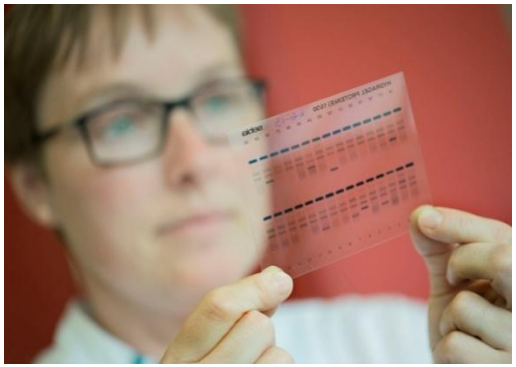
Rondzending M-proteïne diagnostiek

SKML nabespreking, sectie HIM



13 april 2017

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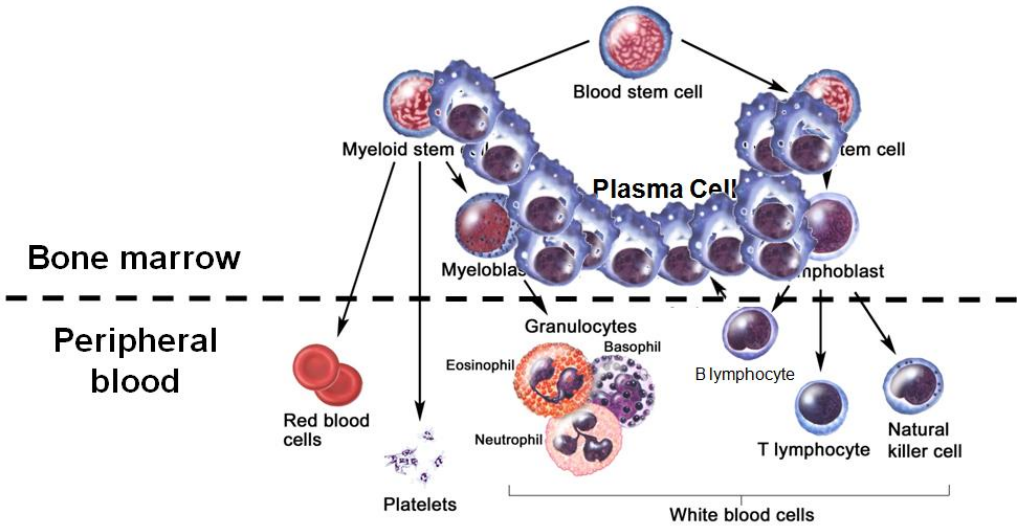
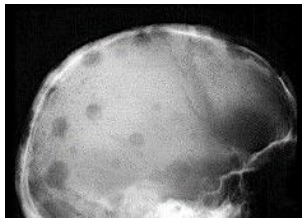
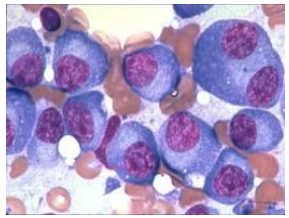
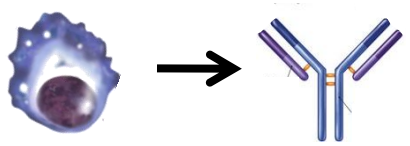
Monoclonal gammopathy; multiple myeloma

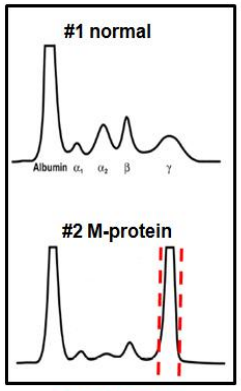




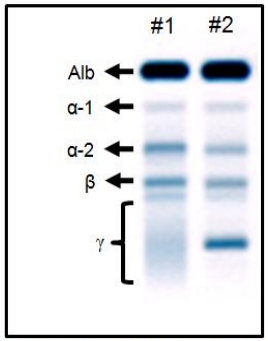




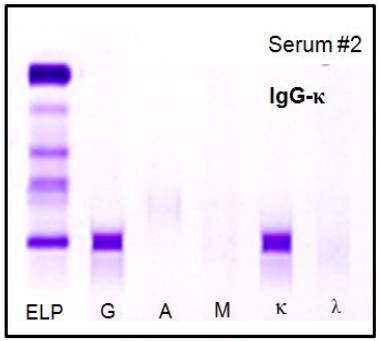
Gel electrophoresis



Densitometry

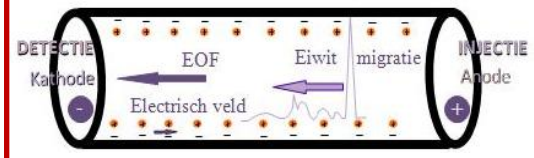


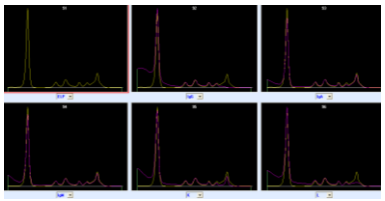
Serum protein electrophoresis



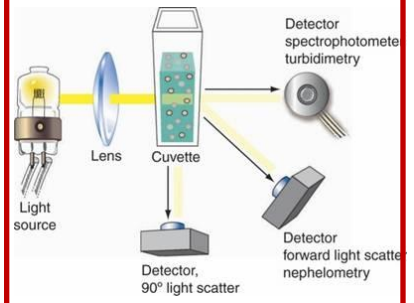
Immunofixation

Capillary electrophoresis



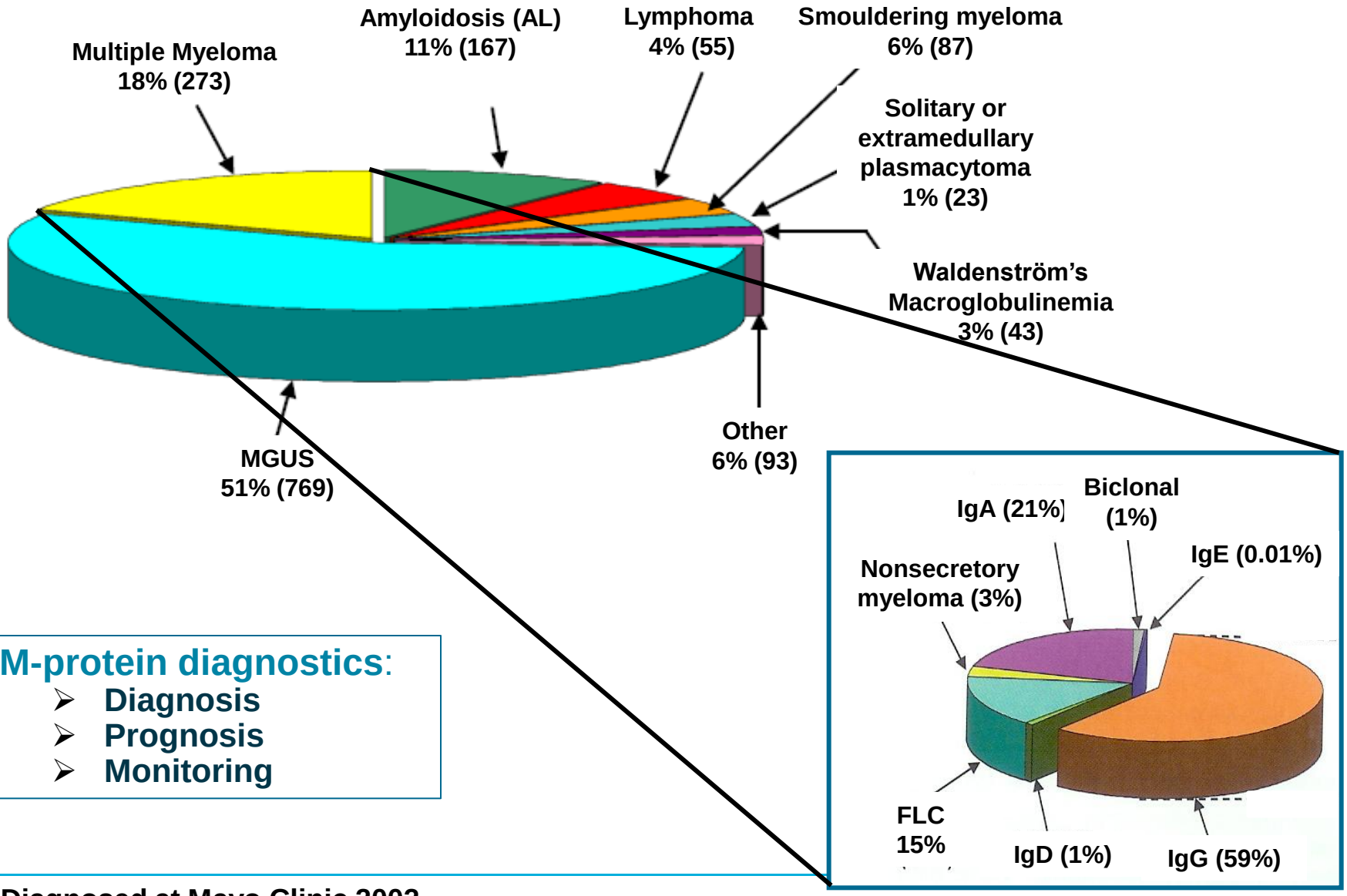


Immunochemistry



Nephelometry / Turbidimetry

Monoclonal gammopathies



M-protein diagnostics:

