GCAL® NEPH

GCAL® NEPH Calprotectin on the Siemens Healthineers BN™ II, Atellica® NEPH 630 and BN ProSpec® Systems

SMN 10873737

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 GCAL® NEPH Immunoassay is an immunonephelometric 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 GCAL® NEPH Immunoassay is intended for use on automated clinical analysers by laboratory professional users. Used in conjunction with other laboratory findings and clinical assessments, GCAL® NEPH 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 Ca2+ 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, 2]. 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 [16] 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 UV280 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 GCAL® NEPH Immunoassay is a particle-enhanced nephelometric immunoassay (PENIA). The lithium heparin plasma or serum sample is mixed with GCAL® NEPH 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	
GCAL® NEPH Calprotectin • R1 Supplement (2.0 mL) • 3 x R2 Reagent (1.9 mL)	SMN 10873737 (REF 1701)
Products required, but not provided	
GCAL® NEPH Calibrator (3 x 0.6 mL)	SMN 10873735 (REF 1712)
GCAL® NEPH Controls (2 levels, each 3 x 1.1 mL)	SMN 10873736 (REF 1719)
N Diluent (Siemens Healthineers)	REF OUMT65
BN II Evaporation Stoppers (optional) (Siemens Healthineers)	REF OVLE21
BN™ II System, Atellica® NEPH 630 System or BN ProSpec® System (Siemens Healthineers)	

All GCAL® NEPH products are ready for use.

Composition

Reaction Buffer 1 (R1, 2.0 mL inactive ingredient): GCAL® NEPH Supplement. R1 is a MOPS [3-(N-Morpholino)-propane sulfonic acid] buffered saline, containing avian proteins and preserved with ProClin® 950.
Reaction Buffer 2 (R2, 1.9 mL active ingredient): GCAL® NEPH Reagent. R2 contains a purified immunoglobulin fraction directed against human calprotectin, which is covalently attached to latex 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 local distributor or 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 GCAL® NEPH Immunoassay must be stored at 2-8 °C. The expiry date is printed on the labels. The in-use stability of the GCAL® NEPH reagents was found to be at least 7 days using a BN™ II and 5 weeks using a BN ProSpec® instrument performed as an on board study based on the CLSI guideline EP25 [17].

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

All results refer to the validation of the GCAL® NEPH Immunoassay on a BN™ II instrument at one instrument site with 3 lots of reagents, unless otherwise stated.

Measuring range

The measuring range of the GCAL® NEPH Immunoassay was found to be 0.4-10.6 mg/L in lithium heparin plasma and serum samples. The exact measuring range is specific to the calibrator, 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 analytical sensitivity of the GCAL® NEPH Immunoassay was tested in a study including 4 samples per tested concentration based on the CLSI guideline EP17 [18]. 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 GCAL® NEPH Immunoassay was measured as 0.42 mg/L in both lithium heparin plasma and serum samples.

Precision

Precision of the GCAL® NEPH Immunoassay was tested in a 20-day precision study based on the CLSI guideline EP05 [21]. 3 serum and 2 lithium heparin plasma pools and 2 controls were measured 40 times with 2 replicates (n=80). Total imprecision from 3 lots was below 6.4 % for all samples and controls with calprotectin concentration ≥ 1.0 mg/L: and below 10.4 % for all samples and controls with calprotectin concentration <1.0 mg/L. See table for detail results from one representative lot.

Sample ID	Mean [mg/L]	Within run CV [%]	Within lab CV [%]	Between run CV [%]	Between day CV [%]
P1	0.94	2.48	5.67	1.44	4.89
P2	7.79	3.76	6.31	0.00	5.07
S1	0.52	4.16	8.29	3.44	6.29
S2	1.79	1.80	4.41	0.72	3.96
S3	17.23	2.65	4.29	1.25	3.13
CL	0.98	2.85	5.61	0.36	4.82
CH	5.03	2.94	3.83	0.00	2.45

Analytical specificity and limitations

Interference was tested in a study based on the CLSI guideline EP07 [23]. As the antibodies in the GCAL® NEPH Immunoassay are of avian origin, there is no interference due to Rheumatoid Factor in the samples [15]. The potential interferents listed in the table below were spiked into one human lithium heparin plasma sample and one human serum sample, using one lot. No clinically relevant difference was detected at the tested interferent concentrations.

Potential interferents	Concentration with no interference
Haemoglobin	8 g/L
Intralipid	10 g/L
Bilirubin	0.6 mg/L

Linearity

The linearity range of the GCAL® NEPH Immunoassay was found as 0.33-21.4 mg/L in lithium heparin plasma and 0.37-20.6 mg/L in serum samples in a linearity study with 11 samples using a protocol based on the CLSI guideline EP06 [19].

