GCAL®

Gentian GCAL® Calprotectin Reagent Kit

REF 1201 and REF 1202

For *in vitro* diagnostic use by laboratory professionals.

This document describes the general use of the product above. For instrument specific settings, please refer to the application notes available upon request to marketing@gentian.com.

Intended purpose

The Gentian Calprotectin Immunoassay is an immunoturbidimetric assay intended for the *in vitro* quantitative determination of calprotectin, a neutrophilic protein that is a marker of inflammation, in human lithium heparin plasma and serum samples. The Gentian Calprotectin Immunoassay is intended for use on automated clinical analysers by laboratory professional users. Used in conjunction with other laboratory findings and clinical assessments, Gentian Calprotectin is intended to be used as an aid in detection and assessment of inflammation and inflammatory response to infections.

Summary and explanation of test

Calprotectin is a heterodimeric protein S100A8/A9 with a molecular mass of 24 kDa, consisting of the two Ca^{2+} binding proteins S100A8 and S100A9 (also termed myeloid related proteins 8 and 14 (MRP8 and MRP14). Calprotectin is predominantly found in the neutrophils where it accounts for approximately 50 % of the cytosol's protein content [1]. Neutrophil granulocytes are one of the first responders to inflammation and bacterial infection [3]. Calprotectin is released from activated neutrophils after which its main biological effects are sequestering of ions [3] and binding to Toll-like receptor 4 (TLR4) and Receptor of Advanced Glycation Endproducts (RAGE) triggering an inflammatory response [1, 2, 4]. Calprotectin increases in the blood within hours up to 100-fold (in response to bacteria or endotoxin [5]) and it is considered to be an important inflammation marker [1, 2, 5-7].

Calprotectin indicates phagocyte activation more sensitively than conventional parameters of inflammation [4]. Consequently, there is a strong correlation to the inflammation of various acute and chronic disorders, making this protein a sensitive parameter for the assessment of disease activity and response to treatment in individual patients [4].

Calibrator standardisation

No international standard is available for calprotectin. Therefore, traceability is established according to section 5.6 in ISO 17511 [20] where the highest metrological entry level is the manufacturer's selected measurement procedure. The calibrator is traceable to a highly pure recombinant calprotectin solution, value assigned by total protein determination by UV_{280} and known extinction coefficient. A working calibrator of the pure recombinant material in calibrator matrix is used with the manufacturer's standing measurement procedures to assign value to the product calibrators via a published value transfer protocol [8].

Assay principle

The Gentian Calprotectin Immunoassay is a Particle-Enhanced Turbidimetric Immunoassay (PETIA). The lithium heparin plasma or serum sample is mixed with calprotectin immunoparticles. Calprotectin from the sample and the anti-calprotectin antibodies from the immunoparticles solution bind to form aggregates that increase the turbidity of the solution. The degree of turbidity is proportional to the concentration of calprotectin, which can be quantified via an established standard calibration curve.

Assay kit components

Products provided	
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
Other available products (not included)	
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.

Composition

Reaction Buffer 1 (R1, 54 mL inactive ingredient): Gentian Calprotectin Assay Buffer. R1 is a MOPS [3-(N-Morpholino)-propane sulfonic acid] buffered saline, containing avian proteins and preserved with ProClin® 950.

Reaction Buffer 2 (R2, 9 mL active ingredient): Gentian Calprotectin Immunoparticles. R2 contains a purified immunoglobulin fraction directed against human calprotectin, which is covalently attached to polystyrene nanoparticles. The solution is preserved with ProClin® 950.

Hazards identification

Hazard pictograms (CLP): GHS07

Signal word (CLP): Warning
Contains: 2-methylisothiazol-3(2H)-one

Hazard statements (CLP):
H317 - May cause an allergic skin reaction.
Precautionary statements (CLP):
P280 - Wear eye protection, protective gloves, protective clothing.
P302+P352 - IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313 - If eye irritation persists: Get medical advice/attention.
P333+P313 - If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 - Take off contaminated clothing and wash it before reuse.

To obtain the SDS (safety data sheet), please contact Gentian at marketing@gentian.com.