- Diagnosis
- Prognosis
- Monitoring

Diagnosed at Mayo Clinic 2002
Dimopoulos et al. Blood 2011

M-proteine rondzendingen

Periode 2014.3 t/m 2016.4

- 4 rondzendingen per jaar, 75 deelnemers
- Elke rondzending 3 monsters (A,B,C), soms met casus
- Invoer en rapportage via Qbase

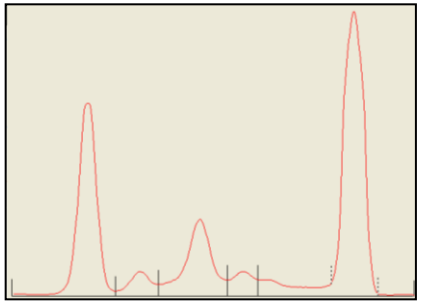
Inventarisatie:

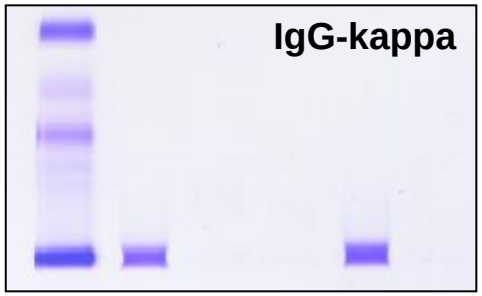
- Typering M proteine
- Kwantificering M proteine
- Kwantificering totaal eiwit, Ig's
- Soms kwantificering Vrije Lichte Ketens (VLK)
- Soms urine of cryobepaling
- Soms additionele vraag

Wat is er rondgestuurd in deze periode:

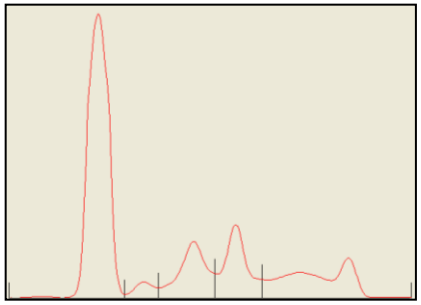
IgG-K	7x
IgG-L	2x
IgM-K	7x
IgA-K	5x
IgA-L	2x
VLK-K	3x
Geen MPR	7x
Urine BJK	1x
Cryo	2x

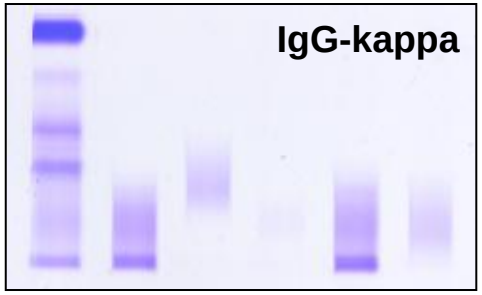
Verschillen in VC afhankelijk van het M-proteine



