Application Note
Guideline for Determination of Cystatin C in Serum/Plasma on ABX Pentra 400

General information

Intended use
The Application Note is intended for the quantitative determination of cystatin C in human sample material by turbidimetry on ABX Pentra 400 (1, 2).

Measuring range
Approximately 0.4-7.0 mg/L depending on the specific lot of the calibrator. In case of post dilution the range can be expanded to 0.4-14 mg/L.

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

Instrument settings
Instrument programming is performed according to “Instrument Settings” on page 3.

Reagents

<table>
<thead>
<tr>
<th>Code</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td>Cystatin C Immunoparticles (ERM-DA471/IFCC Standardized)</td>
</tr>
<tr>
<td>Reaction buffer</td>
<td>Dako Turbidimetry/Nephelometry Reaction Buffer 9</td>
</tr>
<tr>
<td>Calibrator</td>
<td>Cystatin C Calibrator (ERM-DA471/IFCC Standardized)</td>
</tr>
<tr>
<td>Controls</td>
<td>Cystatin C Control Set (ERM-DA471/IFCC Standardized)</td>
</tr>
<tr>
<td>Diluent</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 performed automatically by the instrument.

Reaction buffer (R1)
The reaction buffer is ready for use. On board stability is 12 weeks.

Antibody (R2)
The immunoparticle solution is ready for use.
Stability at 2-8°C: See specification sheet and expiry on the label.
On board stability is 12 weeks.
Capacity: 1 mL immunoparticle solution is equivalent to approximately 27 cuvette readings of standards or samples.
The dead volume of the reagent bottle should be added when calculating the required amount of reagent.

Calibration stability
It is recommended to recalibrate every 8 weeks, when reagent lots change or quality control results fall outside the range as established by the individual laboratory.

Trouble shooting
If performance is unacceptable, try to recalibrate. Check reagents and procedure. If the problem persists, please contact instrument supplier or Dako Technical Support.
Performance Data

Sensitivity
An OD value of approximately 0.30 on ABX Pentra 400 corresponds to a concentration around 7.0 mg/L cystatin C.

Detection limit
The detection limit is estimated to 0.10 mg/L.

Precision
The precision was estimated by using two controls and two serum cystatin C levels by ANOVA analysis of 6 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></td>
<td>Within run</td>
<td>Between run</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Cystatin C Control 1, Code X7913</td>
<td>4.27</td>
<td>0.023</td>
<td>0.067</td>
<td>0.071</td>
</tr>
<tr>
<td>Cystatin C Control 2, Code X7913</td>
<td>1.21</td>
<td>0.021</td>
<td>0.024</td>
<td>0.031</td>
</tr>
<tr>
<td>Low human serum sample</td>
<td>1.46</td>
<td>0.017</td>
<td>0.017</td>
<td>0.024</td>
</tr>
<tr>
<td>High human serum sample</td>
<td>4.06</td>
<td>0.038</td>
<td>0.038</td>
<td>0.054</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 0.4-7.0 mg/L.

Security range
No antigen excess is found for cystatin C concentrations below 11 mg/L.

Interference
No interference is found at concentrations up to 10 g/L of hemoglobin, 600 mg/L of bilirubin, 16 g/L of intralipid and 1200 IU/mL of rheumatoid factor.

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

References
2. ABX Pentra 400 manual(s).
**Instrument Settings**

<table>
<thead>
<tr>
<th>Instrument Setting</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Name</strong></td>
<td>Cystatin C*</td>
</tr>
<tr>
<td><strong>Channel</strong></td>
<td>990*</td>
</tr>
<tr>
<td><strong>Code</strong></td>
<td>CYSC*</td>
</tr>
<tr>
<td><strong>Local Code</strong></td>
<td>LX004*</td>
</tr>
</tbody>
</table>

**General Parameters**

- **Sample Type**: Serum / Plasma
- **Number of Reagents**: Reagent 2
- **Reagent Short Name**: CYSC*
- **Reagent Number**: 990*
- **On Board Stability (days)**: --
- **Enable**: [√]
- **Modified on**: --

**Automatic Rerun**

- **Post Dilution**: 2.0
- **Post Concentration**: --
- **Enable**: [√]

**Result**

- **Unit**: mg/L
- **Decimal Position**: 2

**Calibration Parameters**

- **Pre-dilution**: [√]
- **Calibrator Diluent**: PHYS
- **Factor 1 - Factor 8**: 18.0, 9.0, 5.0, 3.0, 2.0, 1.0
- **Run(s)**: 2
- **Dev_Rep (%)**: 10.0
- **Dev_C (%)**: --
- **Calibrator used**: X7912*

**Analysis parameters**

- **Cleaner Solution**: [H20]
- **Wavelength (nm)**: 550
- **Before**: --
- **After**: --

**Analysis Sequence**

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Reagent Needle</th>
<th>Volume (µL)</th>
<th>Sample Needle</th>
<th>Volume (µL)</th>
<th>H2O Vol (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R1</td>
<td>154.0</td>
<td>SAMPLE</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>10</td>
<td>--</td>
<td>--</td>
<td>R2</td>
<td>34.0</td>
<td>10.0</td>
</tr>
<tr>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
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</tr>
</tbody>
</table>

**Calculation parameters**

- **Correlation Factor**: 1.00000
- **Slope**: 0.00000
- **Intercept**: 0.00000
- **Relation Limit**: [√]

**Diluent**

- NaCl solution 154 mmol/L (0.9% w/v)
- R1: Code S2361
- R2: Code LX004

*Defined by the customer.*

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**Manual Patient Validation**

- **Pre-dilution Checks**: [√]
- **Reagent Limit Absorbance Check**: Reagent Range low, Reagent Range High
- **Reagent Blank Limit Absorbance Check**: Blank Range - Low limit, Blank Range - High limit
- **On Request**: [√]
- **Low limit Check**: --
- **High Limit Check**: --
- **Time Validity**: --
- **Relative variation check**: --
- **Reaction Limit Absorbance Check**: --
- **First point**: --
- **Last point**: --

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**Definition Step A**

<table>
<thead>
<tr>
<th>Calculation Type</th>
<th>End point</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Reading</td>
<td>Last Reading</td>
</tr>
<tr>
<td>Cycle</td>
<td>Cycle</td>
</tr>
</tbody>
</table>

**Deviation check**

- **First point**: SD
- **Last point**: SD Factor