UMC St Radboud

Deelnemersbespreking SKML – sectie HIM

Vrije Lichte Keten assays: nieuwe ontwikkelingen

13 december 2012

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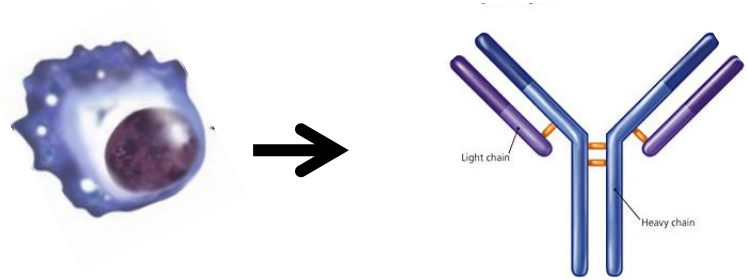
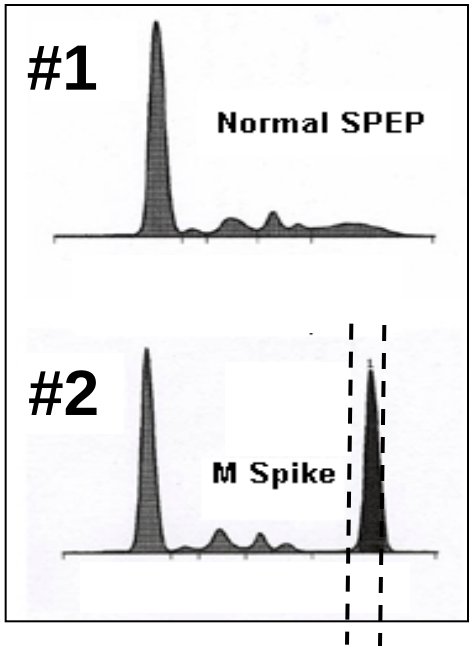
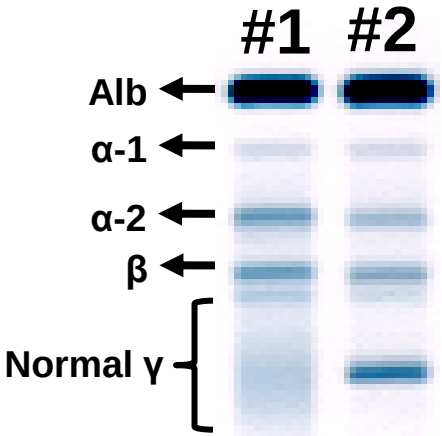
M-proteine diagnostiek

