**Application Note**  
**Guideline for Determination of Cystatin C in Serum/Plasma on Cobas MIRA Plus**

**General information**

**Intended use**  
The Application Note is intended for the quantitative determination of cystatin C in human serum, heparinized plasma and EDTA plasma by turbidimetry on Cobas MIRA Plus (1, 2).

**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 to 0.4-15 mg/L.

**Reference interval**  
For individuals 1-50 years: 0.55-1.15 mg/L.  
For individuals >50 years: 0.63-1.44 mg/L.  
It is recommended to determine the reference interval for the local population.

**Instrument settings**  
Instrument programming is performed according to the “Instrument Settings” on page 8.

**Reagents**

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td>DakoCytomation Cystatin C Immunoparticles</td>
</tr>
<tr>
<td>Reaction buffer</td>
<td>DakoCytomation Reaction Buffer 9</td>
</tr>
<tr>
<td>Calibrator</td>
<td>DakoCytomation Cystatin C Calibrator</td>
</tr>
<tr>
<td>Controls</td>
<td>DakoCytomation Cystatin C Control Set</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 ºC.  
Stable for at least 6 months at –20 ºC.  
Frozen samples should preferably be thawed at 37 ºC and mixed well before analysis.

**Calibrator**  
Dilution of standards is performed automatically by the instrument.

**Reaction buffer**  
The reaction buffer is ready for use.

**Antibody**  
The immunoparticle solution is ready for use.  
Stability at 2-8 ºC: See the specification sheet and expiry on the label.  
Capacity: 1 mL immunoparticle solution is equivalent to approximately 20 cuvette readings of standards or samples.  
The dead volume of the reagent bottle should be added when calculating the required amount of reagent.

**Stability**  
After transfer of the DakoCytomation Cystatin C Immunoparticles and the DakoCytomation Reaction Buffer 9 to appropriate containers, on-board stability is 24 hours.

**Calibration stability**  
It is recommended to recalibrate every 90 days on Cobas MIRA Plus, or when reagent lots change or quality control results fall outside the range stated on the Analytical Value Sheet or outside the range established by the individual laboratory.

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

Detection Limit
The detection limit is determined as the lowest concentration giving a signal higher than the signal of the blank plus 3 standard deviations. Detection limit is determined to 0.08 mg/L.

Precision
Precision was estimated using two controls and three serum cystatin C levels according to guidelines in the NCCLS Document EP5-A (3).

<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 day</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Cystatin C Control 1, code No. X0973</td>
<td>4.17</td>
<td>0.069</td>
<td>0.045</td>
<td>0.083</td>
</tr>
<tr>
<td>Cystatin C Control 2, code No. X0973</td>
<td>1.09</td>
<td>0.013</td>
<td>0.014</td>
<td>0.020</td>
</tr>
<tr>
<td>Low human serum pool</td>
<td>0.55</td>
<td>0.016</td>
<td>0.000</td>
<td>0.013</td>
</tr>
<tr>
<td>Medium human serum pool</td>
<td>1.90</td>
<td>0.042</td>
<td>0.000</td>
<td>0.042</td>
</tr>
<tr>
<td>High human serum pool</td>
<td>5.71</td>
<td>0.064</td>
<td>0.057</td>
<td>0.086</td>
</tr>
</tbody>
</table>

Accuracy
A recovery of cystatin C of 85–115% can be expected for DakoCytomation Cystatin C Control 1, code No. X0973, and DakoCytomation Cystatin C Control 2, code No. X0973.

Linearity
The assay is linear in the range 0.4-13 mg/L (the highest concentration tested).

Security range
No antigen excess is found for cystatin C concentrations below 113 mg/L (the highest concentration tested).

Interference
No interference is found at concentrations up to 10 g/L of hemoglobin, 600 mg/L of bilirubin, 15 g/L of triglyceride, 10 g/L of intralipid, and 1200 IU/mL of rheumatoid factor.
All drugs described in reference 4 were investigated according to the recommendations in reference 4. No interference was observed. Possible interference from monoclonal and polyclonal antibodies used in the treatment of transplant patients has not been evaluated.

Method comparison
Determination of cystatin C according to this Application Note was compared with a commercially available nephelometric assay. Data are available on request.

References
### Instrument Settings (Software Version 9215)

<table>
<thead>
<tr>
<th>P2 TESTS ROUTINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYSC</td>
</tr>
</tbody>
</table>

**GENERAL**
- MEASUREMENT MODE: ABSORB
- REACTION MODE: D-R-S-SR1
- CALIBRATION MODE: LOGIT/LOG5
- REAGENT BLANK: REAG/DIL
- CLEANER: NO
- WAVELENGTH: 550 nm
- DECIMAL POSITION: 2
- UNIT: mg/l

**ANALYSIS**
- DILUENT: DIL1
  - FACTOR: 4.00
  - TIME: NO
  - STD: MAIN DIRECT
  - MAIN STD: (4.00 \times C_{\text{cal}}) mg/l
  - POSES: 1
  - FACT.STD-1: 4.00 2: 6.00 3: 9.00 4: 15.00 5: 33.00 6: 75.00 7: NO 8: NO
- POST DIL. FACTOR: 2.00
- CONC.FACTOR: 1.50
- SAMPLE CYCLE: 3
- VOLUME: 10.0 µl
- DILUENT NAME: H2O
  - VOLUME: 5.0 µl
- REAGENT CYCLE: 1
- VOLUME: 225 µl
- START R1 CYCLE: 1
- VOLUME: 50.0 µl
- DILUTION NAME: H2O
  - VOLUME: 5.0 µl

**CALCULATION**
- SAMPLE LIMIT: NO
- REAC. DIRECTION: INCREASE
- CHECK: ON
- ANTIGEN EXCESS: NO
- CONVERS. FACTOR: 1.00000
- OFFSET: 0.00000
- TEST RANGE LOW: ON
- HIGH: ON
- NORM. RANGE LOW: NO
- HIGH: NO
- NUMBER OF STEPS: 1
- CALC. STEP A: ENDPOINT
- READINGS FIRST: T2 LAST: 16

**CALIBRATION**
- CALIB. INTERVAL: ON REQUEST
- REAG. RANGE LOW: NO
  - HIGH: NO
- BLANK RANGE LOW: NO
  - HIGH: NO
- STANDARDS**
  1: 7.36 2: 4.91 mg/l
  3: 3.27 4: 1.96 mg/l
  5: 0.89 6: 0.39 mg/l
  7: NO 8: NO
- REPLICATE: DUPL
- DEVIATION: 5.0 %
- CORRECTION STD: NO
- CONTROL
  - CS1 POS: NO
  - CS2 POS: NO
  - CS3 POS: NO

**Notes**
- DIL 1 – Saline
- REAGENT – DakoCytomation Reaction Buffer 9, code No. S2361.
- START R1 – DakoCytomation Cystatin C Immunoparticles, code No. LX002, undiluted.

[*] The concentration is calculated as the factor times the calibrator value for the specific lot of DakoCytomation Cystatin C Calibrator, code No. X0974, (C_{\text{cal}}, is stated in mg/L on the X0974 Analytical Value Sheet).

[**] These values are not input. They are calculated automatically by the instrument from the "MAIN STD" and "FACT.STD" in the "ANALYSIS" section of the instrument settings. The values vary slightly with the calibrator value.