

Application Note for the Gentian Calprotectin Immunoassay on the AU5800¹

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 <ul style="list-style-type: none"> R1 Assay Buffer (54 mL) R2 Immunoparticles (9 mL) 	REF 1201
Gentian GCAL [®] Calprotectin Reagent Kit <ul style="list-style-type: none"> 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 0.40 mg/L.

Linearity

The linearity range of the Gentian GCAL[®] Calprotectin Immunoassay was found to be 0.38-20.26 mg/L in a linearity study based on the CLSI guideline EP06 [3].

Security zone

No antigen excess effect in samples below 100 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 100 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]. 2 serum pools (S1-2), 1 lithium heparin plasma pool (S3) and 2 controls were measured 5 times with 5 replicates (n=25).

Sample ID	Mean [mg/L]	Within run CV [%]	Between run CV [%]	Total CV [%]
S1	0.93	3.62	3.86	5.29
S2	9.13	0.87	1.41	1.65
S3	12.75	0.88	0.58	1.05
CL	1.05	3.35	1.25	3.57
CH	9.66	0.90	1.20	1.49

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 103-119 %.

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
43	0.56-20.33	Intercept	0.08	[0.03, 0.11]
		Slope	1.04	[1.02, 1.06]
		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

- Harmonised analytical measuring range across Beckman Coulter instruments

Date of issue

2024-11-26

Instrument Settings for the Gentian GCAL[®] Calprotectin Immunoassay on the AU5800¹ (serum/plasma)

Reagent ID: 254

Parameters			Specific Test Parameters				
General	LIH	ISE	HbA1c		Calculated Test	Range	
Test Name:		CAL1G ▾	<	>	Type: Serum*** ▾	Operation Yes ▾	
Sample Volume	4.0	μL	Dilution	0	μL	OD Limit	
Pre-Dilution Rate	1	▾	Diluent Bottle	OutSide	▾	Min.OD Max.OD	
Rgt. Volume	R1(R1-1)	170	μL	Dilution	0	μL	Reagent OD Limit
	R1-2		μL	Dilution		μL	1 st Low High
							Last Low High
	R2(R2-1)	25	μL	Dilution	15	μL	Dynamic Range Low High
Common Rgt. Type	None		Name	None		Correlation Factor A	B
Wavelength	Pri 660	▾nm	Sec.	None	▾nm	Factor for Maker A	B
Method	END	▾					
Reaction Slope	+	▾				Onboard Stability Period	Day Hour
Measuring Point1 1 st	9		Last	15		LIH Influence Check	No ▾
Measuring Point2 1 st			Last			Lipemia	+ ▾
Linearity Limit		%				Icterus	+ ▾
Lag Time Check		▾				Hemolysis	+ ▾

Parameters			Specific Test Parameters				
General	LIH	ISE	HbA1c		Calculated Test	Range	
Test Name: <input type="text" value="CAL1G"/>			Type: <input type="text" value="Serum***"/>				
Value/Flag: <input type="text" value="*"/>			Level		Low <input type="text" value="-99999.99"/>	High <input type="text" value="99999.99"/>	
Specific Ranges:			From	To			
	Sex	Year	Month	Year	Month	Low	High
<input type="checkbox"/> 1.	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>
<input type="checkbox"/> 2.	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>
<input type="checkbox"/> 3.	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>
<input type="checkbox"/> 4.	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>
<input type="checkbox"/> 5.	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>
<input type="checkbox"/> 6.	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>	<input type="text" value="*"/>
7.	Standard demographics					<input type="text" value="*"/>	<input type="text" value="*"/>
8.	Not within expected values					<input type="text" value="*"/>	<input type="text" value="*"/>
Panic Value		Low	<input type="text" value="*"/>	High	<input type="text" value="*"/>	Unit	<input type="text" value="mg/dL"/>
						Decimal Places	<input type="text" value="2"/>

Parameters		Calibration Parameters			
Calibrators		Calibration Specific			
General		ISE			
Test Name: <input type="text" value="CAL1G"/> < > Type <input type="text" value="Serum***"/> Cuvette <input type="text"/>					
<input type="checkbox"/> Use Serum Cal.					
Calibration Type: <input type="text" value="6AB"/> Formula: <input type="text" value="Spline"/> Counts: <input type="text" value="2"/>					
<Calibrator Parameters>					
	Calibrator	OD	Conc	Range	
				Low High	
Point 1:	<input type="text" value="Cal 1"/>	<input type="text"/>	<input type="text" value="**"/>	<input type="text"/>	<input type="text"/>
Point 2:	<input type="text" value="Cal 2"/>	<input type="text"/>	<input type="text" value="**"/>	<input type="text"/>	<input type="text"/>
Point 3:	<input type="text" value="Cal 3"/>	<input type="text"/>	<input type="text" value="**"/>	<input type="text"/>	<input type="text"/>
Point 4:	<input type="text" value="Cal 4"/>	<input type="text"/>	<input type="text" value="**"/>	<input type="text"/>	<input type="text"/>
Point 5:	<input type="text" value="Cal 5"/>	<input type="text"/>	<input type="text" value="**"/>	<input type="text"/>	<input type="text"/>
Point 6:	<input type="text" value="Cal 6"/>	<input type="text"/>	<input type="text" value="**"/>	<input type="text"/>	<input type="text"/>
Point 7:	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Point 8:	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Point 9:	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Point 10:	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<Point Cal. For		No. of Correction Points		<input type="text"/>	Use Master Curve
Master Curve>					<input type="text"/>
				<input type="checkbox"/> Lot Calibration	
	Calibrator	OD	Conc	OD Range	
				Low High	
Point-1	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Point-2	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
MB Type Factor:		1-Point Calibration Point		<input type="text"/>	<input type="checkbox"/> with Conc-0
Slope Check <input type="text" value="None"/>					
Allowance Range Check					
<input type="checkbox"/> Reagent Blank <input type="text"/>					
<input type="checkbox"/> Calibration <input type="text"/>					
Advanced Calibration Operation <input type="text" value="No"/>					
Interval (RB/ACAL) <input type="text"/>					
Stability					
Reagent Blank <input type="text" value="7"/> Day <input type="text" value="0"/> Hour					
Calibration <input type="text" value="7"/> Day <input type="text" value="0"/> Hour					

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