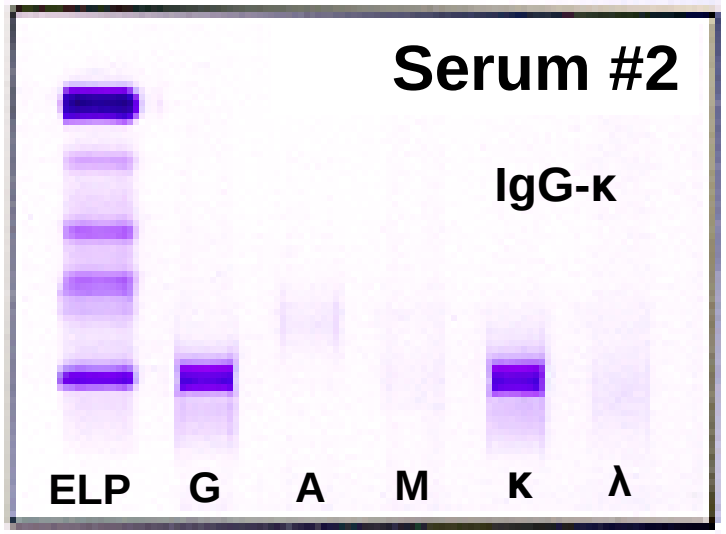




Densitometrie

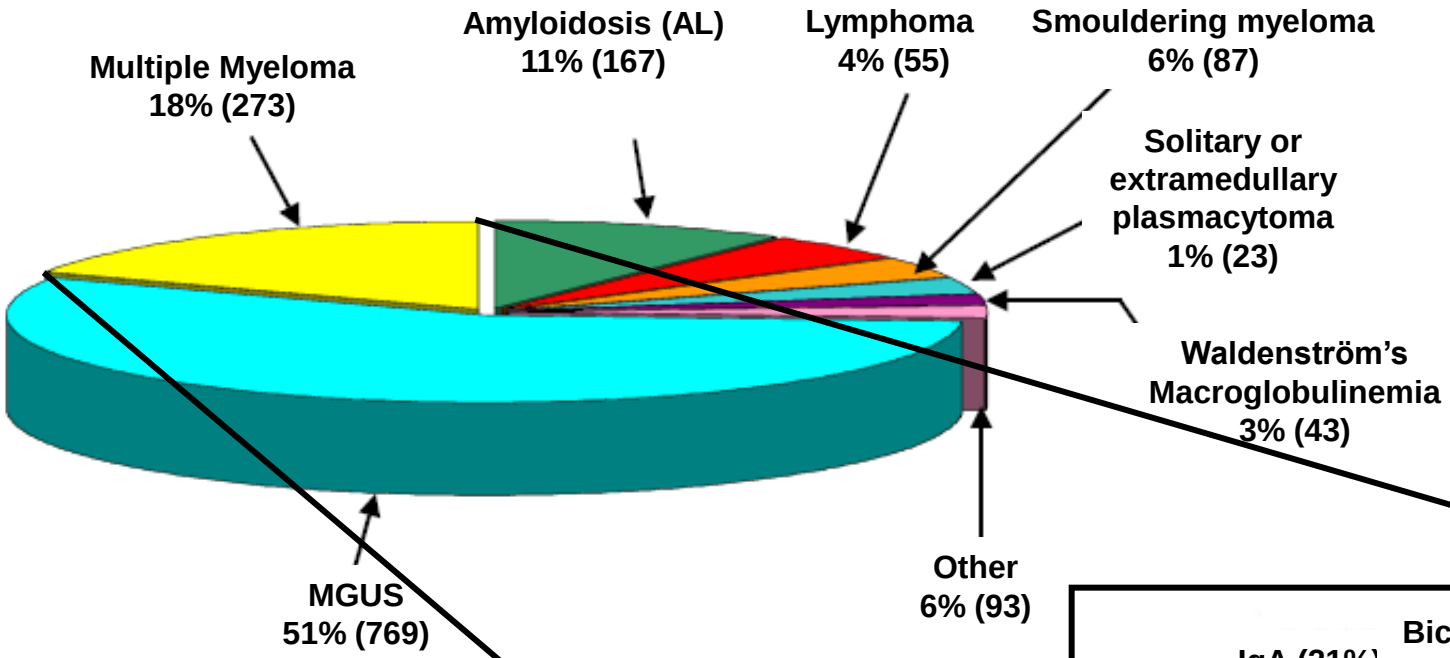


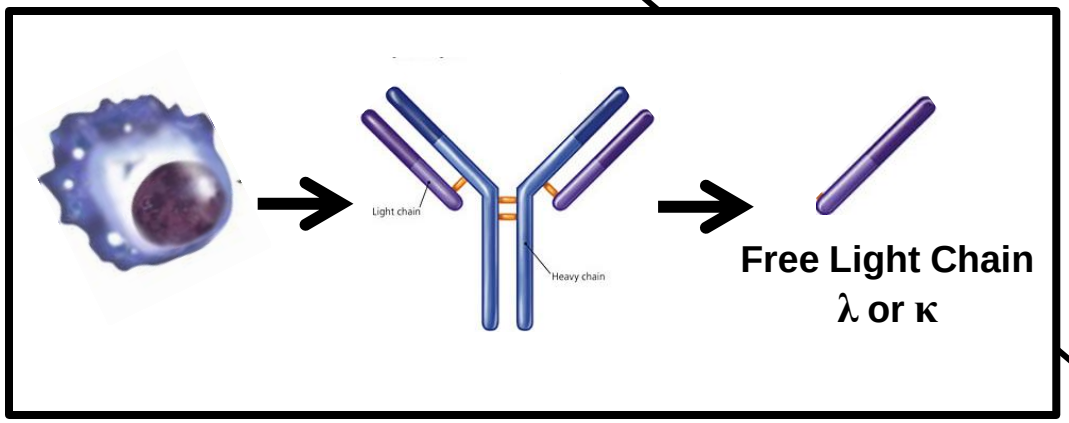
Eiwit electroforese

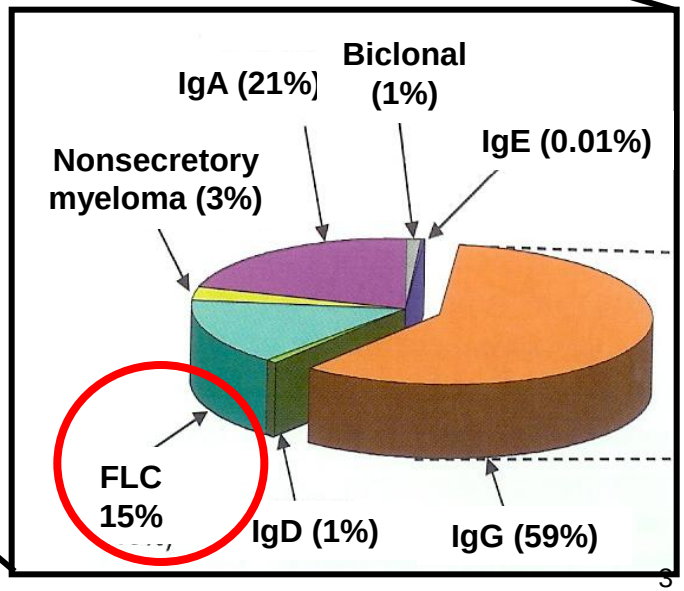


Immunofixatie

Monoclonal gammopathies

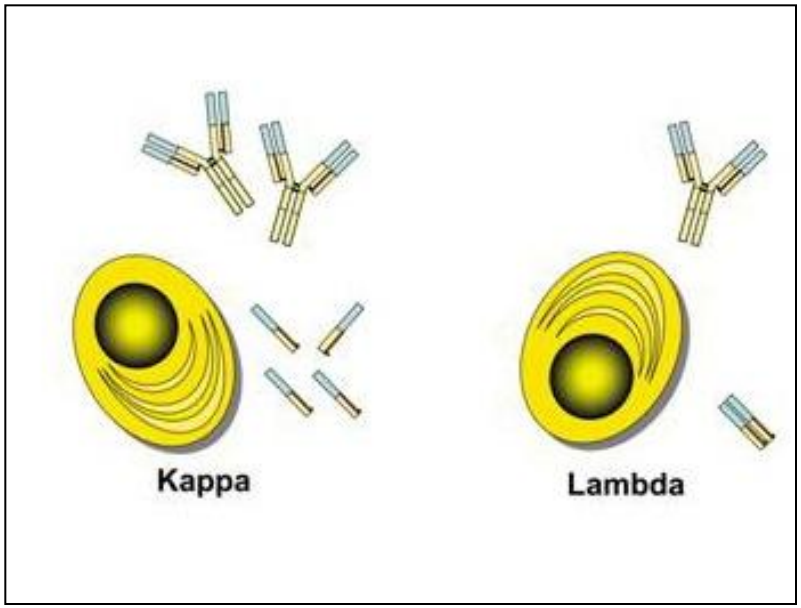


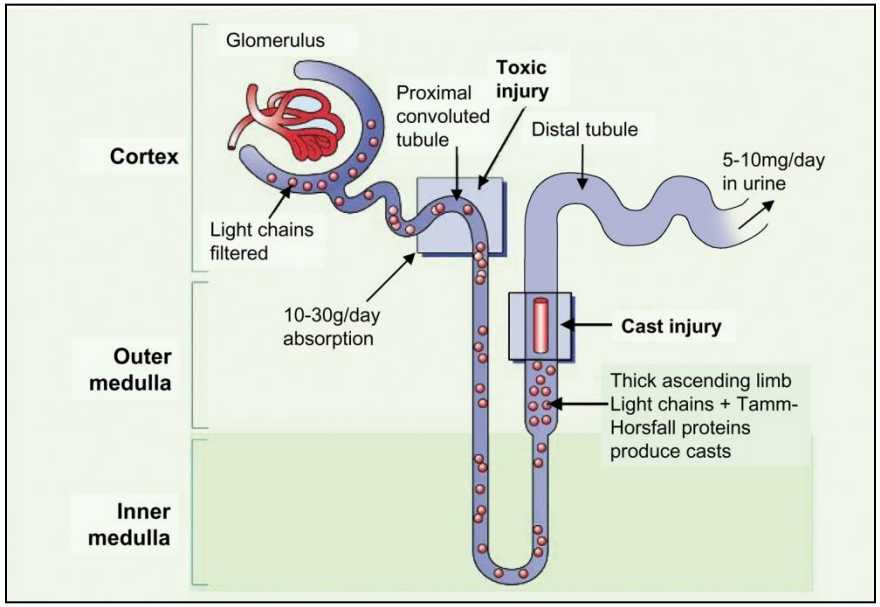




Diagnosed at Mayo Clinic 2002

Free Light Chains





Bone marrow and lymphoid organs

Produced 500 mg/day

Kidney

Capacity to absorb and metabolise 10-30 gram/day

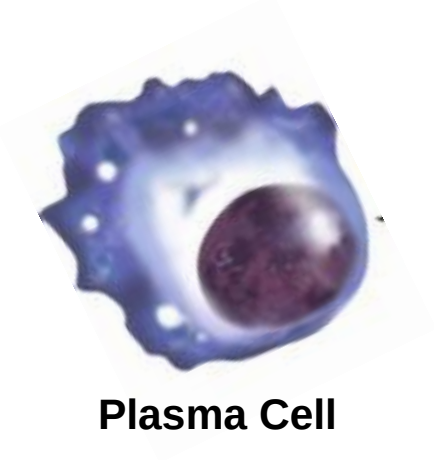
$T^{1/2}$ varies from hrs to 2-3 days (renal function)

