Application Note
General Guideline for Determination of IgG in Serum/Plasma

General information

Intended use
The Application Note is intended for the quantitative determination of immunoglobulin G (IgG) 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 2.5-50 g/L depending on the specific lot of the calibrator. In case of post-concentration or -dilution the range can be expanded.

Reference interval
7-16 g/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>Antibody</td>
<td>DakoCytomation Polyclonal Rabbit Anti-Human IgG</td>
</tr>
<tr>
<td>Reaction buffer</td>
<td>DakoCytomation Reaction Buffer 1</td>
</tr>
<tr>
<td>Diluent</td>
<td>DakoCytomation Dilution Buffer 1</td>
</tr>
<tr>
<td>Calibrator</td>
<td>DakoCytomation Human Serum Protein Calibrator</td>
</tr>
<tr>
<td>Controls</td>
<td>DakoCytomation Human Serum Protein Low Control</td>
</tr>
<tr>
<td></td>
<td>DakoCytomation Human Serum Protein High Control</td>
</tr>
</tbody>
</table>

Samples
Human serum, heparin-plasma or EDTA-plasma.
Stable for 7 days at 2-8 ºC.
Stable for 3 months at –20 ºC (if only frozen once) (3).
Frozen samples should be thawed at 37 ºC 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 28 days at 2-12 ºC.

Antibody
Predilute the antibody in accordance with your final setting.
If in rare cases the prediluted antibody appears slightly turbid, filtration through a 0.22 µm membrane filter is recommended.
Stability of undiluted antibody: See expiry on the label.
Stability of prediluted antibody: 28 days at 2-8 ºC.
On board stability: 28 days at 2-12 ºC.

Capacity: 1 mL of prediluted antibody is equivalent to approximately 10 cuvette readings of standards or samples when 100 µL diluted antibody solution 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 28th day or when reagent lots change, a new antibody dilution is prepared, the antibody dilution is filtered, 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 911

**Sensitivity**
An OD value of approximately 0.53 on Hitachi 911 corresponds to an IgG concentration around 50 g/L.

**Detection limit**
The detection limit is estimated to 0.22 g/L.

**Precision**
The precision was estimated by testing at 3 different Immunoglobulin G (IgG) levels by ANOVA analysis of 6 runs each with a new calibration and 6 determinations in each run.

<table>
<thead>
<tr>
<th>Sera</th>
<th>IgG Mean value (g/L)</th>
<th>Standard deviation (g/L)</th>
<th>Total CV (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Within run</td>
<td>Between run</td>
<td>Total</td>
</tr>
<tr>
<td>Human serum low</td>
<td>8.53</td>
<td>0.142</td>
<td>0.097</td>
<td>0.172</td>
</tr>
<tr>
<td>Human serum medium</td>
<td>18.14</td>
<td>0.275</td>
<td>0.049</td>
<td>0.280</td>
</tr>
<tr>
<td>Human serum high</td>
<td>28.42</td>
<td>0.447</td>
<td>0.271</td>
<td>0.522</td>
</tr>
</tbody>
</table>

**Accuracy**
A recovery of IgG of 90-110% can be expected for DakoCytomation Human Serum Protein Low Control, code No. X 0939, and DakoCytomation Human Serum Protein High Control, code No. X 0940.

**Linearity**
The assay is linear in the range of 3.2-52 g/L.

**Security range**
No antigen excess is found for IgG concentrations up to 300 g/L.

**Interference**
No interference is found at concentrations up to 10 g/L of hemoglobin, 600 mg/L of bilirubin and 25 g/L of triglyceride. All drugs described in reference 4 were investigated according to the recommendations in reference 4. No interference was observed.

**Method comparison**
Determination of IgG 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 IgG

* Parameter | Suggestion
--- | ---
Light path (mm) | 10
Incubation time (s) | 120
Sample dilution (Dil. factor) | 20
Volume of prediluted sample (µL) | 10
Sample volume (µL) (=Neat) | 0.50
Diluent+Flush (for Sample+Reag) (µL) | 280
Reaction buffer volume (µL) | 600
PEG-conc. in Reac. buffer (%) | 5
PEG-conc. in incubation volume (%) | 3.4
Incubation volume (µL) | 890
Antibody dilution (Ab dil. factor) | 3
Volume of diluted antibody (µL) | 90
Antibody volume (µL) (=Neat) | 30
Diluent+Flush (for Antibody) (µL) | 20
Total volume (µL) | 1000
Reaction time (s) | 300
Total analysis time (s) | 420

| Ratio | Sample vol.(Neat) | Antibody vol.(Neat) | 0.017 | [0.016 - 0.019] |
| Relation | PEG - conc. in incub. vol.(%)* Sample vol.(Neat) | Incub. vol. | 0.0019 | [0.0015 - 0.0027] |
| Ratio | Sample vol.(Neat) | Total vol. | 0.00050 | [0.00034 - 0.00061] |
| PEG-conc. in Total volume (%) | 3.0 | [2.8 - 3.4] |

### Standards

<table>
<thead>
<tr>
<th>Dilution factor</th>
<th>f_i Relative conc. factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1 (Highest conc.)</td>
<td>4.65</td>
</tr>
<tr>
<td>Std 2</td>
<td>6.25</td>
</tr>
<tr>
<td>Std 3</td>
<td>9.52</td>
</tr>
<tr>
<td>Std 4</td>
<td>16.67</td>
</tr>
<tr>
<td>Std 5</td>
<td>28.57</td>
</tr>
<tr>
<td>Std 6 (Lowest conc.)</td>
<td>100.00</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_i is the relative concentration factor used to calculate the relative concentration (RC_i) of IgG in the standards from a specific lot of calibrator (C(Cal)); RC_i = f_i x C(Cal).