

Application Note for the Gentian Calprotectin Immunoassay on the DxC 700 AU¹

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 S <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 performed as an on board study based on 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 a DxC 700 AU instrument at 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 Calprotectin Immunoassay was found to be 0.45 mg/L in serum and 0.48 mg/L in lithium heparin plasma.

Linearity

The linearity range of the Gentian GCAL[®] Calprotectin Immunoassay was found to be 0.44-24.53 mg/L in lithium heparin plasma and 0.45-19.16 mg/L in serum in a linearity study based on the CLSI guideline EP06 [3].

Security zone

No antigen excess effect in samples below 106 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 106 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 (P1-3) and 3 serum pools (S1-3), 2 controls 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 | 17.60 | 2.98 | 17.85 |
| P2 | 5.17 | 1.10 | 1.58 | 1.93 |
| P3 | 13.04 | 0.67 | 0.70 | 0.97 |
| S1 | 0.85 | 5.37 | 3.75 | 6.55 |
| S2 | 4.63 | 1.43 | 0.61 | 1.55 |
| S3 | 13.06 | 1.99 | 0.00 | 1.99 |
| CL | 1.02 | 4.89 | 2.42 | 5.45 |
| CH | 10.09 | 0.87 | 0.36 | 0.94 |

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 96-114 %.

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 on DxC 700 AU instrument were compared using Passing-Bablok regression with results from the AU480 instrument (Beckman Coulter) in a study based on the CLSI guideline EP09 [9].

| n | Range of samples [mg/L] | Term | Coefficient | 95 % CI |
|----|-------------------------|----------------|-------------|--------------|
| 50 | 0.30-22.40 | Intercept | 0.07 | [0.03, 0.11] |
| | | Slope | 0.97 | [0.94, 0.99] |
| | | 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 and 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 DxC 700 AU¹ (serum/plasma)

Reagent ID: 254

| General | | LIH | | ISE | | Calculated Test | | Range | |
|---|--|--|--------------------------------|--|----------------------------|---|--------------------------------------|--------|-------------------------------------|
| Test Name: <input type="text" value="CAL1G"/> | | Test No: <input type="text"/> | | Type: <input type="text" value="Serum***"/> | | Operation: <input type="text" value="Yes"/> | | | |
| Sample Volume | <input type="text" value="3.0"/> μL | Dilution | <input type="text" value="0"/> | μL | OD Limit | | | | |
| Pre-Dilution Rate | <input type="text" value="1"/> | | | | Reagent OD Limit | Min. OD | <input type="text"/> | Max OD | <input type="text"/> |
| Reagent Volume | R1 (R1-1) | <input type="text" value="150"/> μL | Dilution | <input type="text" value="0"/> μL | 1 st | Low | <input type="text" value="-2.0000"/> | High | <input type="text" value="2.0000"/> |
| | R1-2 | <input type="text"/> | Dilution | <input type="text"/> | Last | Low | <input type="text" value="-2.0000"/> | High | <input type="text" value="2.0000"/> |
| | R2 (R2-1) | <input type="text" value="22"/> μL | Dilution | <input type="text" value="0"/> μL | Analytical Measuring Range | Low | <input type="text" value="0.48"/> | High | <input type="text" value="19.16"/> |
| Common Reagent | Type | <input type="text" value="None"/> | Name | <input type="text" value="None"/> | Correlation Factor | A | <input type="text" value="1"/> | B | <input type="text" value="0"/> |
| | Wavelength | Pri: <input type="text" value="660"/> nm | Sec | <input type="text" value="None"/> nm | Manufacturer Factor | A | <input type="text" value="1"/> | B | <input type="text" value="0"/> |
| Method | <input type="text" value="END"/> | | | | | | | | |
| Reaction Slope | <input type="text" value="+"/> | | Onboard Stability Period | | | <input type="text" value="28"/> Day | <input type="text" value="0"/> Hour | | |
| Measuring Point-1 | 1st | <input type="text" value="9"/> | Last | <input type="text" value="15"/> | LIH Influence Check | <input type="text" value="No"/> | | | |
| Measuring Point-2 | 1st | <input type="text"/> | Last | <input type="text"/> | Lipemia | <input type="text"/> | | | |
| Linearity Limit | <input type="text"/> | | | | Icterus | <input type="text"/> | | | |
| Lag Time Check | <input type="text"/> | | | | Hemolysis | <input type="text"/> | | | |

| General | | LIH | | ISE | | Calculated Test | | Range | |
|---|------------------------------------|--------------------------------|--------------------------------|---|--------------------------------|---------------------------------------|--------------------------------|-----------------------------------|---|
| Test Name: <input type="text" value="CAL1G"/> | | Test No: <input type="text"/> | | Type: <input type="text" value="Serum***"/> | | | | | |
| Value/Flag | <input type="text" value="Value"/> | Level | Low | <input type="text" value="-99999.99"/> | High | <input type="text" value="99999.99"/> | | | |
| Specific Ranges | | | | | | | | | |
| | Sex | Year | Month | Year | Month | Other Type | 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="None"/> | <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="None"/> | <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="None"/> | <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="None"/> | <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="None"/> | <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="None"/> | <input type="text" value="*"/> | <input type="text" value="*"/> | |
| 7: | Standard demographics | | | | | | | | |
| 8: | Not within expected values | | | | | | | | |
| Critical Limits | Low | <input type="text" value="*"/> | | High | <input type="text" value="*"/> | | Unit | <input type="text" value="mg/L"/> | Decimal Places <input type="text" value="2"/> |

¹DxC 700 AU is a registered trademark of Beckman Coulter
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| | | | | |
|--|---|--|---|--|
| Calibrators | General | ISE | | |
| Test Name: <input type="text" value="CAL1G"/> | | Type: <input type="text" value="Serum***"/> | | |
| <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> | | | Slope Check <input type="text" value="None"/> | |
| | Calibrator | OD | Conc | Range |
| | | | | Low High |
| Point-1 | GCAL Calibrator Level 1 | | ** | -2.0000 2.0000 |
| Point-2 | GCAL Calibrator Level 2 | | ** | -2.0000 2.0000 |
| Point-3 | GCAL Calibrator Level 3 | | ** | -2.0000 2.0000 |
| Point-4 | GCAL Calibrator Level 4 | | ** | -2.0000 2.0000 |
| Point-5 | GCAL Calibrator Level 5 | | ** | -2.0000 2.0000 |
| Point-6 | GCAL Calibrator Level 6 | | ** | -2.0000 2.0000 |
| Point-7 | | | | |
| MB Type Factor <input type="text"/> | 1-Point Calibration Point <input type="text" value="None"/> | <input type="checkbox"/> with Conc-0 | | |
| | | | | Interval (RB) <input type="text"/> |
| | | | | Interval (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 |
| | | | | <input type="checkbox"/> Reagent Blank <input type="text"/> |
| | | | | <input type="checkbox"/> Calibration <input type="text"/> |
| | | | | Advanced Calibration |
| | | | | Operation <input type="text" value="No"/> |

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