Quantitative measurement of immunoglobulins and free light chains using mass spectrometry

Martijn M. VanDuijn[1], Joannes F.M. Jacobs[2], Ron A. Wevers[3], Udo F. Engelke[3], Irma Joosten[2] and Theo M. Luider[1]

1: Laboratory of Neuro-Oncology and Clinical & Cancer Proteomics, Department of Neurology, Erasmus MC, Rotterdam, The Netherlands. 2: Laboratory of Medical Immunology, Department of Laboratory Medicine, Radboudumc, Nijmegen, The Netherlands. 3: Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboudumc, Nijmegen, The Netherlands.

Supporting information

Figure S-1. Computation of analyte concentration from SRM data

Figure S-2. Depletion efficacy for IgA and IgG

Figure S-3. Quality control data for methionine oxidation and missed cleavages

Figure S-4. Comparisons between patient sFLC data from SRM, Freelite and N-latex tests

Table S-1. Peptides measured in the SRM assay and associated data

Table S-2. Data from healthy control sera

Table S-3. Comparison between SRM, nephelometry and serum protein electrophoresis

Table S-4 Comparison between SRM and certified data for intact immunoglobulins in CRM reference serum.

$$[Lambda] = \frac{[YAASSYLSLTPEQWK] + [SYSCQVTHEGSTVEK]}{2}$$

$$[Kappa] = \frac{\begin{array}{c}([VYAGEVTHQGLSSPVTK] + [LYACEVTHQGLSSPVTK] + [VYACEVTHQGLSSPVTK]) \\ +[TVAAPSVFIFPPSDEQLK] + [SGTASVVCLLNNFYPR]\end{array}}{3}$$

