Application Note  
General Guideline for Determination of  
C-Reactive Protein in Serum/Plasma

General information

Intended use  
The Application Note is intended for the quantitative determination of C-reactive protein (CRP) 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 0.2-15 mg/L depending on the specific lot of the calibrator. In case of post-concentration or -dilution of the sample the range can be expanded.

Reference interval  
0-0.5 mg/L (2). 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.

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagents</td>
<td>Antibody</td>
</tr>
<tr>
<td></td>
<td>Reaction buffer</td>
</tr>
<tr>
<td></td>
<td>Calibrator</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diluent</td>
</tr>
</tbody>
</table>

Samples  
Human serum, heparin-plasma or EDTA-plasma. Stable for 5 days at 2-8 °C. The use of previously frozen samples is not recommended.

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 8 weeks at 2-12 °C.

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

Capacity: 1 mL immunoparticle suspension corresponds to approximately 10 cuvette readings of standards or samples when 100 µL immunoparticle suspension is used per test. The dead volume of the reagent bottle should be considered when calculating the required amount of reagents.

Calibration stability  
It is recommended to recalibrate every 8 weeks or when reagent lots change 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 DakoCytomation Technical Service.
An Example of Performance Data on Hitachi 917

**Sensitivity**
An OD value of approximately 0.35 on Hitachi 917 corresponds to a CRP concentration around 14 mg/L.

**Detection limit**
The detection limit is estimated to 0.09 mg/L.

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>CRP 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>Between day</td>
<td></td>
</tr>
<tr>
<td>C-Reactive Protein Low Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Sensitive), Code No. X0971</td>
<td>3.26</td>
<td>0.026</td>
<td>0.040</td>
<td>0.057</td>
</tr>
<tr>
<td>C-Reactive Protein High Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Sensitive), Code No. X0972</td>
<td>11.62</td>
<td>0.031</td>
<td>0.109</td>
<td>0.110</td>
</tr>
<tr>
<td>Human serum pool A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.87</td>
<td>0.032</td>
<td>0.022</td>
<td>0.016</td>
</tr>
<tr>
<td>Human serum pool B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.06</td>
<td>0.021</td>
<td>0.075</td>
<td>0.000</td>
</tr>
<tr>
<td>Human serum pool C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.33</td>
<td>0.035</td>
<td>0.077</td>
<td>0.035</td>
</tr>
</tbody>
</table>

**Accuracy**
A recovery of CRP of 85-115% can be expected for DakoCytomation C-Reactive Protein Low Control (Sensitive), code No. X0971, and DakoCytomation C-Reactive Protein High Control (Sensitive), code No. X0972.

**Linearity**
The assay is linear in the range 0.2-60 mg/L.

**Security range**
No antigen excess is found for CRP concentrations below 500 mg/L.

**Interference**
No interference is found at concentrations up to 10 g/L of hemoglobin, 25 g/L of triglyceride, 1200 IU/mL of rheumatoid factor, 275 mg/L (~325 µmol/L) of conjugated bilirubin and 320 mg/L (~550 µmol/L) of nonconjugated bilirubin. Bilirubin above the mentioned concentrations reduces the signal significantly (false low results).

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

**References**
Suggestion for Instrument Settings for Human C-Reactive Protein

Special Recommendations

A primary wavelength of approximately 450 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) | 84
Antibody volume (µL) (=Neat) | 84
Diluent+Flush (for Antibody) (µL) | 26
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) | 

Relation

PEG conc. in incub.vol.(%)*Samplevol.(Neat) | 0.013 [0.01 - 0.013]
PEG conc. in incub.vol.(%) | 0.0100 [0.0085 - 0.0102]
Incub.vol. | 

Ratio

Sample vol.(Neat) | 0.77 [0.76 – 0.78]
Total vol. | 

PEG conc. in Total volume (%) | 

Standards

<table>
<thead>
<tr>
<th>Dilution factor</th>
<th>f, Relative conc. factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1 (Highest conc.)</td>
<td>2.13</td>
</tr>
<tr>
<td>Std 2</td>
<td>6.29</td>
</tr>
<tr>
<td>Std 3</td>
<td>16.61</td>
</tr>
<tr>
<td>Std 4</td>
<td>54.95</td>
</tr>
<tr>
<td>Std 5</td>
<td>90.91</td>
</tr>
<tr>
<td>Std 6 (Lowest conc.)</td>
<td>161.29</td>
</tr>
</tbody>
</table>

* For further explanations to the individual parameters please refer to “Introduction to DakoCytomation General Application Notes”, order no. 30134.Intro, which is available on request or at www.dakocytomation.com.

[] Indicates an acceptable range found on various instruments.

f, Is the relative concentration factor used to calculate the relative concentration (RC(i)) of CRP in the standards from a specific lot of calibrator (C(Cal)); RC(i) = f x C(Cal) .