Cystatin C Immunoassay

General Application Note

Intended Use
The Gentian Cystatin C Immunoassay is an in vitro diagnostic test for quantitative determination of cystatin C in human serum and plasma. The measurement of cystatin C is used in the diagnosis and treatment of renal disease.

This application note is intended for instruments for which no specific guideline exists. Performance on an instrument chosen by the customer should be validated.

Measuring range
The overall measuring range of cystatin C concentration for Gentian Cystatin C Immunoassay is 0.4-8.0 mg/L. The exact range is dependent on the calibrator value, which is lot specific, and the detection limit of the analyser.

Reference intervals
Gentian followed the CLSI Guideline, C28-A2 to determine the transferability of the reference interval. The reference interval was determined from a population of ostensibly healthy subjects with no history of renal disease. A total of 136 samples obtained from 58 males and 78 females ranging in age from 20 to 80 years were tested. The reference interval was calculated non-parametrically and was determined to be 0.51-1.05 mg/L. This represents the central 95% of the population. It is recommended that every laboratory should determine a local reference interval since values may vary depending on the population tested.

In a separate study involving 850 healthy children (46% boys, 54% girls) in the age from 5 to 15 years, the reference range 0.51 - 1.05 mg/L was confirmed in all ages down to 5 years of age [5]. For any other instrument the reference interval should be in accordance with the interval obtained on the Architect ci8200.

GFR prediction calculation
Several cystatin C based prediction equations for calculation of GFR for adults and children have been published. It should be noted that these formulas were evaluated with different Cystatin C assays (particle-enhanced nephelometric immunoassay PENIA or particle-enhanced turbidimetric immunoassay PETIA) and may reveal inaccurate GFR results if an inappropriate combination of formula and assay is used. For calculation of GFR from cystatin C values measured with the Gentian assay the following prediction equation is recommended using mg/L as the unit factor [4]:

\[
\text{GFR} = \frac{79.901}{\text{Cystatin C (mg/L)}}^{1.4389}
\]

Specimen
Required sample material is human serum, heparinized plasma and EDTA plasma. It is recommended to analyse the samples as fresh as possible. Sample stability testing showed that cystatin C in serum and plasma samples are stable for 14 days at room temperature (8-25°C), 21 days if stored at 2-8°C and stored at below -20°C for at least 10 years [6]. Mix samples well before analysing. The samples can be shipped without special cooling and must then be analysed within 14 days after shipment.

Assay Buffer (R1)
The Gentian Cystatin C Assay Buffer is the Reaction Buffer R1. The buffer is ready for use.

Immunoparticles (R2)
The Gentian Cystatin C Immunoparticles are ready for use.

Calibrator
The calibrator is ready to use and a dilution series for calibration curve establishment is prepared automatically by the instrument.

All reagents are stable until the expiry date given on the labels when stored at 2-8°C.

Calibrator Standardisation
Gentian Cystatin C Calibrator is standardised against the international calibrator standard ERM-DA471/IFCC.

Calibration Stability
The established calibration curve is stable for 4 weeks on Architect ci8200. When reagent lots are changed or measured control values are outside the assigned range given in the Analytical Value Sheet, a recalibration is recommended.

On board Stability
In appropriate bottles, Gentian Cystatin C Immunoparticles and Assay Buffer are stable for at least 9 weeks when stored at 2-8°C on board the analyser.

Procedure
Application Parameter Set Up
A defined application for the Gentian Cystatin C immunoassay must be installed in accordance with the general instrument settings given below. For instructions consult the instrument manual. Title the application CysC.

Loading of Reagents
Transfer Gentian Cystatin C Immunoparticles (REF1014) to reagent bottle used for R2, mix well before transfer. Transfer Gentian Cystatin C Assay Buffer (REF1007) to reagent bottle used for R1. For diluent loading, see Instrument Settings below. Reagents shall be stored at 2-8°C when not in use.

Calibration curve establishment
When the reagents (R1 and R2) and the calibrator are loaded onto the instrument, a 6 point calibration curve for Gentian Cystatin C Immunooassay can be established, for instructions consult the instrument manual. Saline is used as diluent for the calibrator dilution series. The set up procedure for calibrator dilution series is described in the general instrument settings below. Calibrator values are lot dependent. Do always check the dilution series concentrations when a new calibration is performed. The stability of a calibration curve is normally 4 weeks.
QC Controls
The Gentian cystatin C controls low and high must be assayed each day before any samples are assayed in order to validate the calibration curve. The controls have an assigned value range that must be met before measuring samples. The assigned values are given in the Analytical Value sheet provided with the controls. If the control values are not valid, repeat the control measurements. If the calibration cannot be performed without error, or valid control values cannot be reproduced, contact the instrument manufacturer for support.

Measuring Patient Samples
When a valid calibration has been performed and the control values are within the valid range, serum or plasma samples may be measured. Check that minimum sample is present and assay the samples according to the instructions given in the Instrument Manuals.

Results
The results are calculated automatically by the analyser and are presented in mg/L.