$$[IgG] = \frac{[DTLMISR] + ([GPSVFPLAPSSK] + [GPSVFPLAPCSR])}{2}$$

$$[IgA] = \frac{[SVTCHVK] + [YLTWASR]}{2}$$

$$[IgE] = [GSGFFVFSR]$$

$$[IgM] = \frac{[VTSTLTIK] + [YVTSAPMPEPQAPGR]}{2}$$

Figure S-1. Computation of concentrations from SRM data. Peptide concentrations are determined based on the signal ratio between the analyte and the known amount of synthetic isotope labelled standard. Sequence variants are summed before averaging. All computations are based on molar concentrations, conversion to mg/L is done before plotting where required.
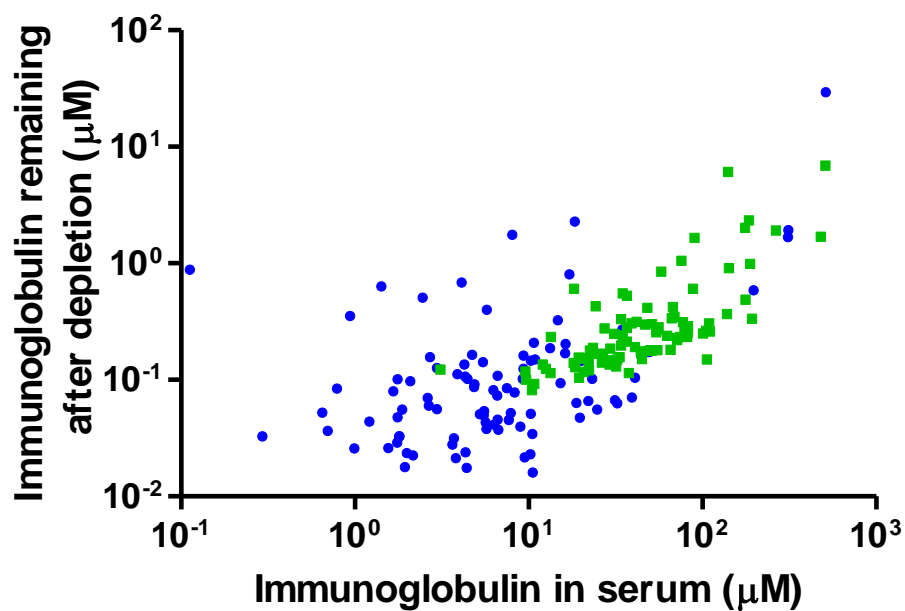
Figure S-2. Efficacy of the depletion of intact immunoglobulins by Protein A/G beads plus anti-IgA. Shown is the concentration of IgG (green) and IgA (blue) in the serum sample plotted against the concentration remaining after depletion with the beads.
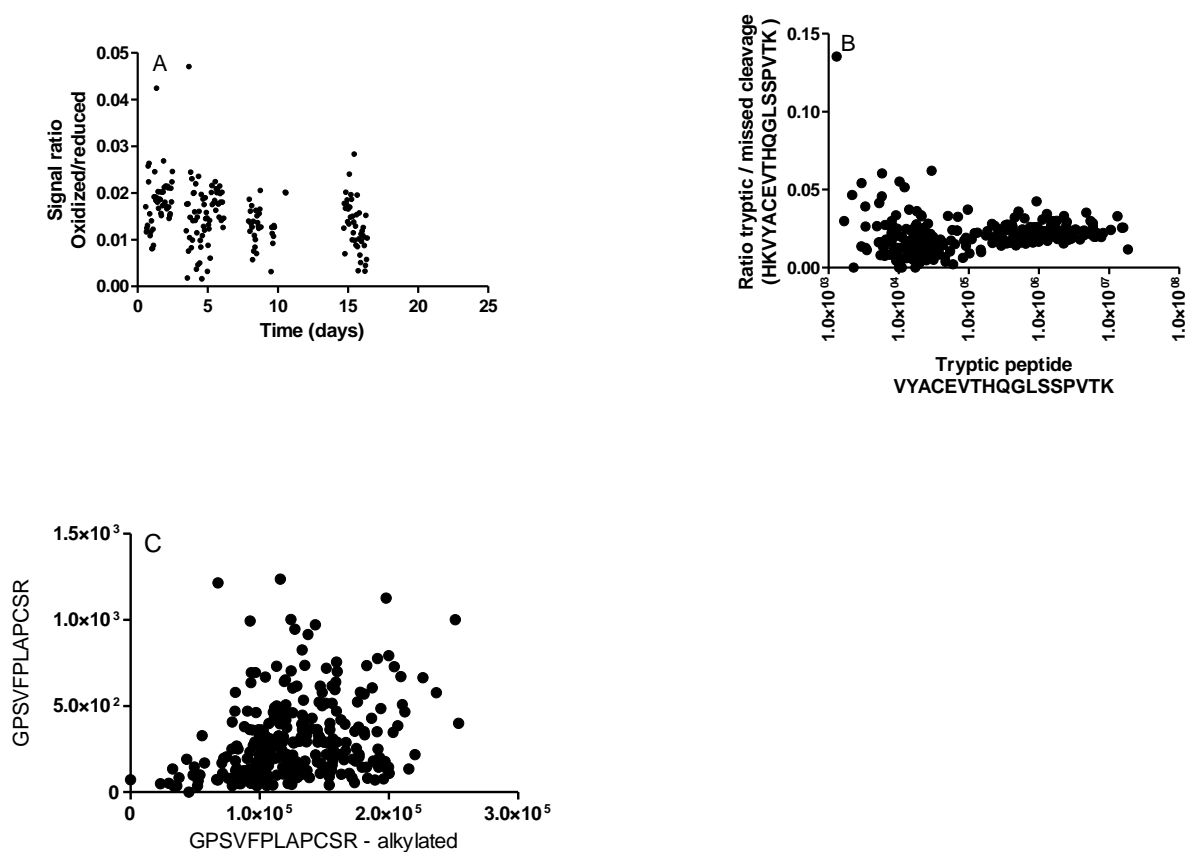
Figure S-3. Quality control metrics. A. Oxidation artifacts. Shown are the ratio of the uncalibrated signals of the peptide DTLMISR and its counterpart containing an oxidized methionine residue. The X-axis show the time elapsed after sample processing, during which samples were stored in a LC vial at 4°C. B. Missed cleavage after digestion. Shown are the uncalibrated signal of the peptide VYACEVTHQGLSSPVTK, and its ratio to the signal of the peptide HKVYACEVTHQGLSSPVTK which contains two additional amino acids due to a missed cleavage by trypsin. C. Alkylation efficiency. Cysteines are alkylated with iodoacetamide during sample preparation. The alkylated product which is used for the SRM assay is shown on the X-axis, remaining un-alkylated peptide is shown on the y-axis.
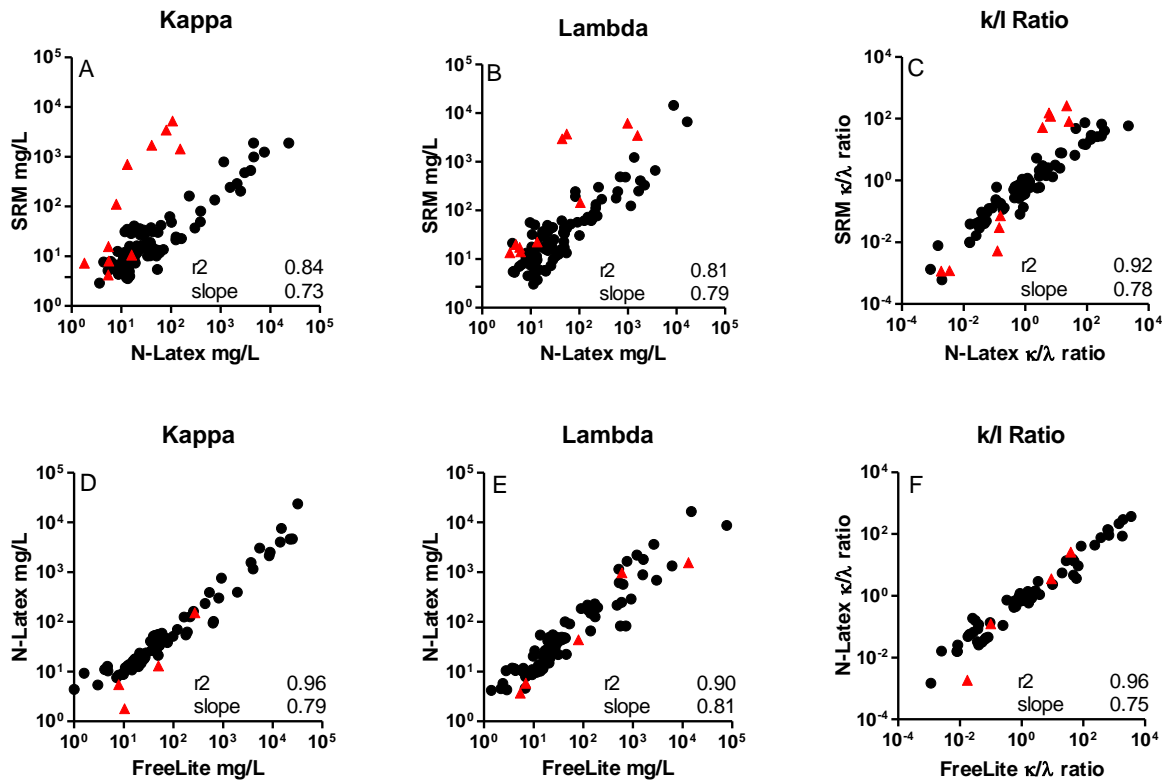
Figure S-4. Comparison between the SRM test for sFLC and the commercial N-Latex assay for κ and for λ sFLC (A-C), as well as between the commercial Freelite and N-Latex assays (D-F). These figures complement those in Figure 4, red markers indicate samples with undepleted intact immunoglobulin classes. Shown are the results for those samples from the cohort that have data available from both tests. The LLoQ of the SRM method is shown by an additional tick on the Y-axis.