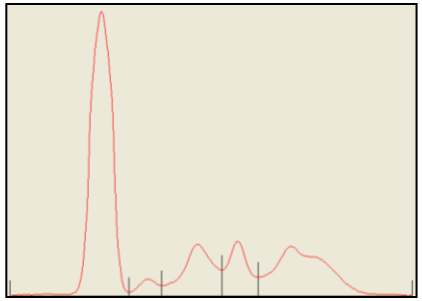


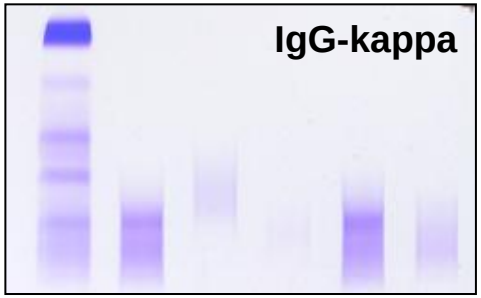
>95% IgG-kappa
Gem: 42.4 g/L
VC: 13 %



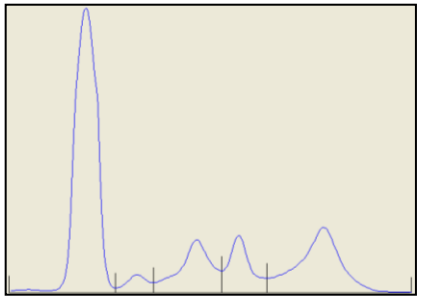


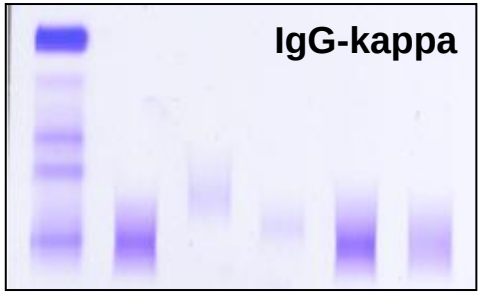
>95% IgG-kappa
Gem: 2.7 g/L
VC: 29 %





92% IgG-kappa
Gem: 5.5 g/L
VC: 40 %



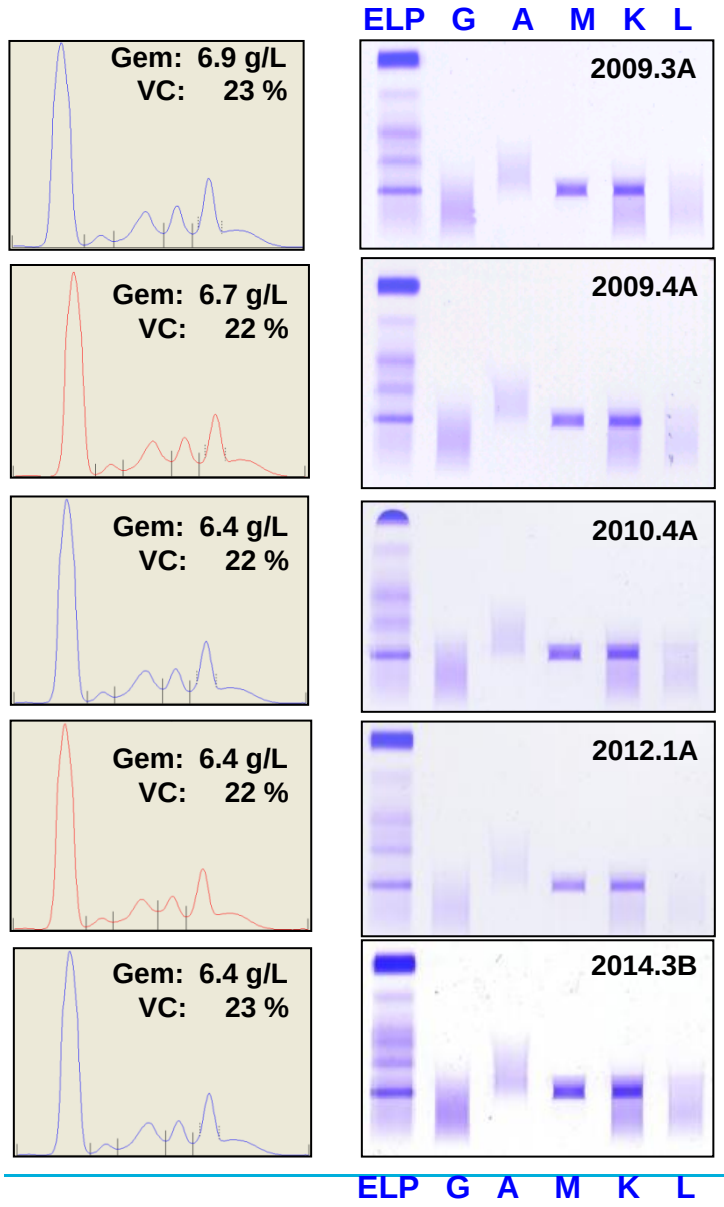


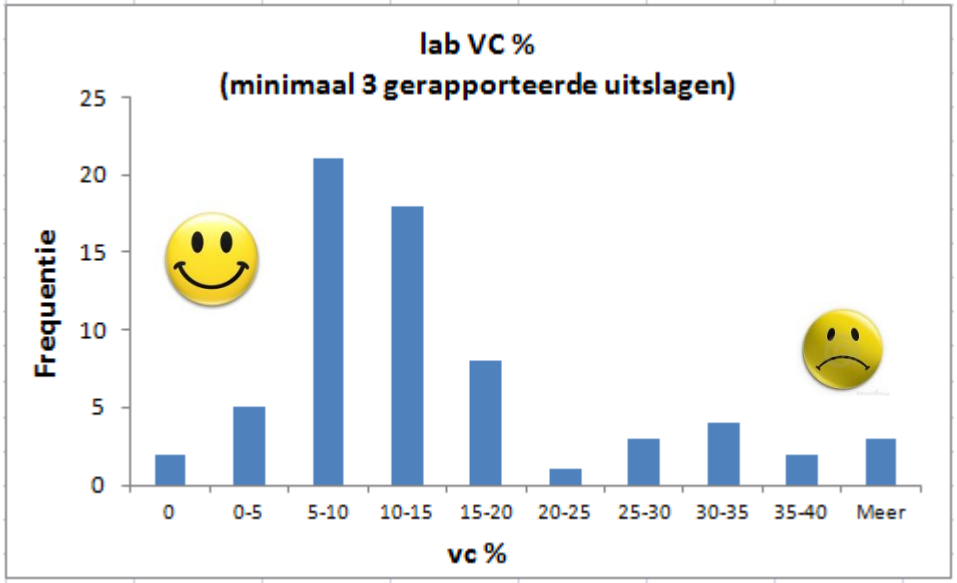
>95% IgG-kappa
Gem: 2.5 g/L
VC: 51 %

ELP G A M κ λ

Data gepresenteerd tijdens SKML nabespreking 2014

Reproduceerbaarheid van M-spike





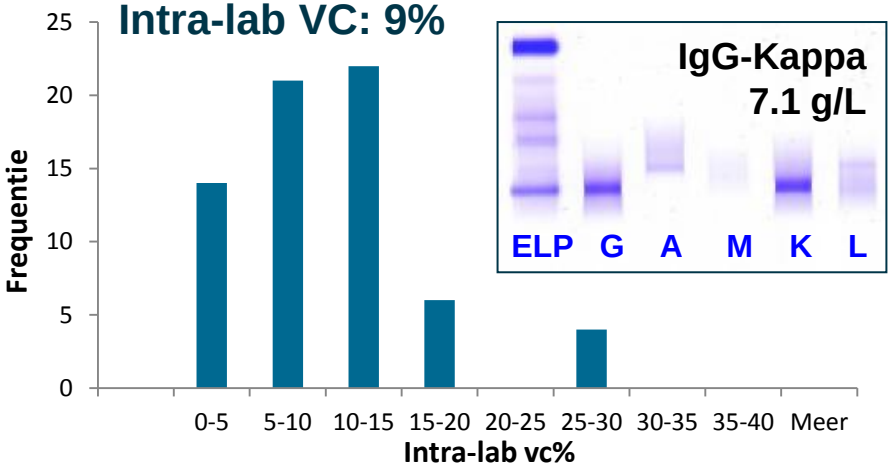
Gemiddelde VC binnen 1 lab over minimaal 3 metingen: 14 %