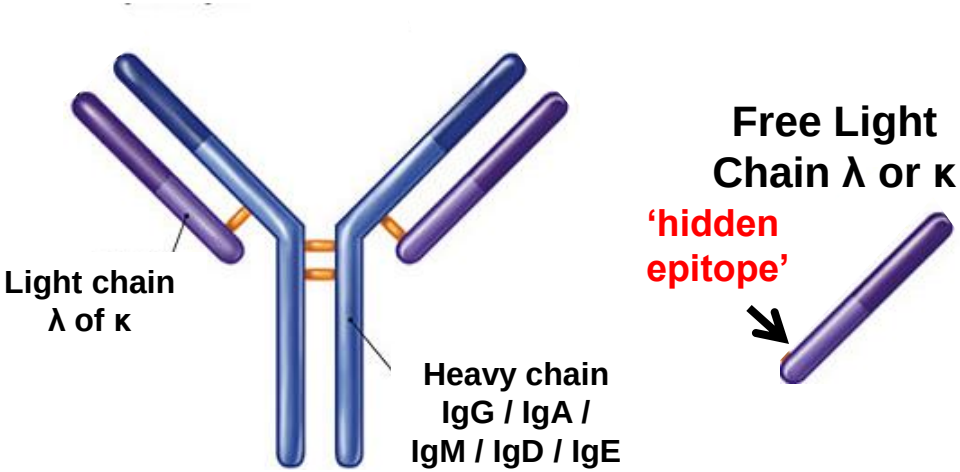
FLC normal ranges (when measured with Freelite reagents)

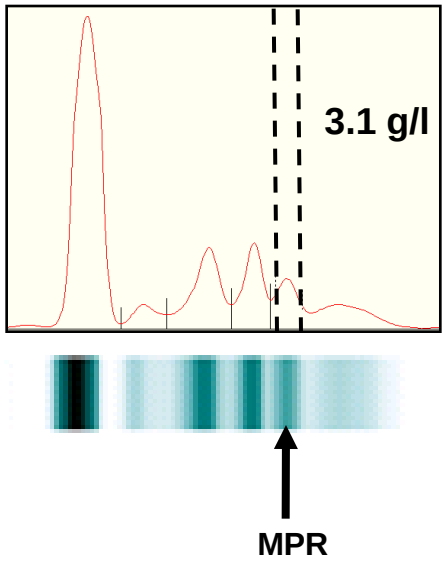
Kappa: 3.3 – 19.4 mg/L
Lambda: 5.7 – 26.3 mg/L
Kappa/Lambda ratio: 0.26 – 1.65

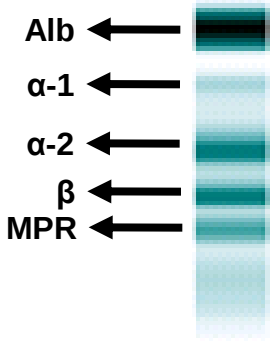
Bradwell. sFLC analysis 6th edition

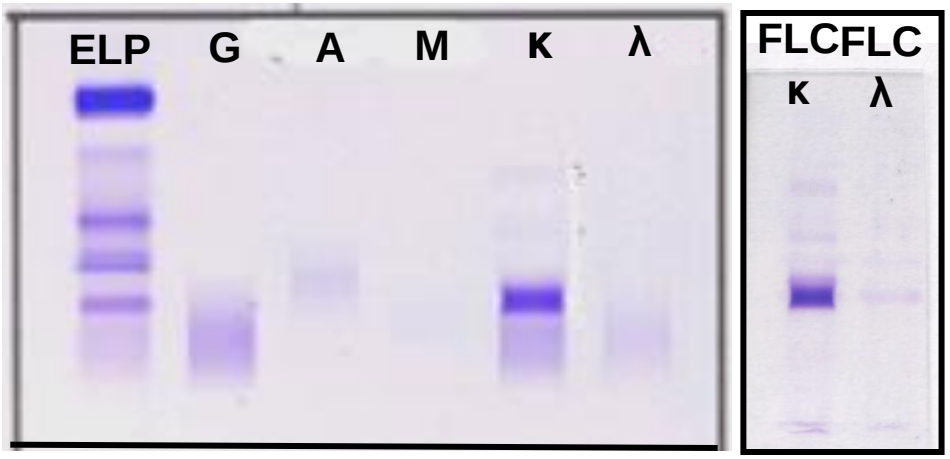
Free Light Chain diagnostics











Free Light Chain diagnostics

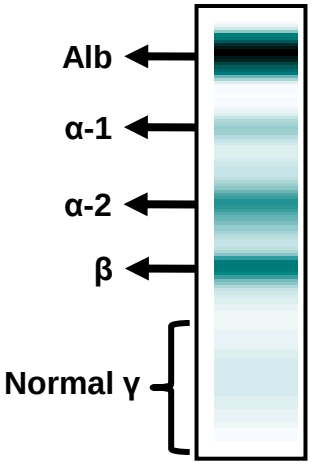
Case:

Man, 56 years old.
Bone pain & compression # spine

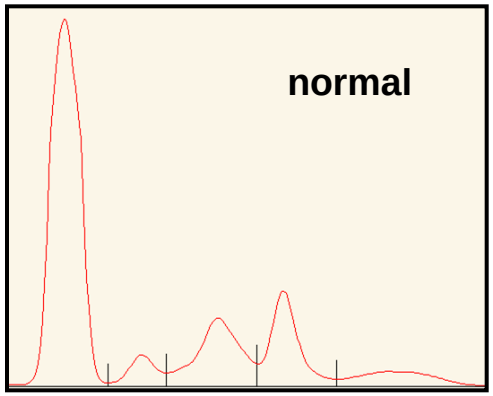
Lab: **Ca ↑↑, Hb ↓**

X-ray: **multiple lytic lesions**

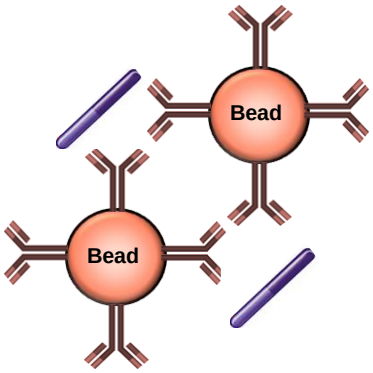
BM biopsy: **58% plasma cells**



SPE



Nephelometry (Freelite)