Limitations
The materials must not be used past expiration date. Interference studies must be a part of the instrument validation. But for a variety of analysers, no interference is detected for this product with triglycerides, haemoglobin, Intralipid or bilirubin at tested concentrations. There is no interference detected with the drugs tested on recommendation from Sonntag and Scholer [1]. There is no RF interference present because the antibodies are made from avian (chicken) [3]. Several cystatin C based prediction equations for calculation of GFR for adults and children have been published. It should be noted that these formulas were evaluated with different Cystatin C assays (particle-enhanced nephelometric immunoassay PENIA or particle enhanced turbidimetric immunoassay PETIA) and may reveal inaccurate GFR.

Performance Characteristics
All results refer to the validation of Gentian Cystatin C Immunoassay on Abbott Architect\textsuperscript{1} ci8200.

Detection Limit
Limit of Quantification is defined as: The lowest actual amount of an analyte that can be reliably detected and at which the total error means the requirements for accuracy.

Limit of Detection is defined as: The smallest amount of an analyte that the method can reliably detect to determine presence or absence of the analyte.

Gentian Cystatin C Immunoassay has a Limit of Detection of 0.031 mg/L and a Limit of Quantification of 0.33 mg/L.

Precision
The Gentian Cystatin C Immunoassay was used in a 5-day precision study designed in accordance with CLSI protocol EP5-A. 4 serum pools and 2 control levels were measured on the Architect\textsuperscript{1} ci8200.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value (mg/L)</th>
<th>Within-Run CV (%)</th>
<th>Total CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low human serum pool</td>
<td>0.69</td>
<td>1.51</td>
<td>2.81</td>
</tr>
<tr>
<td>High human serum pool</td>
<td>5.71</td>
<td>1.12</td>
<td>4.18</td>
</tr>
<tr>
<td>Medium human serum pool</td>
<td>3.38</td>
<td>1.93</td>
<td>3.75</td>
</tr>
<tr>
<td>Medium human serum pool</td>
<td>1.35</td>
<td>0.97</td>
<td>2.67</td>
</tr>
<tr>
<td>Low Control</td>
<td>0.88</td>
<td>1.42</td>
<td>3.72</td>
</tr>
<tr>
<td>High Control</td>
<td>3.58</td>
<td>0.71</td>
<td>1.41</td>
</tr>
</tbody>
</table>

Linearity
Gentian Cystatin C Immunoassay on Architect\textsuperscript{1} ci8200 is linear in the range of 0.3-8.8 mg/L. Concentrations outside this range have not been tested.

Analytical Recovery
For the Gentian Cystatin C Immunoassay on Architect\textsuperscript{1} ci8200 a recovery of 100-107% can be expected.

Interference
No interference is detected with Triglycerides (12.5 mmol/mL) Haemoglobin (8 g/L), Intralipid (11 g/L) or Bilirubin (420 mg/L) on Architect\textsuperscript{1} ci8200.

There is no interference detected with the drugs tested on recommendation from Sonntag and Scholer [1]. The interference study was designed in accordance with the protocol EP7-A from CLSI [2]. There is no RF interference present in the Gentian Cystatin C Immunoassay because the antibodies are made from avian (chicken) [3].

Shipping Damage
Please notify Gentian AS if this product is received damaged.

References

Manufacturer:
Gentian AS, PO BOX 733, N-1509 Moss, Norway

EC REP Authorized European Representative in EU:
Gentian AS, PO BOX 733, N-1509 Moss, Norway
TEL: +47 99 33 99 05
FAX: +47 69 24 09 62
http://www.gentian.no

Symbols Key

1\textsuperscript{A} A registered trademark of Abbott Diagnostics
The following reaction mode is recommended:

1. Mixing of (diluted) sample and reaction buffer.
2. Incubation (until stable readings are obtained).
3. Addition of the immunoparticle reagent.
4. Blanking (first reading) immediately after mixing all reagents.
5. Measuring signals during reaction until an end-point is obtained.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Suggestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation time before adding Immunoparticles</td>
<td>120 sec</td>
</tr>
<tr>
<td>Sample dilution</td>
<td>1</td>
</tr>
<tr>
<td>Sample Volumes</td>
<td>3 µl</td>
</tr>
<tr>
<td>Assay Buffer Volume</td>
<td>220 µl</td>
</tr>
<tr>
<td>Immunoparticle Volume</td>
<td>45 µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>268 µl</td>
</tr>
<tr>
<td>Reaction Time</td>
<td>300 sec</td>
</tr>
<tr>
<td>Total analysis Time</td>
<td>420 sec</td>
</tr>
<tr>
<td>Wavelength</td>
<td>550 nm</td>
</tr>
</tbody>
</table>

Calibrator Standards  Rel. dilution factor

| Standard 1 (lowest) | 0.0313         |
| Standard 2          | 0.0625         |
| Standard 3          | 0.125          |
| Standard 4          | 0.25           |
| Standard 5          | 0.5            |
| Standard 6 (highest)| 1.0            |

Spline regression is the preferred math model.