Application Note
General Guideline for Determination of Cystatin C in Serum/Plasma

General information

Intended use
The Application Note is intended for the quantitative determination of cystatin C in human sample material by instruments for which no specific guideline exists (1). Performance on an instrument chosen by the customer should be validated.

Measuring range
Approximately 0.4-7.5 mg/L depending on the specific lot of the calibrator. In case of post-concentration or -dilution the range can be expanded.

Reference interval
0.59-1.03 mg/L. It is recommended to determine the reference interval for the local population.

Instrument settings
Guidelines for instrument programming are presented in “Suggestion for Instrument Settings...” on page 3.

Reagents

<table>
<thead>
<tr>
<th>Code</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>LX004</td>
<td>Dako Cystatin C Immunoparticles (ERM-DA471/IFCC Standardized)</td>
</tr>
<tr>
<td>S2361</td>
<td>Dako Reaction Buffer 9</td>
</tr>
<tr>
<td>X7912</td>
<td>Dako Cystatin C Calibrator (ERM-DA471/IFCC Standardized)</td>
</tr>
<tr>
<td>X7913</td>
<td>Dako Cystatin C Control Set (ERM-DA471/IFCC Standardized)</td>
</tr>
<tr>
<td></td>
<td>NaCl solution 154 mmol/L (0.9% w/v)</td>
</tr>
</tbody>
</table>

Samples
Human serum, heparin-plasma or EDTA-plasma. Stable for 2 days at 2-8 °C. Stable for at least 3 months at –20 °C. Frozen samples should preferably be thawed at 37 °C and mixed well before analysis.

Calibrator
Dilution of standards is either performed automatically by the instrument as indicated or done manually.

Reaction buffer
The reaction buffer is ready for use. On board stability is 12 weeks at 2-12 °C.

Antibody
The immunoparticle solution is ready for use. Stability at 2-8 °C: See the Specification Sheet and the expiry date on the label. On board stability: 12 weeks at 2-12 °C.

Calibration stability
It is recommended to recalibrate every 8 weeks or when the reagent lot changes, a new antibody dilution is prepared or quality control results fall outside the range as established by the individual laboratory. However, the calibration stability should be validated on the individual instrument.

Trouble shooting
If performance is unacceptable, try to recalibrate. Check reagents and procedure. If the problem persists, please contact instrument supplier or Dako Technical Service.
An Example of Performance Data on Hitachi 917

**Sensitivity**
An OD value of approximately 0.50 on Hitachi 917 corresponds to a cystatin C concentration of ~7.5 mg/L.

**Detection limit**
The estimated detection limit is 0.02 mg/L.

**Precision**
The precision was estimated by ANOVA analysis of data using two controls and two serum cystatin C levels in five runs, each with a new calibration and 6 determinations in each run.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cystatin C Mean value (mg/L)</th>
<th>Standard deviation (mg/L)</th>
<th>Total CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystatin C Control 1, Code X7913</td>
<td>4.39</td>
<td>0.054</td>
<td>0.030</td>
<td>0.062</td>
</tr>
<tr>
<td>Cystatin C Control 2, Code X7913</td>
<td>1.13</td>
<td>0.008</td>
<td>0.016</td>
<td>0.018</td>
</tr>
<tr>
<td>Low human serum sample</td>
<td>0.96</td>
<td>0.008</td>
<td>0.013</td>
<td>0.015</td>
</tr>
<tr>
<td>High human serum sample</td>
<td>5.21</td>
<td>0.019</td>
<td>0.032</td>
<td>0.037</td>
</tr>
</tbody>
</table>

**Accuracy**
A recovery of cystatin C of 90–110% can be expected for Dako Cystatin C Control 1, Code X7913, and Dako Cystatin C Control 2, Code X7913.

**Linearity**
The assay is linear in the range 0.4-7.5 mg/L.

**Security range**
No antigen excess is found for cystatin C concentrations below 22 mg/L.

**Interference**
No interference is found at concentrations up to 5 g/L of haemoglobin, 600 mg/L of bilirubin, 16 g/L of intralipid and 1600 IU/mL of rheumatoid factor.

**Method comparison**
Determination of cystatin C according to this Application Note was compared with other commercial turbidimetric assays. Data are available on request.

**References**
Suggestion for Instrument Settings for Human Serum Cystatin C

Special Recommendations
A primary wavelength of approximately 546 nm and a secondary wavelength of approximately 700 nm should be used.

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.

Parameter | Suggestion
--- | ---
Light path (mm) | 10
Incubation time (s) | 120
Sample dilution (Dil. factor) | 1
Volume of prediluted sample (µL) | 5
Sample volume (µL) (=Neat) | 5
Diluent-Flush (for Sample+Reag) (µL) | 0
Reaction buffer volume (µL) | 385
PEG-conc. in Reac. buffer (%) | 1
PEG-conc. in incubation volume (%) | 0.987
Incubation volume (µL) | 390
Antibody dilution (Ab dil. factor) | 1
Volume of diluted antibody (µL) | 85
Antibody volume (µL) (=Neat) | 85
Diluent-Flush (for Antibody) (µL) | 25
Total volume (µL) | 500
Reaction time (s) | 300
Total analysis time (s) | 420

Ratio

| Sample vol. (Neat) | 0.06 | [0.05 - 0.06] |
| Antibody vol. (Neat) | 0.013 | [0.09 - 0.013] |
| PEG - conc. in incub. vol. (%) * Sample vol. (Neat) | 0.0100 | [0.0085 - 0.0105] |
| Incub. vol. | 0.77 | [0.70 - 0.80] |

Relation

| PEG-conc. in Total volume (%) | 1.00 |
| Std 1 (Highest conc.) | 1.00 |
| Std 2 | 1.43 |
| Std 3 | 2.57 |
| Std 4 | 5.21 |
| Std 5 | 9.35 |
| Std 6 (Lowest conc.) | 18.18 |

Agilent Technologies Singapore (International) Pte Ltd.
No. 1 Yishun Avenue 7
Singapore, 768923
Tel.: +44 161 492 7050
www.agilent.com

[ ] Indicates an acceptable range found on various instruments.
f_i is the relative concentration factor used to calculate the relative concentration (RC_i) of cystatin C in the standards from a specific lot of calibrator (C_{Cal}):

\[ RC_i = \frac{f_i \times C_{Cal}}{C_{Cal}} \]