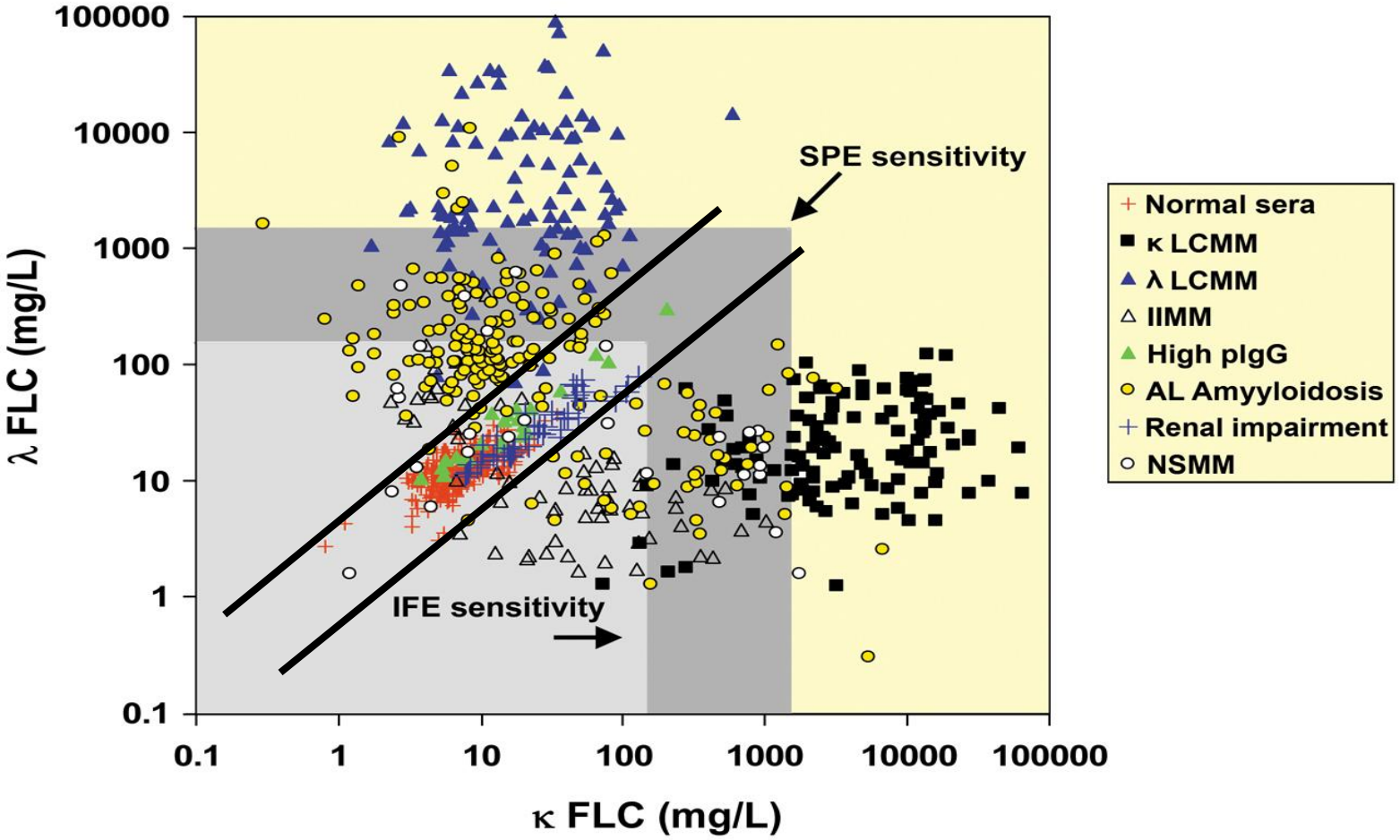
	REF values	*Our Case*
Free kappa:	**3,3 – 19,4 mg/l**	**410 mg/l**
Free lambda:	**5,7 – 26,3 mg/l**	**1.9 mg/l**
Ratio:	**0,26 – 1,65**	**219**

Bradwell et al. Clin Chem 1999

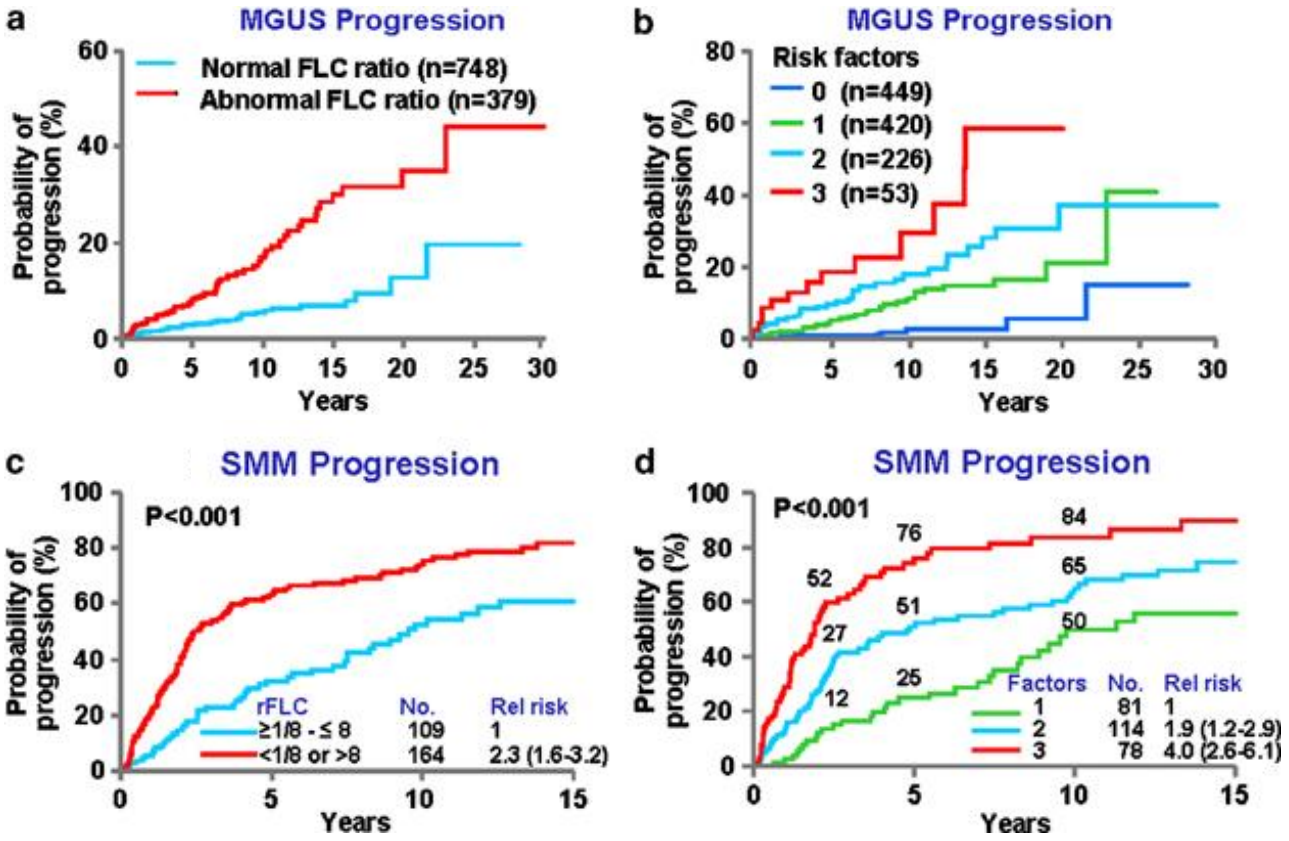
Binding Site

For increased sensitivity: nephelometric FLC analysis (Freelite)

