.	* ▽	*	*	*	*	*	*
4.	* ▽	*	*	*	*	*	*
5.	* ▽	*	*	*	*	*	*
6.	* ▽	*	*	*	*	*	*
7. No demographics						*	*
8. Not within expected values						*	*

Panic Value

Low	High
*	*

Unit mg/L Decimal Places *

Calibration Specific

General | ISE

Test Name: CAL1G ▽ | < | > | Type: Serum*** ▽ | ☐ Use Serum Cal.

Calibration Type: 6AB ▽ | Formula: Spline ▽ | Counts: 2

<Calibrator Parameters> | Slope Check: None ▽

	Calibrator	OD	Conc	Factor Range Low	Factor Range High
Point 1:	1 ▽		**	-2.0000	2.5000
Point 2:	2 ▽		**	-2.0000	2.5000
Point 3:	3 ▽		**	-2.0000	2.5000
Point 4:	4 ▽		**	-2.0000	2.5000
Point 5:	5 ▽		**	-2.0000	2.5000
Point 6:	6 ▽		**	-2.0000	2.5000
Point 7:	▽				
Point 8:	▽				
Point 9:	▽				
Point10:	▽				

Allowable Range Check
☐ Reagent Blank
☐ Calibration

Advanced Calibration
Operation: * ▽
Interval (RB/ACAL): * ▽

<Point Cal. For Master Curve> | No. of Correction Points ▽ | Use Master Curve ▽ | ☐ Lot Calibration

	Calibrator	OD	Conc	OD Range Low	OD Range High
Point 1:					
Point 2:					

Stability
Reagent Blanks: 7 Day 0 Hour
Calibration: 7 Day 0 Hour

MB Type Factor: | 1-Point Calibration Point: None ▽ | ☐ With CONC-0

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
***Valid for both serum and lithium heparin plasma