Vervolg: Reproduceerbaarheid M-proteine M-spikes

2014.2B; 2015.4C; 2016.4B

Inter-lab VC: 22%

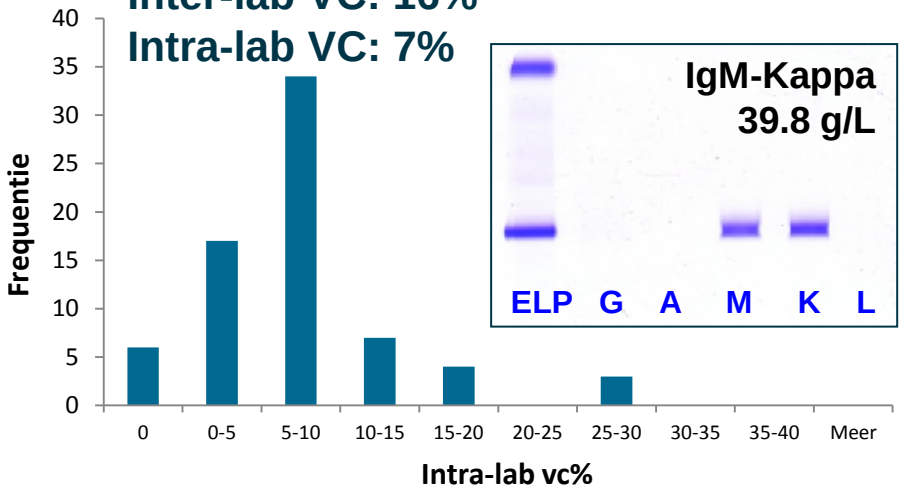
Intra-lab VC: 9%



2013.2A; 2014.4A; 2015.3C

Inter-lab VC: 16%

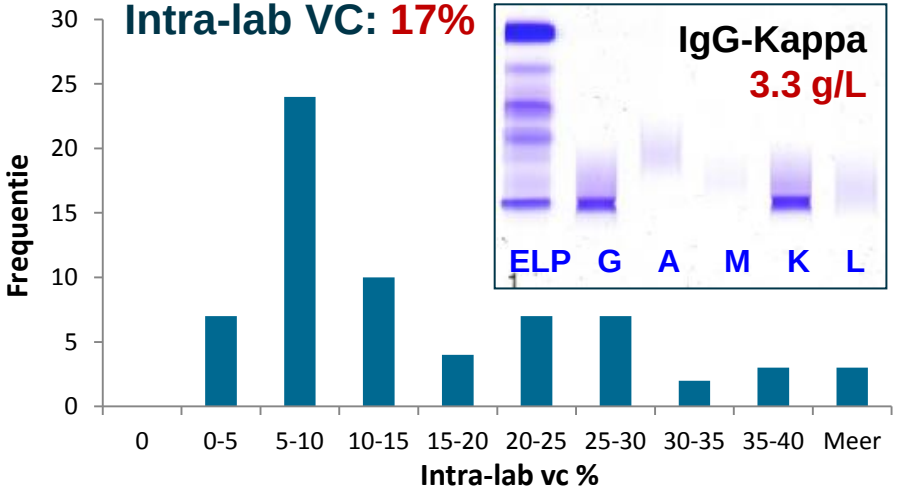
Intra-lab VC: 7%



2014.3A; 2015.2A; 2016.4C

Inter-lab VC: 24%

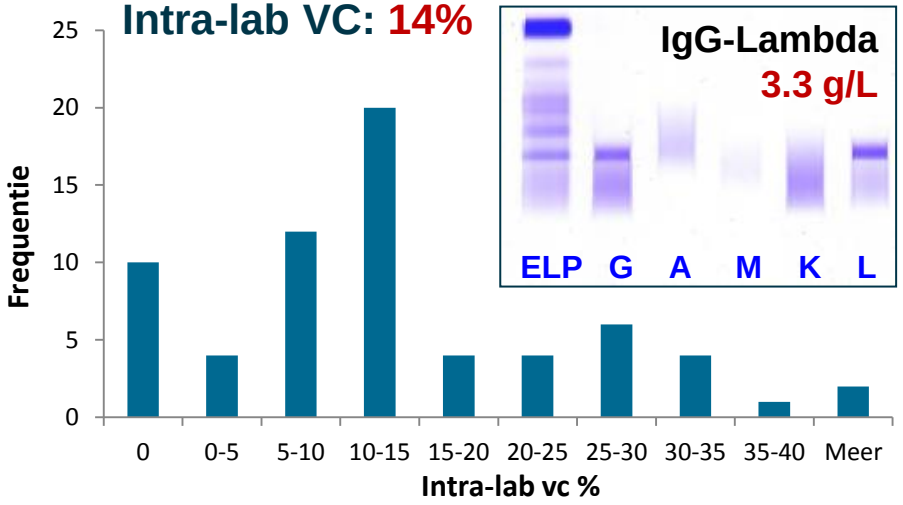
Intra-lab VC: 17%



2014.1B; 2014.4B; 2015.2C

Inter-lab VC: 37%

Intra-lab VC: 14%



VC% M-proteine diagnostiek: en hoe daarmee om te gaan in kliniek

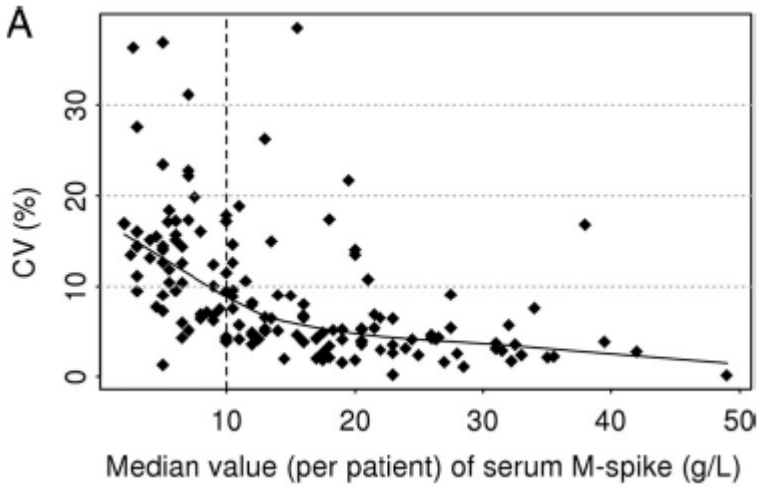


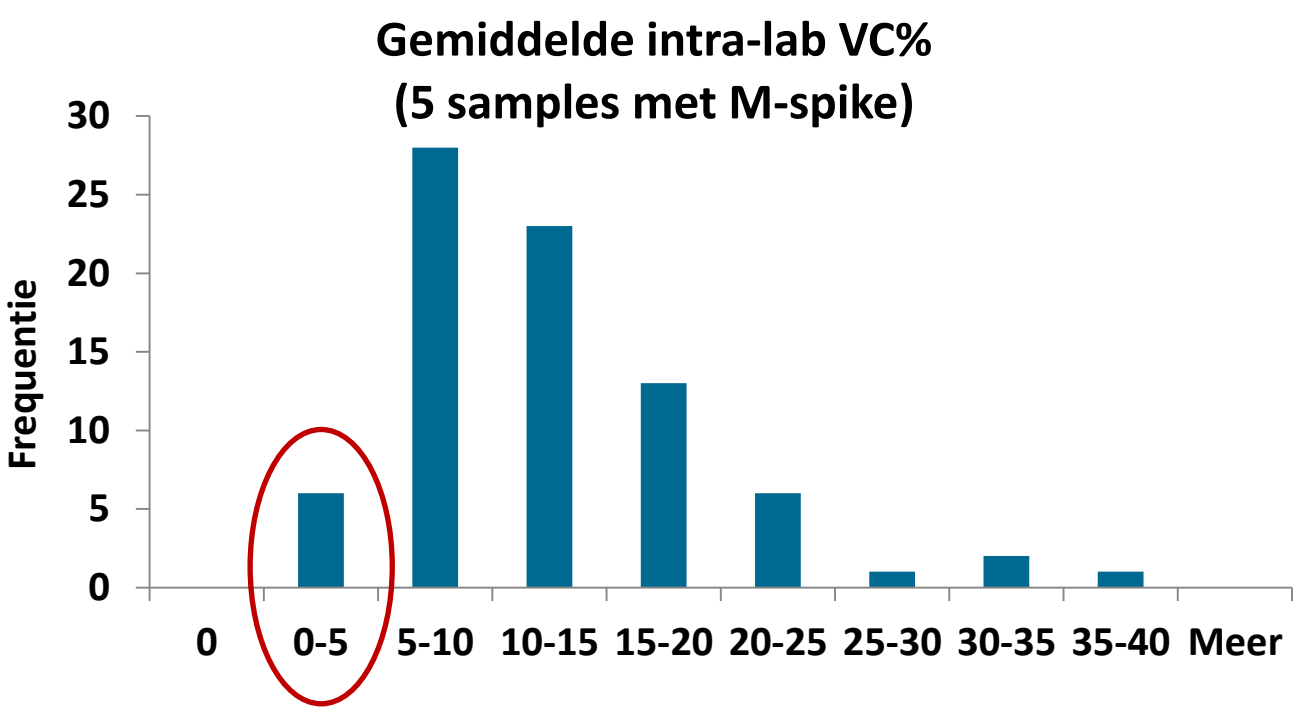
Table 3. Percentage decrease needed in each assay for statistical significance at various probability thresholds.

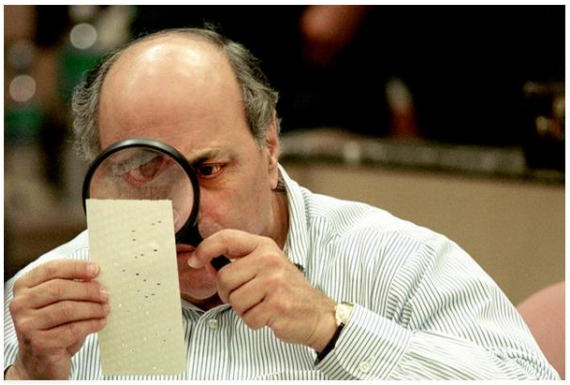
Test	n	Total CV	$P = 0.5$	$P = 0.8$	$P = 0.9$	$P = 0.95$
Serum M-spike	158	11.6	10.5	19.0	23.6	27.5
Measurable serum M-spike[a]	90	8.1	7.4	13.7	17.2	20.1
Involved immunoglobulin (IgG)	148	13.0	11.7	21.0	26.1	30.3
Urine M-spike	25	35.8	28.9	47.7	56.5	62.9
iFLC	158	34.9	28.3	46.9	55.6	62.0
Measurable iFLC[b]	52	28.4	23.7	40.2	48.3	54.5
uFLC	157	45.2	35.0	55.9	65.1	71.4
rFLC	157	36.4	36.4	57.7	66.9	73.2

[a] Values >10 g/L.
[b] Values >100 mg/L.

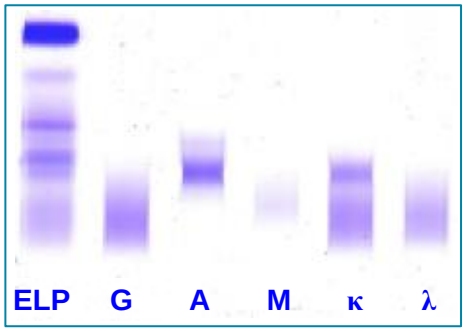
Katzmann et al. Clin Chem 2011

And the winner is….