Method	Kappa	Lambda	Diagnostic requirement
SPE	500-2,000mg/L	500-2,000mg/L	monoclonal band
IFE	150-500mg/L	150-500mg/L	monoclonal band
FLCs Nephel.	1.5mg/L	3.0mg/L	abnormal κ/λ ratio



Freelite assay: FLC conc. correlate to prognosis and disease activitiy



Rajkumar et al. Blood 2005

Dispenzieri et al. Blood 2008

Correlation with prognosis for MM: Snozek et al. Leukemia 2008

Correlation with prognosis for Amyloidosis: Palladini et al. JCO 2012

Correlations with other lympoproliferative disorders (review): Charafeddine et al. Am J Clin Pathol 2012

Correlation with disease activity in autoimmune disorder: Gottenberg et al. Ann Rheum Dis 2007

Freelite assay (The Binding Site): in the clinic

	Kappa	Lambda	Diagnostic requirement
SPE	500-2,000mg/L	500-2,000mg/L	monoclonal band
IFE	150-500mg/L	150-500mg/L	monoclonal band
FLCs	1.5mg/L	3.0mg/L	abnormal κ/λ ratio

Advantages:

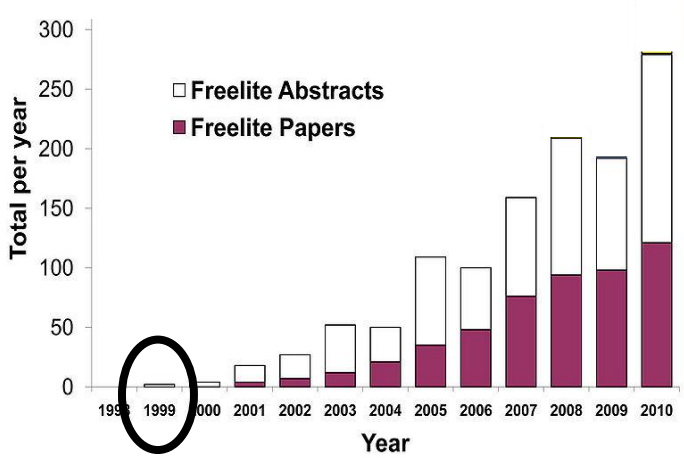
Freelite™

1) Earlier diagnosis

2) Improved monitoring (international response-criteria)

3) Associated with prognosis (international consensus)

4) High through-put

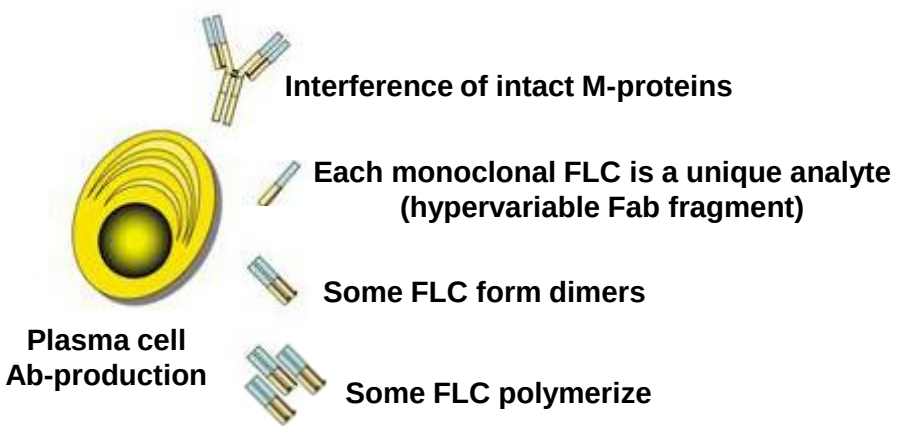


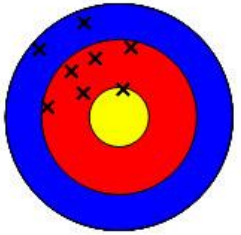
Bradwell et al. 1999 Clin Chem 'immunoassay for quantification of FLC in serum'

Durie et al. 2006 Leukemia 'international uniform response criteria for MM'

Freelite assay: analytical issues

- **Linearity problems**
- **Antigen excess**
- **Imprecision**
- **Non-accurate**

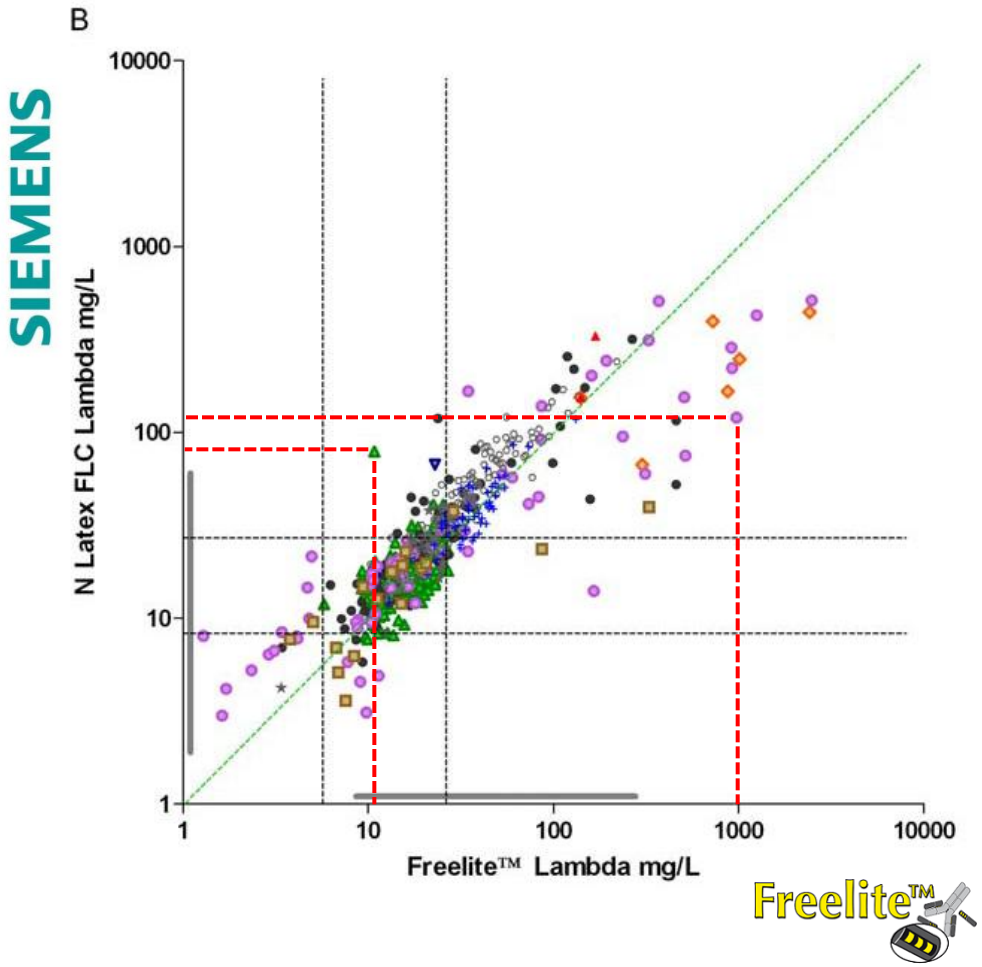


	Accurate	Inaccurate (systematic error)
Precise		
Imprecise (reproducibility error)		