Warnings and precautions

1. Contains substances from human or animal origin and should be considered as potentially infectious material. Handle with caution and discard following local regulations.
2. Reagents containing MOPS/Tween (R1) and EDTA (R2) can be irritating to eyes, respiratory tract and skin. Handle with due caution and do not ingest.

3. R1 contains avian proteins. Handle with due caution to avoid allergic skin reaction.
4. Exposure may result in irritation of skin and eyes.
5. Avoid contact with incompatible materials.
6. Avoid exposure to heat and direct sunlight.

Additional handling instructions

1. This test is for *in vitro* use only and must be handled by laboratory professionals.
2. Use only validated and approved instrument applications.
3. Do not use products after the expiration date has passed.
4. Do not mix reagents of different lots or interchange caps of reagents, controls, calibrators, and lots.
5. Tighten caps carefully back on after use of reagents, calibrators, and controls to avoid evaporation.

Reagent storage and stability

All products provided for the Gentian Calprotectin Immunoassay must be stored at 2-8 °C. The expiry date is printed on the labels. Using an Architect c4000 instrument (Abbott), the in-use stability of the Gentian GCAL® Calprotectin Reagent Kit was found to be at least 4 weeks performed as an on board study based on the CLSI guideline EP25 [14].

Specimen collection and handling

Required sample material is lithium heparin plasma or serum. Gentian recommends lithium heparin plasma and non-gel tubes. It is recommended to analyse the samples as fresh as possible. Serum samples should await 30 minutes before processing. Centrifuge sample within 2 hours of blood collection and transfer the plasma or serum fraction immediately to another tube. Do not use gel and non-gel tubes interchangeably. Sample stability testing showed that calprotectin was stable for at least 48 hours after centrifugation at 2-8 °C. Mix samples well before analysing.

Performance characteristics

The results refer to the main validation of the Gentian Calprotectin Immunoassay on an Architect c4000 instrument (Abbott) at one instrument site with 3 lots of reagents, controls and calibrators, unless otherwise stated. For the instrument specific performance characteristics, please refer to the instrument specific application notes.

Measuring range

The measuring range of the Gentian Calprotectin Immunoassay is approximately 0.4 - 20.3 mg/L in both lithium heparin and serum samples, with a security zone of up to 95 mg/L. The exact measuring range is calibrator- and instrument specific, please refer to the analytical value sheet for the lot specific calibrator values (available on www.gentian.com) and the instrument specific application notes.

Analytical sensitivity

The analysis of the analytical sensitivity of the Gentian Calprotectin Immunoassay was tested in a study based on the CLSI guideline EP17 [15]. 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 was measured to be 0.31 mg/L in lithium heparin plasma and 0.30 mg/L in serum samples. For the instrument specific LoQ, please refer to the instrument specific application notes.

Precision

The total precision of the Gentian Calprotectin Immunoassay was measured with a CV of <4.0 % for samples >1 mg/L in a study based on the CLSI guideline EP05 [16]. For the instrument specific total precision, please refer to the instrument specific application notes.

Analytical specificity and limitations

No interference is detected for this product with haemoglobin, intralipid, or bilirubin at the tested concentrations in a study based on the CLSI guideline EP07 [17]. For the instrument specific interference, please refer to the instrument specific application notes. There is no interference detected with the drugs tested on the recommendations from Sonntag and Scholer [9]. As the antibodies in the Gentian Calprotectin Immunoassay are of avian origin, there is no interference due to Rheumatoid Factor in the samples [10]. Cross reactivity with MRP8, MRP14 and S100A12 was found to be <1 % in a study based on the CLSI guideline EP07.

Method comparison

Results obtained with the Gentian Calprotectin Immunoassay on the Architect c4000 instrument (Abbott) were compared with results from the IDK® Calprotectin ELISA Test™ (Immundiagnostik) on the Multiskan 60 spectrophotometer instrument (Thermo Fisher) in a study with 1 lot of reagents based on the CLSI guideline EP09 [18]. Due to lack of international calibrators or reference procedures, only coefficient of determination R^2 was evaluated.

Sample type	n	Range of samples [mg/L]	R^2 Coefficient
Li-Hep plasma	78	0.7 - 31.9	0.83
Serum	99	0.6 - 27.8	0.89

Assay procedure

Application notes

Applications of the Gentian Calprotectin Immunoassay have been established on several clinical chemistry analysers. Detailed, validated application notes describing the procedures for installation and analysis on specific instruments are available upon request to marketing@gentian.com. For instructions on how to install a new application, consult the instrument manual. Maintenance, operation and precautions must be handled in accordance with the specific instrument manual.