Protein	Peptide sequence	SI standard included	Used for quantitation	Agreement between NMR and aminoacid analysis	Q1 mass SRM transitions	Q3 masses SRM transitions	Known polymorphisms not included in SRM	Polymorphism frequency (ExAC)	Reference polymorphism
IgA	SVTCHVK	✓			415.7	644.3; 322.7;684.3			
	SAVQGPPER	✓	✓		470.7	683.3;355.3;498.3	SAVQGPPDR	3.40E-01	http://exac.broadinstitute.org/variant/14-106174261-C-G
	YLTWASR	✓	✓	0.9	448.7	620.3;519.3;277.2	YLFWASR	3.00E-02	http://exac.broadinstitute.org/variant/14-106053461-T-A
IgG	GPSVFPLAPSSK	✓	✓		593.8	846.5;699.4;418.2			
	GPSVFPLAPCSR	✓	✓	0.6	644.3	947.4;800.4;519.2			
	DTLMISR	✓	✓	1.3	418.2	619.4;506.3;375.2			
	NQVSLTCLVK	✓			581.3	919.5;802.5;342.2			
IgG1	THTCPCPAPELLGGPSVFLFPPKPK				948.8	1253.2;1173.1			
IgG2	CCVECPPCPAPPVAGPSVFLFPPKPK				970.1	1100.6;839.4;519.2			
IgG3	CPAPELLGGPSVFLFPPKPK				717.7	912; 685.9			
IgG4	YGPPCPSCPAPEFLGGPSVFLFPPKPK				981.8	1185.1; 908.5			
IgM	VTSTLTIK	✓	✓		431.8	763.4;662.4;201.1			
	YVTSAPMPEPQAPGR	✓	✓	1.1	800.9	1079.5; 851.4	YVTSAPVPEPQAPGR	8.46E-06	http://exac.broadinstitute.org/variant/14-106320663-T-C
Kappa	VVAGEVTHQGLSSPVTK	✓	✓	1.0	591.6	788.5; 444.3; 755.9		6.00E-04	http://exac.broadinstitute.org/variant/2-89156939-A-C
	LYACEVTHQGLSSPVTK	✓	✓	1.0	630.7	788.5; 807.4; 277.2		1.30E-01	http://exac.broadinstitute.org/variant/2-89156948-C-G
	VVACEVTHQGLSSPVTK	✓	✓	0.8	626.0	618.3; 807.4; 263.1			
	TVAAPSVFIFPPSDEQLK	✓	✓		973.5	1320.7; 1060.5;913.5			
	SGTASVVCLLNNFYPR	✓	✓		899.5	1196.6; 810.4; 435.2			
	DSTYSLSSTLTLSK	✓	✓		751.9	1036.6; 949.6; 836.5			
	DSTYSLSNTLTLSK	✓			765.4	1063.6; 976.6; 863.5		2.00E-03	http://exac.broadinstitute.org/variant/2-89156889-C-T
Lambda	YAASSYLSLTPEQWK	✓	✓		872.4	988.5; 788.4; 687.3			
	SYSCQVTHEGSTVEK	✓	✓	1.0	571.3	987.5; 731.3; 251.1			
	VTHEGSTVEK	✓	✓	0.9	543.8	620.3; 494.2; 443.7			
IgD	TPFCPSHTQPLGVYLLTPAVQDLWLR				997.9	1198.7; 1097.6;948			
	VPTGGVEEGLLER				678.4	1058.5; 845.4; 628.8			
	VPAPPSPQPATYTCVVSHEDSR				799.0	1064.5; 1016; 1067.5			
IgE	GSGFFVFSR	✓	✓		502.3	655.4;409.2; 595.3			
	AVHEAASPSQTVQR				740.9	902.5; 815.4; 666.3			
QC	VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSK	✓	✓		1207.2	1036.6; 1490.2; 1446.7			
	HKVYACEVTHQGLSSPVTK				714.4	938.5; 888.9; 1088.5			
	QSNNKYAASSYLSLTPEQWK				772.4	687.3; 755.4; 814.9			
	SYSCQVTHEGSTVEKTVAPTECS				853.0	664.3; 593.2; 983			
	GPSVFPLAPCSR (1)				620.8	900.5; 753.4			
	DTLMISR (2)				431.2	645.4; 532.3			