Tate et al. Clin Biochem Rev 2009

Recently Siemens has introduced a second commercial immunoassay to measure FLC (N Latex assays)

Te Velthuis et al. Clim Chem Lab Med (2011)



Red dotted lines illustrate that differences in FLC concentrations can be observed between the two commercial immunoassays

(up to 10 times !)

Hoedemakers et al. Clim Chem Lab Med 2011.

First (personal and international) experiences in clinical labs with the N latex assays

Compared to Freelite assay:

- **Improved linearity but also STRONG non-linearity in some samples, especially when also intact M-protein is present** (Jacobs et al. Clin Chim Acta 2012)

FLC linearity

Clinica Chimica Acta 412 (2011) 1798–1804

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Effect of sample dilution on serum free light chain concentration by immunonephelometric assay

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Freelite !

FLC linearity in presence of intact monoclonal Ig

Table 1.

M-protein	N Latex FLC kappa (mg/L)							Freelite™ FLC kappa (mg/L)							
Ig (g/L)	01:20	1:100	1:400	1:2,000	1:8,000	1:40,000	% diff	01:20	1:100	1:400	1:2,000	1:8,000	1:40,000	1:160,000	% diff
IgG-K (6)		15.2	16.7				10		25.4	50.6					99
IgG-K (6)		20.6	17.5				-15		21.8	38.4					76
IgG-K (12)		63.6	89	98.7			40		145	225					55
IgG-K (56)		56.9	69.9	83.4			23		65.1	108	<127				66
IgA-K (12)		>110	275	280			2		202	364	385				80
IgA-K (28)		38.5	108	174	<274		181		63.1	168	215				166
IgA-K (28)		21	46.6	74	<274		122		30.4	116	154	<507			282
IgA-K (62)		>110	>438	504	609	<1370	21		10.5	152	461	660	<2530		1348
IgM-K (5)		67.0	74.8	83.8			12		51.5	83.8					63
IgM-K (17)		109	117	127	<274		7		99.3	146	193	<507			47
IgM-K (19)		11.4	30.2	<68.4			165		42.7	70.2	<127				64
IgM-K (19)		>110	358	405	437	<1370	13		>203	>811	833	1060	<2530		27
IgM-K (27)		4.4	17.2	69.8	<274		291		15	27	<127				80
IgM-K (30)		>110	104	140			13		90.7	167	174				84
IgM-K (35)		5.1	19.8	73.4	<274		288		20.7	35.2	<127				70

Jacobs et. al. Clin. Chim. Acta. 2012

First (personal and international) experiences in clinical labs with the N latex assays

Compared to Freelite assay:

- **Improved linearity but also STRONG non-linearity in some samples, especially when also intact M-protein is present** (Jacobs et al. Clin Chim Acta 2012)
- **Build in antigen excess protection**

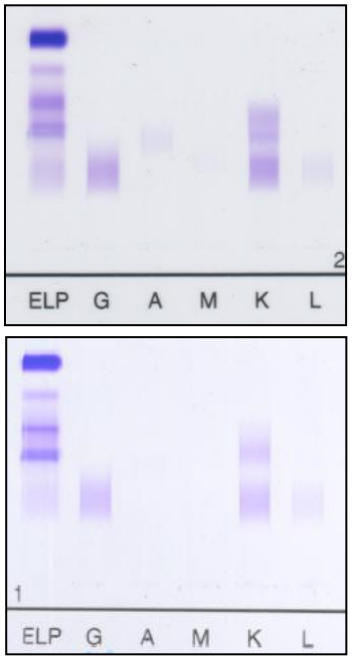
Antigen excess in FLC measurements

Antigen excess

	N latex	Freelite
Kappa	0	2
Lambda	0	0

11.2 g/L
11.5 g/L

N = 93



Highest measured concentration

	N latex	Freelite
Kappa	24 g/L	**32 g/L**
Lambda	13 g/L	78 g/L

See also Murata et. al. 2010. Clin. Chem.

First (personal and international) experiences in clinical labs with the N latex assays

Compared to Freelite assay:

- **Improved linearity but also STRONG non-linearity in some samples, especially when also intact M-protein is present** (Jacobs et al. Clin Chim Acta 2012)
- **Build in antigen excess protection**
- **Higher batch-to-batch precision** (Pretorius et al. Ann Clin Biochem 2012)
- **mAb impose risk of missing FLCclone** (Hutchison et al. BMC Clin Pathol 2012)
- **Reference values are similar but not identical**

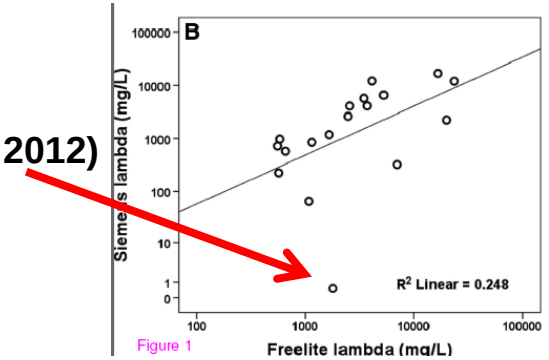


Figure 1

Reference values sFLC

- 28 patients on dialysis for ESRF

	Kappa	Lambda	Ratio
N Latex FLC	196mg/L (80-441)	243mg/L (107-477)	0.48-1.35
Freelite*	195mg/L (81-412)	166mg/L (56-388)	0.79-2.38

Adapted from dr. Jillian Tate, presentation during webinar sept 2012

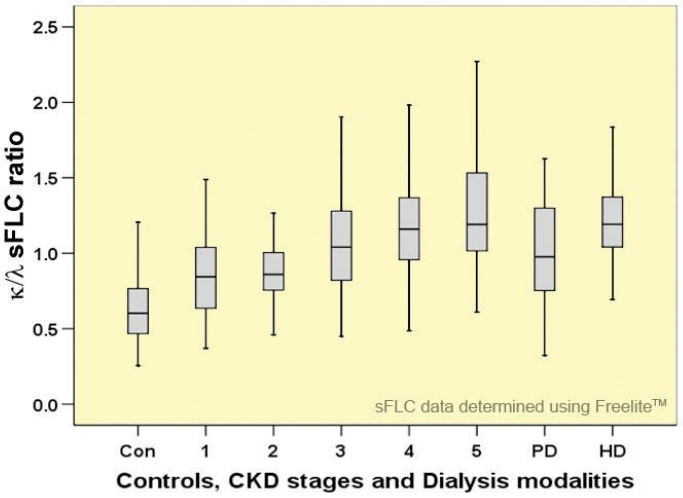
Reference values sFLC

- 28 patien

			Ratio
N Latex FLC	196mg/L (80-441)	243mg/L (107-477)	0.48-1.35
Freelite*	195mg/L	166mg/L	0.79-2.38