Reagent preparation

The reagents are ready for use. Mix the reagents gently before placing them into the assigned reagent positions. The reagent bottles may fit directly into the instrument unless otherwise stated in the application notes.

Establishment of the calibration curve

Please refer to the instruction for use of the Gentian GCAL® Calprotectin Calibrator Kit (REF 1251) available at www.gentian.com.

QC controls

Please refer to the instruction for use of the Gentian GCAL® Calprotectin Control Kit (REF 1219) available at www.gentian.com.

Measuring patient samples

When a valid calibration curve has been established and the control values are within the valid range, the lithium heparin plasma or serum sample may be measured. Ensure that the minimum sample volume is present in the sample cups/tubes and assay the samples according to the instructions given in the instrument manual.

Results

The results are calculated automatically by the instrument for all applications established for the Gentian Calprotectin Immunoassay. The results are presented in mg/L.

Clinical performance

Clinical performance of the Gentian Calprotectin Immunoassay was evaluated in clinical studies [11-13]. Specific cut-offs and clinical performance characteristics are summarized below. Clinical specifications including cut-off and performance characteristics are dependent on sample type and disease area. Therefore, it is recommended that every laboratory should determine local cut-off values since values may vary depending on sample type, population tested and clinical decision point.

Havelka A, et al. Scientific reports. 2020 [11].

Discrimination between patients with bacterial pneumonia and viral respiratory infections

Parameter	Value	95% CI
Cut-off [mg/L]	2.37	
ROC area	0.775	[0.667, 0.861]
Sensitivity [%]	60	[44, 75]
Specificity [%]	79	[63, 90]
LR+	2.9	[1.5, 5.6]
LR-	0.5	[0.3, 0.8]
PPV [%]	84*	
NPV [%]	53*	

Abbreviations: ROC: receiver operation curve, LR+: positive likelihood ratio, LR-: negative likelihood ratio
*Calculated based on prevalence of bacterial infections from study sample: 64% (71/((71+40))

Discrimination between patients with mycoplasma pneumonia and viral respiratory infections

Parameter	Value	95% CI
Cut-off [mg/L]	2.37	
ROC area	0.883	[0.774, 0.952]
Sensitivity [%]	91	[71, 99]
Specificity [%]	77	[67, 93]
LR+	3.9	[2.2, 7.1]
LR-	0.12	[0.03, 0.4]
PPV [%]	70*	
NPV [%]	93*	

Abbreviations: ROC: receiver operation curve, LR+: positive likelihood ratio, LR-: negative likelihood ratio
*Calculated based on prevalence of mycoplasma infections from study sample: 38% (24/((24+40))

The study included of 279 subjects (144 asymptomatic healthy controls, 71 with bacterial infections, 24 with mycoplasma infections and 40 with viral infections). The inclusion criteria for patients in the study were fever of >38 °C and signs and symptoms of respiratory infection.

Sample type: Lithium heparin plasma
Instrument: Mindray BS380

de Guadiana Romualdo LG, et al. J Infect. 2020 [12].

Prediction of mortality in COVID-19 patients

Parameter	Value	95% CI
Cut-off [mg/L]	3.9	
ROC area	0.801	[0.691, 0.894]
Unadjusted OR ratio	13.30	[1.53, 116]

Abbreviations: ROC: receiver operation curve, OR: odds ratio

This study included 66 consecutive patients admitted to the hospital with confirmed SARS-CoV-2 infection. 8 of 66 COVID-19 patients died during the hospital stay and 9 of 66 COVID-19 patients needed mechanical ventilation.

Sample type: Serum
Instrument: Roche Cobas c502

de Guadiana Romualdo LG, et al. Inflamm. Res. 2022 [13].