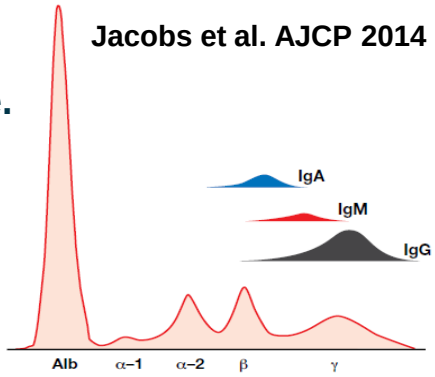


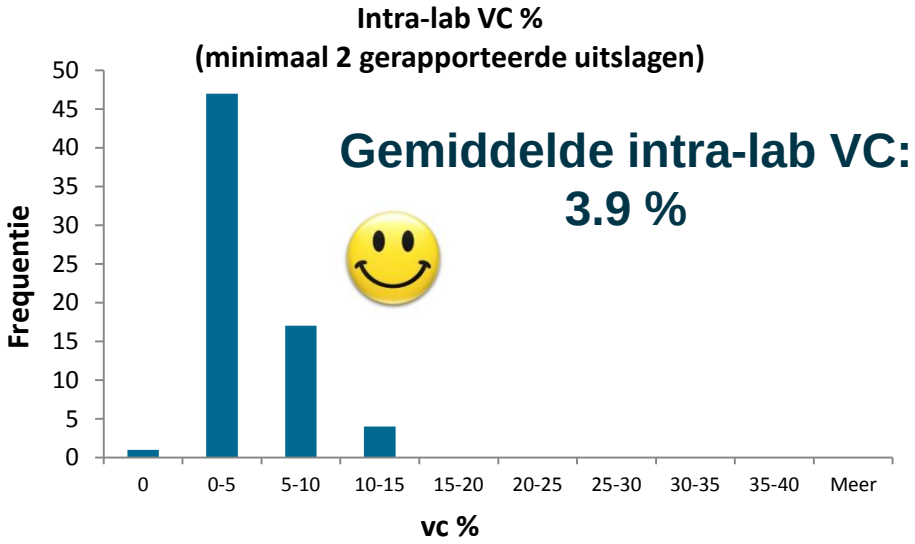
Reproduceerbaarheid van M-proteine in β-gebied



2015.1A / 2015.2B / 2016.2B
94-97% deelnemers detecteert IgA-kappa M-proteine.

Quantificering immunochemisch:
Gemiddeld Totaal IgA: 4.1 g/L (interlab VC 7.3%)





- M-proteine wordt gemist indien ESP niet kritisch bekeken
- Kwantificeren is lastig, spiken is niet mogelijk…
- ‘Second best’ is immunochemisch totaal IgA (cave polyclonaal IgA)
- Alternatief voor quantificeren IgA M-proteine in β-gebied: Hevylite

Dimopoulos et al. Blood 2011
Ruinemans et al. NTVH 2009

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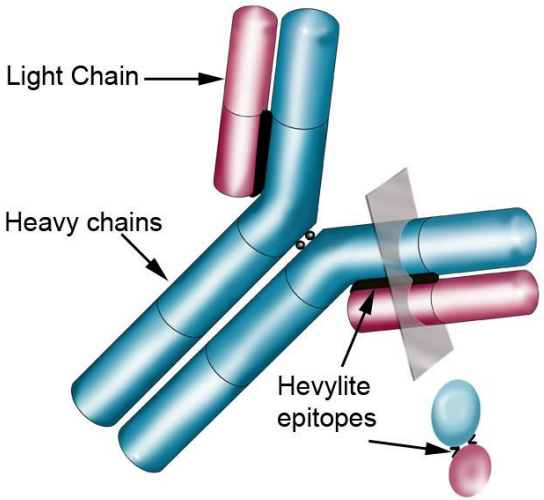
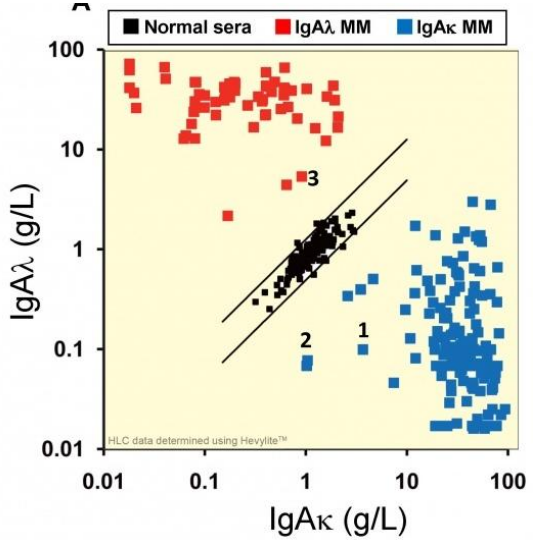
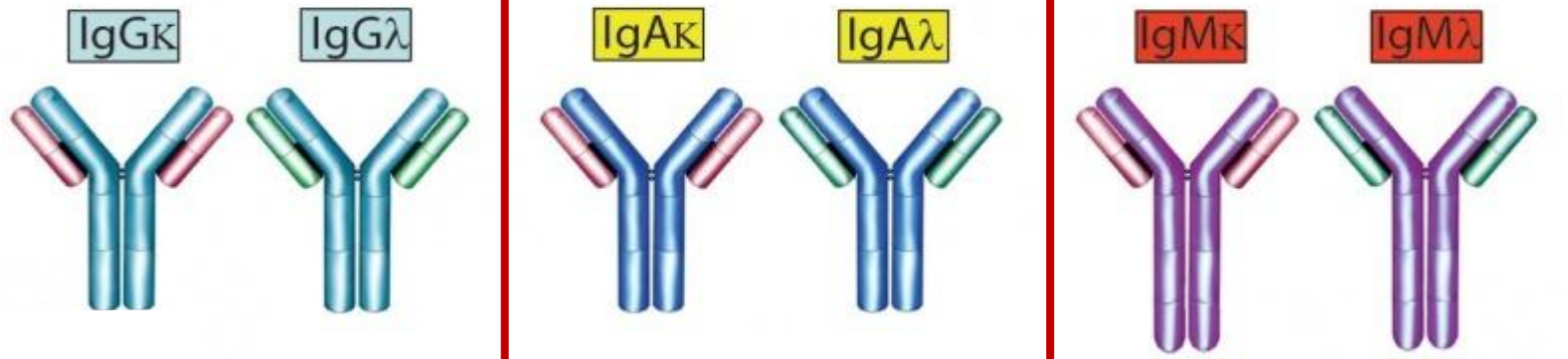
Hevylite reagents







N=109	95% range
IgGκ (g/L)	4.23-12.18
IgGλ (g/L)	2.37-5.91
IgGκ/IgGλ ratio	1.26-3.20

N=191	95% range
IgAκ (g/L)	0.43-2.36
IgAλ (g/L)	0.40-1.73
IgAκ/IgAλ ratio	0.58-2.52

N=118	95% range
IgMκ (g/L)	0.33-1.54
IgMλ (g/L)	0.20-1.10
IgMκ/IgMλ ratio	0.81-2.52

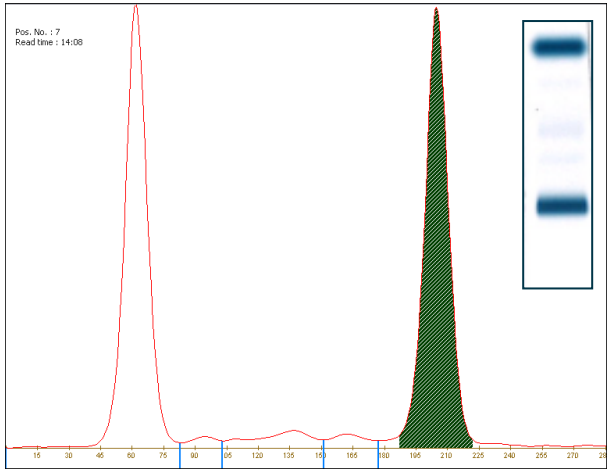
Bradwell et al. Clin Chem 2009
Katzmann et al. Clin Chem 2015

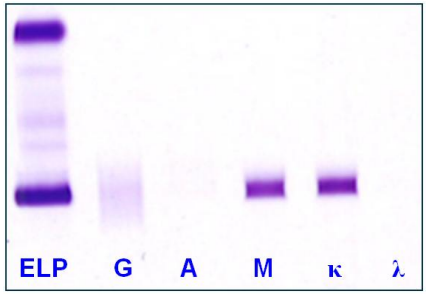
Radboudumc

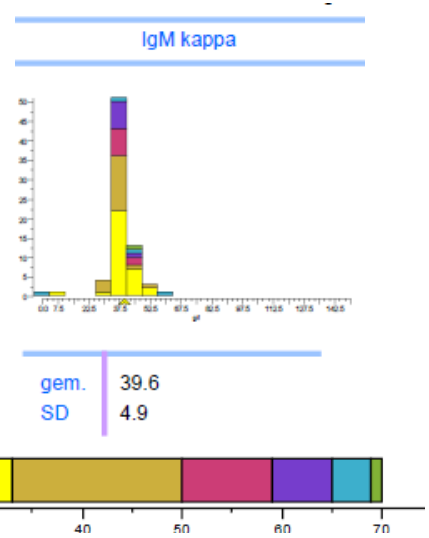
Casus 2015.3C.