"Usual" RR 0.31-1.56
No need for renal RR

"Usual" RR 0.26-1.65
Renal RR 0.37-3.1



Adapted from dr. Jillian Tate, presentation during webinar sept 2012

Summary FLC assays

Assay Characteristic	N Latex (Siemens)	Freelite (The Binding Site)
Antibodies	monoclonal	polyclonal
Measuring range (mg/L)	~1 to > 100,000	~1 to > 100,000
Platforms (AE: Antigen excess testing is available)	BN™II (AE) BN ProSpec® (AE)	SPA-Plus (AE) BNII, ProSpec IMMAGE Olympus Roche Cobas, Integra & Hitachi
Imprecision	Less effect on ratio for low concn. of uninvolved FLC	Effect on ratio for low concn. of uninvolved FLC
Non-linear (some samples)	yes	yes
Prone to overestimation	yes	yes
Reference intervals	K/L ratios 0.31-1.56	K/L ratios 0.26–1.65
Reference intervals – CKD on haemodialysis	K/L ratios 0.31-1.56 (diagnostic range)*	K/L ratios 0.37-3.1**

* higher Lambda FLC observed in CKD;

** Hutchison CA et al. Quantitative assessment of serum and urinary polyclonal free light chains in patients with chronic kidney disease. Clin J Am Soc Nephrol 2008;3:1684-90.

Adapted from dr. Jillian Tate, presentation during webinar sept 2012

Is harmonization possible?

Strong concentration differences observed when compared to Freelite assay

a) Both assays report results in mg/L
b) Which result is correct?! International standard is lacking…
c) Reference values are similar but not identical
d) The above provides a big problem
- For translation of data from literature
- For patients switching from hospital

Standardization is urgently needed (but will be difficult).

Can immunoglobulins and FLC be quantified using mass spectrometry?

Tripple quad MS

MRM = multiple reaction monitoring
(= SRM = selected reaction monitoring)

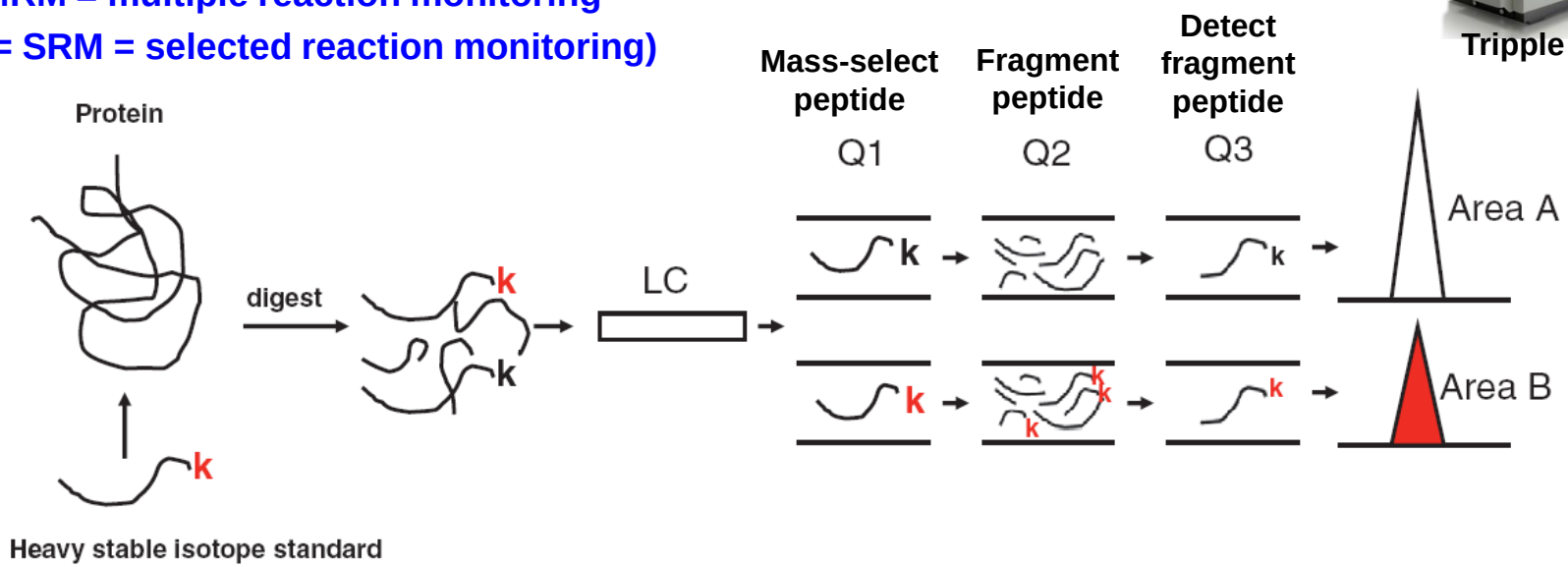
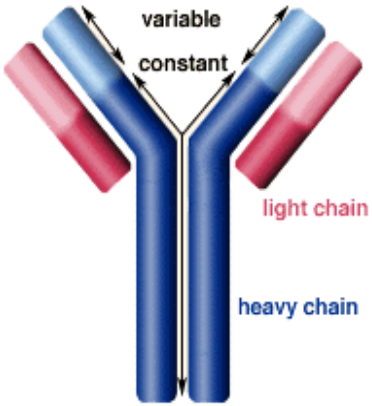


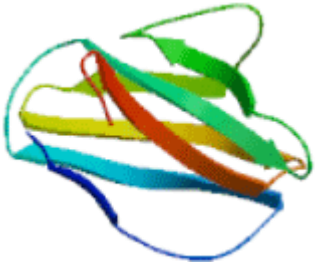
Fig. 2 – Absolute quantitation using MRM-MS in combination with isotope-labeled internal standard.

MRM technique in a triple-quadrupole instrument with stable isotope standards. Proven extremely powerful to accurately quantitate proteins.

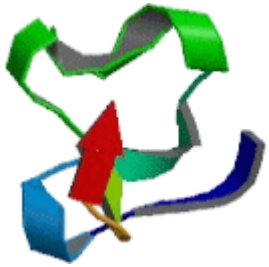
Meng et al. J. of Proteomics 2011

Can MS-suitable peptides from Ig and FLC be selected?