Security zone

No antigen excess effect in samples below 104 mg/L was observed for the GCAL® NEPH Immunoassay in a study based on the CLSI guideline EP34 [20]. Samples with a calprotectin concentration above the highest calibrator and up to 104 mg/L return a value above the highest calibrator and are flagged for rerun with automatic dilution.

Instrument variation

Results obtained with the GCAL® NEPH Immunoassay on BN™ II instrument at 2 different sites were compared using Passing-Bablok regression in a study based on the CLSI guideline EP09 [22]. See table for results from one representative lot.

n	Range of samples [mg/L]	Term	Co-efficient	95% CI
119	0.57-20.65	Intercept	-0.03	[-0.08, 0.03]
		Slope	1.00	[0.97, 1.03]
		R^2	0.97	

Recovery

Recovery was analysed by spiking a low analyte sample with a high analyte sample according to Westgard [14]. The GCAL® NEPH Immunoassay had a recovery of 104-118 %.

Method comparison

Results obtained with the GCAL® NEPH Immunoassay on the BN™ II instrument were compared with results from Gentian Calprotectin Immunoassay (GCAL®) on the Cobas c501 instrument (Roche) in a study based on the CLSI guideline EP09 [22]. Results from 3 different lots and 127 samples, ranging from 0.6-21 mg/L of both lithium heparin plasma and serum samples, showed high equivalence with R^2 ranging from 0.989-0.992 and Recovery ranging from 95-101 %.

Assay procedure

Reagent preparation

The reagents are ready for use. Mix the reagents gently before placing them into the assigned reagent positions. The reagent bottles fit directly into the instrument rack.

Establishment of the calibration curve

Please refer to the instruction for use of the Gentian GCAL® NEPH Calibrator (REF SMN10873735) available at www.gentian.com.

QC controls

Please refer to the instruction for use of the Gentian GCAL® NEPH Controls (REF SMN10873736) 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 GCAL® NEPH Immunoassay. The results are presented in mg/L.

Clinical performance

The GCAL® NEPH Immunoassay is developed with the same antibodies as the Gentian Calprotectin (GCAL®) Immunoassay, thereby targeting the same analyte. GCAL® NEPH is developed to align with reported Gentian Calprotectin (GCAL®) Immunoassay levels, hence results can be directly compared. A detailed methods comparison of GCAL® NEPH and Gentian Calprotectin (GCAL®) Immunoassay has been performed demonstrating equivalence. Therefore, clinical performance for GCAL® NEPH is thereby directly related to results obtained with the Gentian Calprotectin Immunoassay.

Clinical performance of the Gentian Calprotectin (GCAL®) Immunoassay was evaluated in clinical studies [11-12, 25]. 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

Garcia de Guadiana Romualdo L, 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 c502

Garcia de Guadiana Romualdo L, et al. Inflamm. Res. 2022 [25].

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 based on the CLSI guideline on a Cobas c501 (Roche) using the Gentian Calprotectin (GCAL®) Immunoassay. 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 Supplement
R2	R2 Reagent
	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. Garcia de Guadiana Romualdo L, et al. J Infect. 2020:S0163-4453(20)30543-0.
13. Sonntag O, Scholer A. Ann Clin Biochem 2001;38:376-85.
14. Westgard JO. Basic Method Validation, 3rd Edition. 2008; ISBN13: 9781886958258
15. Larsson A, et al. Poultry Science 1993;72:1807-18
16. 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)
17. CLSI. Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI document EP25-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
18. 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
19. CLSI. Evaluation of Linearity of Quantitative Procedures. 2nd ed. CLSI guideline EP06. Clinical and Laboratory Standards Institute; 2020
20. 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.
21. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical Laboratory Standards Institute; 2014
22. CLSI. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. 3rd ed. CLSI guideline EP09c. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
23. CLSI. Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical Laboratory Standards Institute; 2018.
24. 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.

25. Garcia de Guadiana-Romualdo L, et al. Inflamm. Res. 2022;71(1):57-67.

Serious incidents

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

Modification from previous version

- New clinical performance data from 2022, reference 25.
- Added number of the Notified Body to CE mark.
- Added UKCA mark.
- Added chapter “Representatives”.
- Added CLSI references 17-24 and ISO reference 16.
- Added 4-6 in Warnings and Precautions.
- Minor editorial changes and corrections throughout the document.

Date of issue

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