IgM kappa M-proteine

- 65 jarige man meldt zich bij huisarts
- Vermoeidheid, verminderde visus, krachtsverlies en tintelingen in handen en voeten
- Lichamelijk onderzoek: vergrootte milt + div. lymfeklieren → Hematoloog



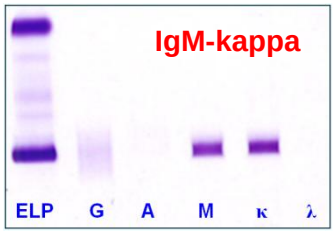


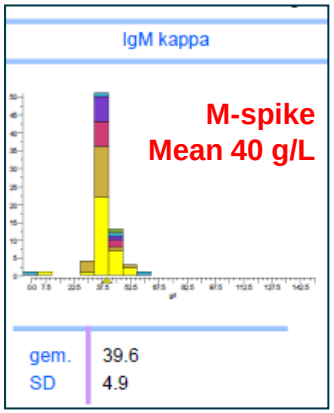


- Diagnose macroglobulinemie (ziekte van Waldenström)
- Klachten doorgaans op basis van:
  - onderdrukt beenmerg (infecties en bloedingen)
  - hyperviscositeit van bloed (verminderde visus, krachtsverlies, perifere neuropathie)
- Behandeling: chemotherapie / rituximab / autologe SCT / plasmaferese

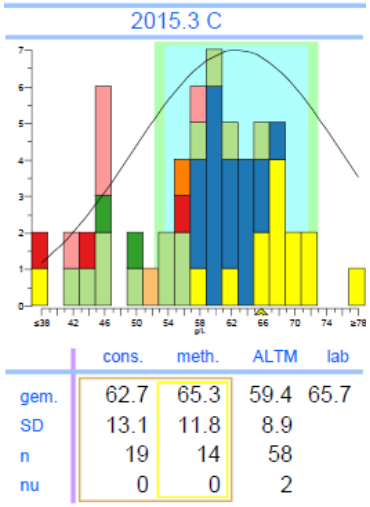
Casus 2015.3C.

Analytische problemen bij hoge concentratie M-proteine



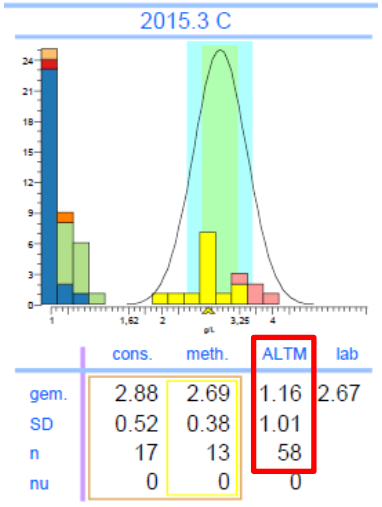


Totaal IgM



Mean 59.4 g/L

Totaal IgG



- **Vaak slechte correlatie M-proteine concentratie: Immunochemisch vs Electrophorese**
- **Hoge concentratie M-proteine kan interfereren met andere analyses:**
  - **Ig-bepalingen, electrolyten, Vrije Lichte Ketens…**

Jelinek and Bachmann. Clin Chemistry 2014
Jacobs et al. Clin Chim Acta 2012
Ruinemans et al. NTVH 2009

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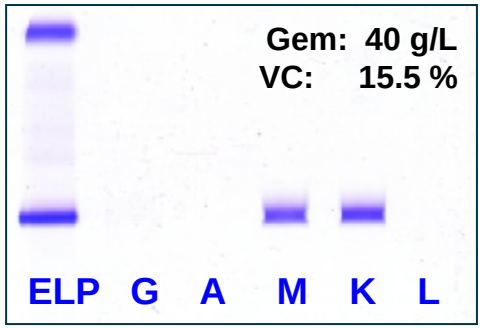
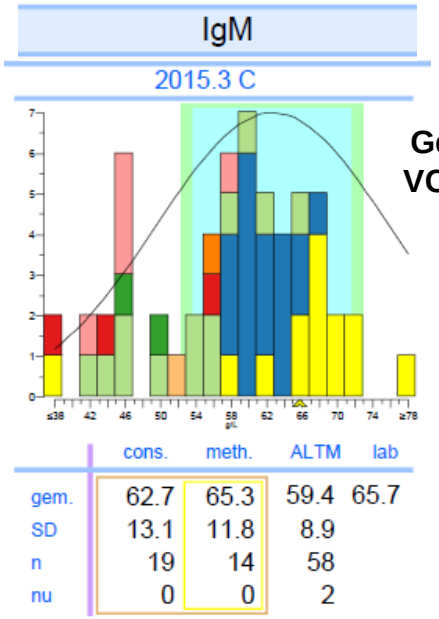
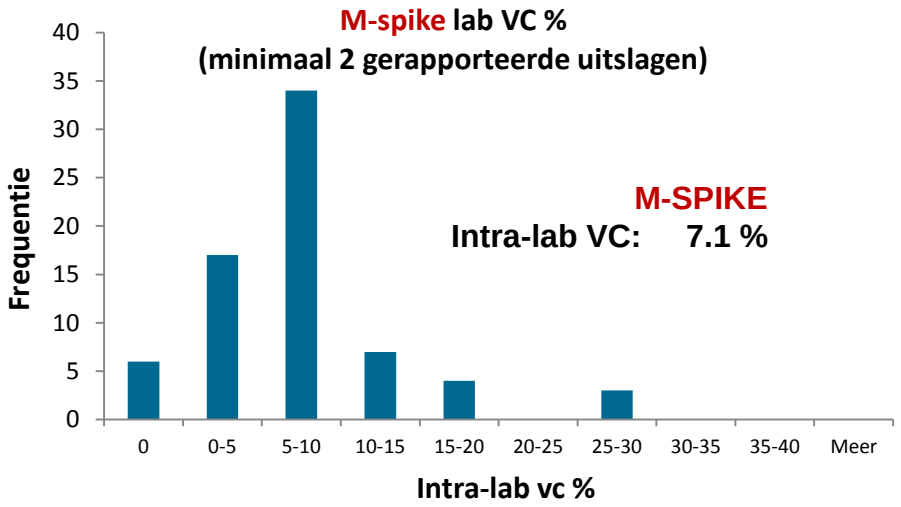
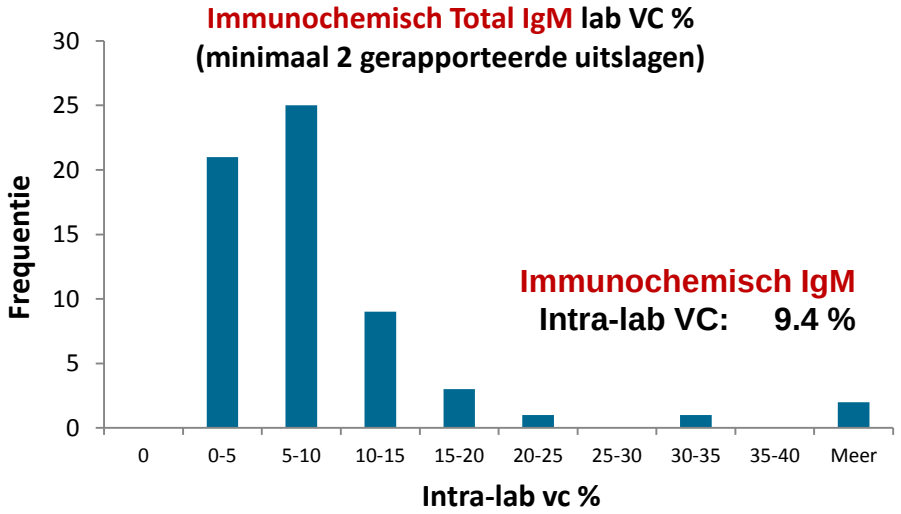
Reproduceerbaarheid van een fors IgM-kappa M-proteine