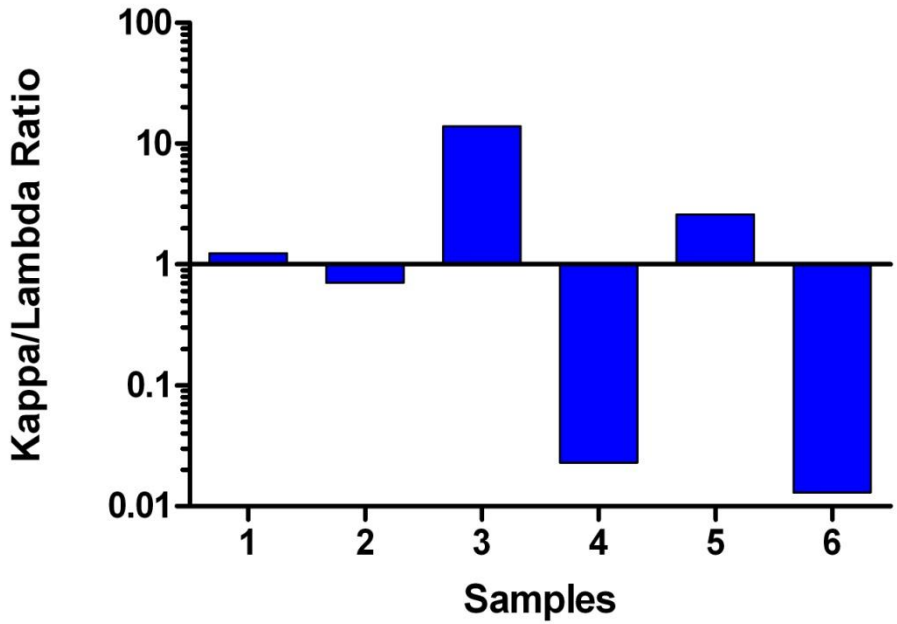
Kappa



Lambda

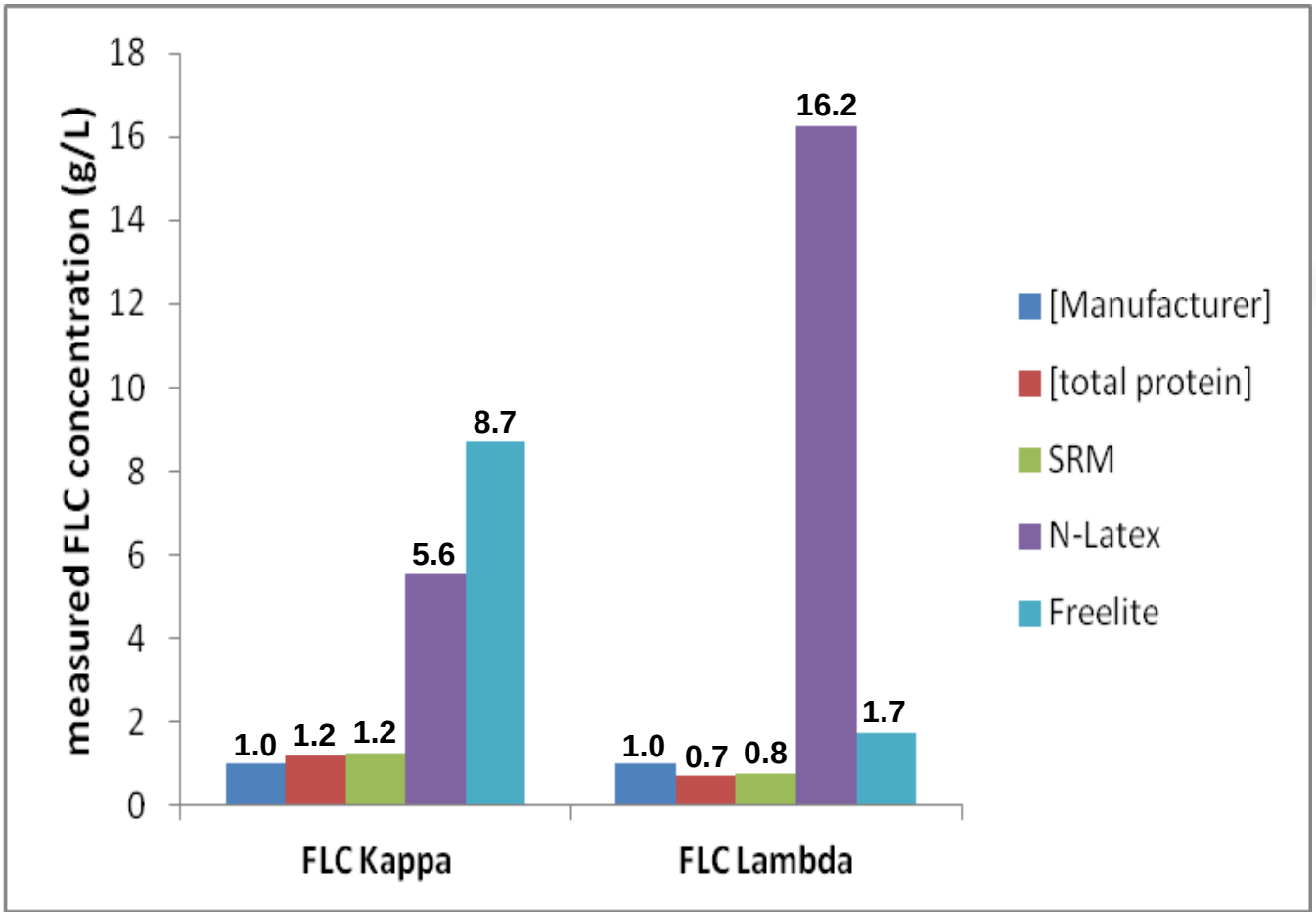
MS-suitable trypsin digestive peptides available in all intact Ig's
BUT ALSO in Light Chains!!!

Quantification of FLC-ratio in pt samples using MRM MS



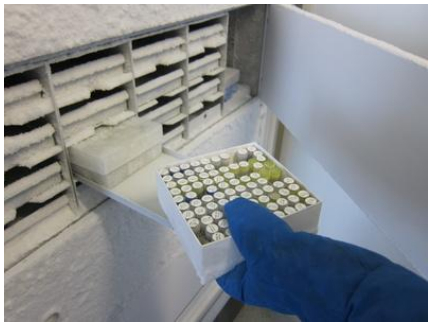
1. Healthy control
2. Pt. on dialysis
3. MM-pt. FLC Kappa
4. MM-pt. FLC Lambda
5. MM-pt. IgG-K (with few FLC K)
6. MM-pt. IgG-L (with abundant FLC L)

Method comparison MRM MS versus immunoassay



Scope

Months 1-2	Months 3-4	Months 5-6
Sample selection from biobank		
	Method validation in large serum sample set	
	Data Analysis	



Aim: reference method for FLC (and Ig) measurements…

Acknowledgements

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Irma Joosten

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Department of Neurology

Martijn van Duijn
Theo Luider

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Relares Grant