(1) Non alkylated on cysteine
(2) Methionine oxidized

Table S-1. List of peptides for which transitions were included in our LC-MS method. For some peptides stable isotope labeled reference peptide were added to the samples, but not all of these were ultimately used for quantitation. Some reference standards were characterized by qNMR; the ratio between this characterization and the manufacturer's data has been noted in the table. The table indicates polymorphisms registered in the ExAC database at a frequency of more than 0.01, as well as those encountered in the samples. If the polymorphism was not accounted for in the SRM method, the sequence has been noted in the table.

	Freelite	N-Latex	SRM
κ (mg/L)	15.3 ± 3.7 (range 9.2-24)	13.1 ± 2.8 (range 8.8-19.1)	5.9 ± 2.5 (range 3.6-11.4)
λ (mg/L)	17.8 ± 7.5 (range 4.3-30.3)	18.4 ± 8.9 (range 8.7-38.2)	7.4 ± 3.2 (range 3-13.5)
κ/λ Ratio	1 ± 0.4 (range 0.6-2.1)	0.8 ± 0.2 (range 0.4-1.2)	0.8 ± 0.2 (range 0.5-1.2)

Table S-2. Summary of results that were obtained with sera from healthy controls (n=15). The control sera resulted in data within the normal range for all analytical platforms. All data shown as mean ± SD.

Sample	Freelite κ	N-Latex κ	SRM κ	SPE κ	Freelite λ	N-Latex λ	SRM λ	SPE λ
1	32.2	23.5	1.9	3.1	NA	0.011	0.032	<
2	22.4	4.6	1.9	2.3	0.008	0.016	0.028	<
3	NA	0.017	0.009	<	77.8	8.7	14.5	14.7
4	0.015	0.014	0.009	<	15.0	16.6	6.6	5.5

Table S-3. Comparison of sFLC data obtained by two commercial nephelometric assays, the SRM method described in this paper, and by serum protein electrophoresis and immunofixation (SPE). All data are shown as g/L sFLC. NA = not assessed; < = below detection limit

	Certificate (g/L)	SRM (g/L)
IgA	1.8	1.4
IgG	9.2	5.9
IgM	0.7	0.5

Table S-4. Concentration of intact immunoglobulins in the CRM reference serum material based on the SRM method, and based on the certified information accompanying the CRM serum.