IgM-kappa: 2013.2A, 2014.4A, 2015.3C

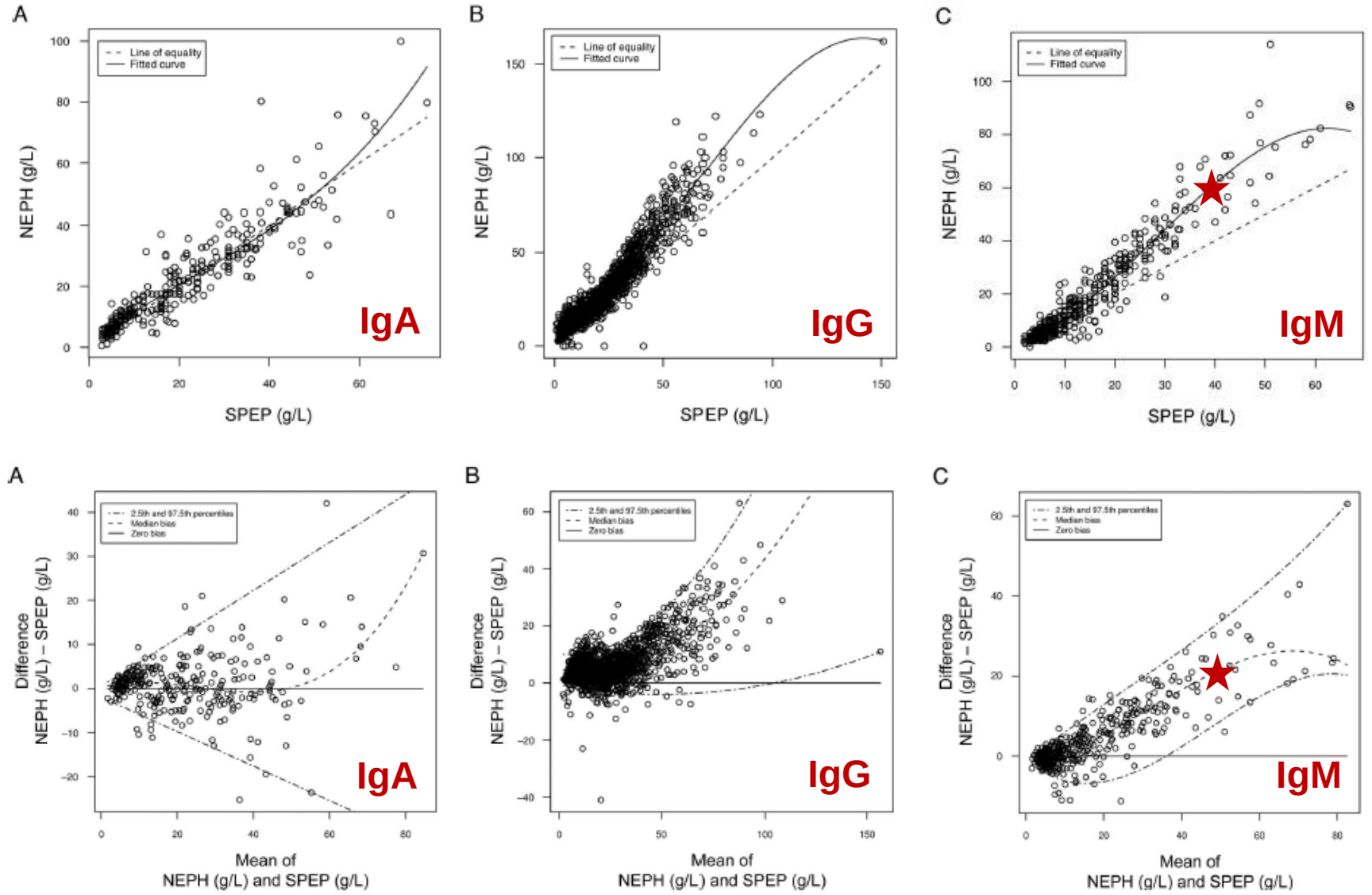








Quantitatieve verschillen M-spike vs Immunochemie



Adapted from Murray et al. Clin Chem 2009

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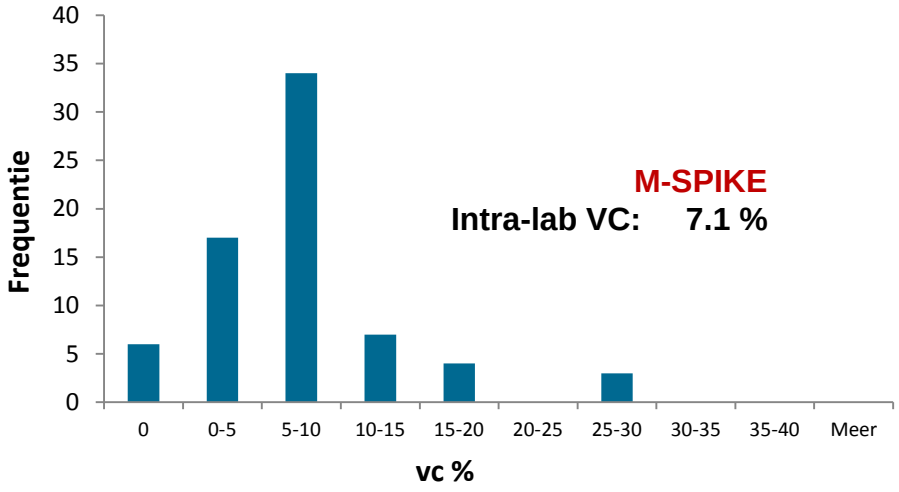
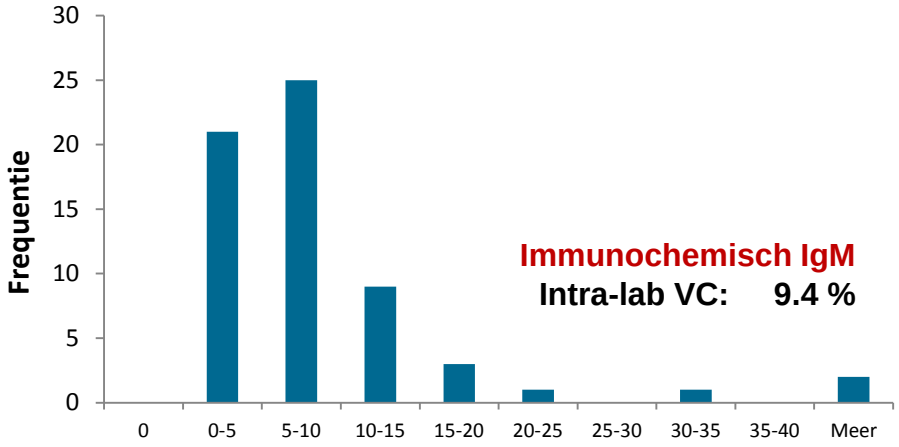
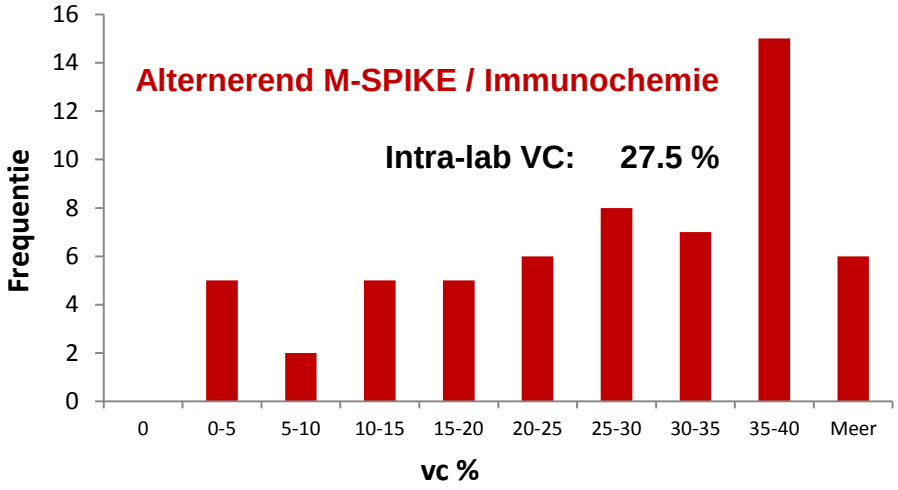
Quantitatieve verschillen M-spike vs Immunochemie