Prediction of need for mechanical ventilation

Parameter	Value	95% CI
Cut-off* [mg/L]	2.98	
ROC area	0.723	[0.652, 0.790]
Sensitivity [%]	73.7	[60.3, 84.5]
Specificity [%]	60.4	[54.9, 65.6]
PPV [%]	23.9	[17.8, 30.9]
NPV [%]	93.2	[89.0, 96.1]

Abbreviations: ROC: receiver operation curve
*Optimal cut-off according to Youden index

Rule out need for mechanical ventilation

Parameter	Value	95% CI
Cut-off* [mg/L]	2.23	
Sensitivity [%]	86.0	[74.2, 93.7]
NPV [%]	94.7	[89.7, 96.4]

Abbreviations: ROC: receiver operation curve
* Optimal cut-off to rule out need for invasive mechanical ventilation

This multicentre study included 395 consecutive patients admitted to the hospitals with confirmed SARS-CoV-2 infection. Of these COVID-19 patients 57 required invasive mechanical ventilation.

Sample type: Serum
Instrument: Roche Cobas c702

Upper reference limit

The calprotectin expected values in a normal adult population were determined in a study using a protocol based on the CLSI guideline C28 [19] on a Cobas c501 (Roche). The reference interval was determined from a population of ostensibly healthy subjects. A total of 416 samples from individuals (52 % males, 48 % females) ranging in age from 16 to 80 years were measured. The samples used were lithium heparin plasma and serum samples using both non-gel and gel tubes (51 lithium heparin non-gel, 163 lithium heparin gel, 51 serum non-gel, 151 serum gel). The upper reference limit was calculated parametrically to represent the upper 97.5 % of the population. It is recommended that every laboratory should determine a local reference limit since values may vary depending on the population tested.

Sample type	Value
Li-Hep plasma non-gel	<0.97 mg/L
Li-Hep plasma gel	<1.69 mg/L
Serum non-gel	<1.41 mg/L
Serum gel	<1.75 mg/L

Symbols key

2°C 8°C	Temperature limit
	Use by date
	Consult instructions for use
	Manufacturer
CE 0123	CE mark with Notified Body number
UK CA	UKCA mark
CH REP	Swiss authorized representative
IVD	*In Vitro* Diagnostic medical device
LOT	Lot number
REF	Catalogue number
UDI	Unique Device Identifier
CONTENTS	Contents
R1	R1 Assay Buffer
R2	R2 Immunoparticles
	Warning

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References

1. Stríz I, Trebichavský I. Physiol Res. 2004;53(3):245-53.
2. Wang S, et al. Front Immunol. 2018;9:1298.
3. Teng TS, et al. J Immunol Res. 2017;2017:9671604.
4. Zackular JP, et al. J Biol Chem. 2015;290(31):18991-8.
5. Johne B, et al. Mol Pathol. 1997;50(3):113-23.
6. Pruenster M, et al. Pharmacol Ther. 2016;167:120-31.
7. Foell D, et al. Clin Chim Acta. 2004;344(1-2):37-51.
8. Blirup Jensen et al. Clin Chem Lab Med 2008:46(10):1470-1479
9. Sonntag O, Scholer A. Ann Clin Biochem 2001;38:376-85.
10. Larsson A, et al. Poultry Science 1993;72:1807-18
11. Havelka A, et al. Scientific reports. 2020;10(1):4208-.
12. de Guadiana Romualdo LG, et al. J Infect. 2020:S0163-4453(20)30543-0.
13. de Guadiana-Romualdo LG, et al. Inflamm. Res. 2022;71(1):57-67.
14. CLSI. Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI document EP25-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
15. 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
16. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical Laboratory Standards Institute; 2014
17. CLSI. Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical Laboratory Standards Institute; 2018.
18. CLSI. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 3rd ed. CLSI guideline EP09c. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
19. CLSI. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI document C28-A3c. Wayne, PA: Clinical Laboratory Standards Institute; 2008
20. EN ISO 17511:2021 In vitro diagnostic medical devices — Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples. (ISO 17511:2020).

Serious incidents

Please notify your manufacturer and competent authority if any serious incidents have occurred in relation to the device.

Modifications from previous version

- New clinical performance data from 2022, reference 13.
- Added number of the Notified Body to CE mark.
- Added UKCA mark.
- Added chapter “Representatives”.
- Added CLSI references 14-19 and ISO reference 20.
- Added 4-6 in Warnings and Precautions.
- Minor editorial changes and corrections throughout the document.

Date of issue

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