Conclusie:

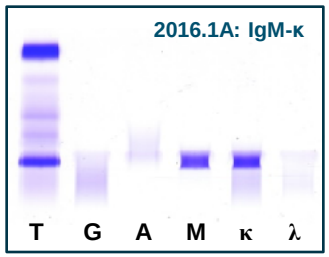
Gebruik niet alternerend M-spike / Immunochemie voor monitoren M-proteine

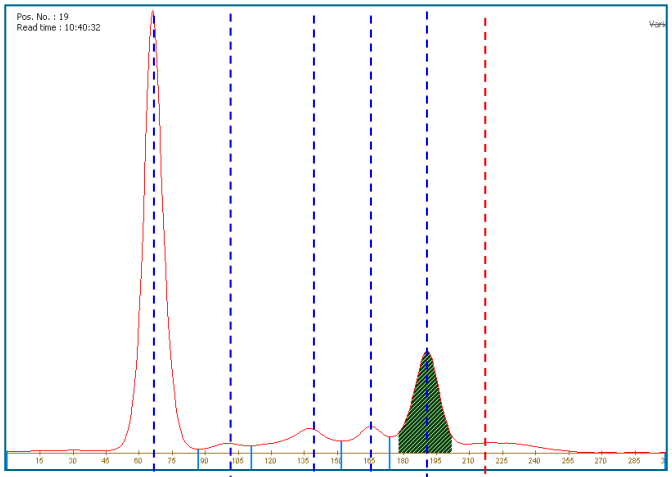






Casus 2016.1A: Vervolgen van een bekend M-proteine



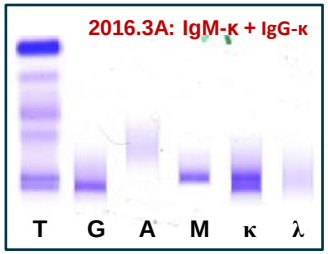


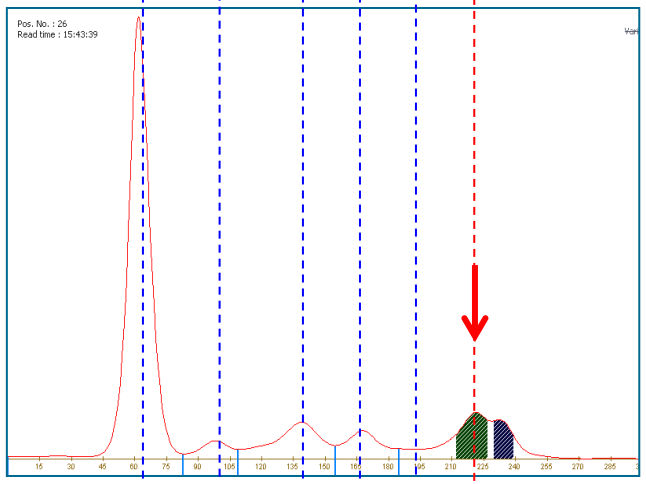
Casus

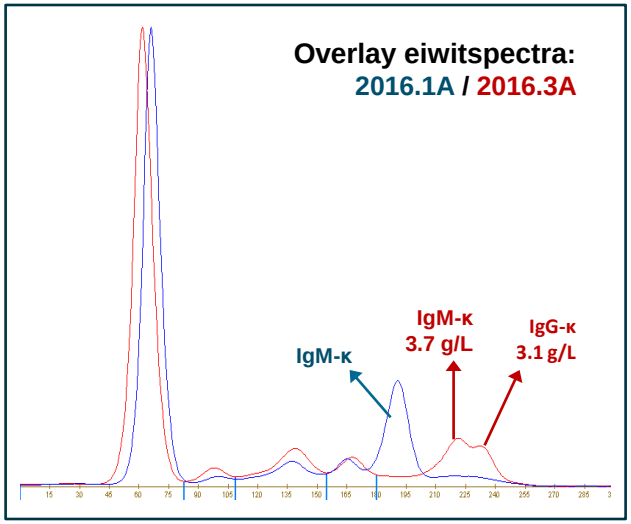
- Man, 65 jaar oud
- Aanhoudende vermoeidheid
- Lymphadenopathie
- Splenomegalie

➢ Diagnose M. Waldenström

- Gecombineerde chemotherapie dexa., rituximab, cyclophos.







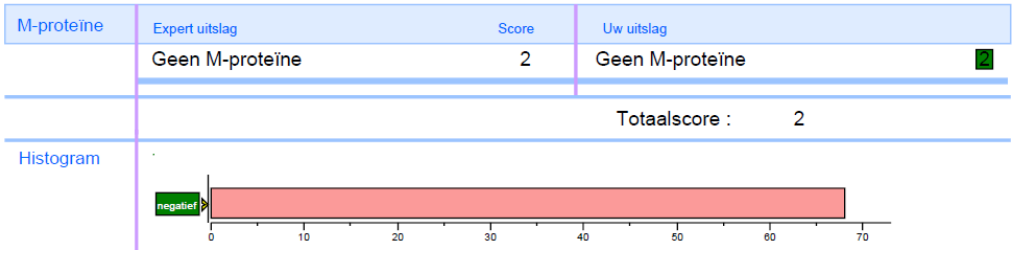
➢ Aan of afwezigheid van oorspronkelijke M-proteine prognostisch belangrijk

➢ Goed archief is belangrijk voor vervolgen van een bekend M-proteine

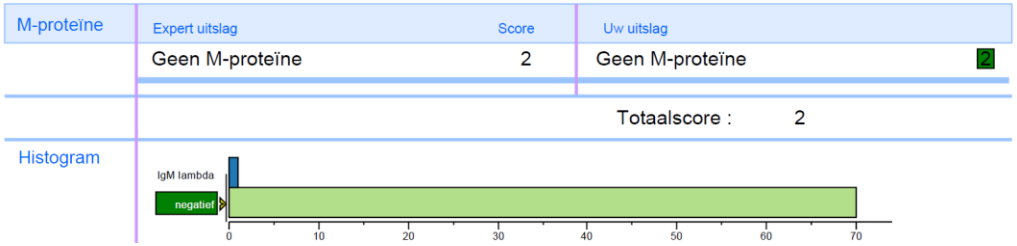
Rapportage bij sera zonder M-proteine (n=7)

➢ **>95 % van de deelnemers rapporteert terecht geen M-proteine**

2016.3B



2015.1B



Hoe duidelijk moet een bandje zijn voordat je rapporteert?

Relevantie van kleine bandjes…?

Laboratory Persistence and Clinical Progression of Small Monoclonal Abnormalities AJCP, 138:609, 2012

David L. Murray, MD, PhD,[1] Justin L. Seningen, MD,[1] Angela Dispenzieri, MD,[1,2] Melissa R. Snyder, PhD,[1] Robert A. Kyle, MD,[1,2] S. Vincent Rajkumar, MD,[2] and Jerry A. Katzmann, PhD[1,2]

- Dysproteinemia Database
- Termed IFE M-proteins
- 439 patients at least one Follow-up
- Median follow-up 3.9 yrs (0.2-13 yrs)
- 3.2% progressed
- About 1% per year

Murray et al AJCP, 138:609, 2012

Type of Clinical Progression in Patients With IFE MGUS

Disease	Sex	Ig Class	Time to Progression, y
Multiple myeloma	M	IgA	1.0
	F	IgG	2.8
	M	IgA	9.9
	M	IgA	2.1
	M	IgG	3.5
	F	IgG	1.7
	F	IgA	1.3
	F	IgA	2.5
Smoldering myeloma	M	IgA	5.1
	M	IgA	4.5
Primary amyloidosis	M	IgG	4.5
Light chain deposition disease	F	IgG	8.9
Extramedullary myeloma	F	IgG	0.4
Lymphoplasmacytic lymphoma	F	IgA	5.6

IFE immunofixation electrophoresis; Ig, immunoglobulin; MGUS, monoclonal gammopathy of undetermined significance.

Samenvatting van dubieuze banden (NTK):

- **84% van patienten persisteerd het M-proteine tijdens follow-up**
- **1% per jaar van de patienten vertoont klinische progressie**
- **8 ‘progressors’ zijn IgA**
- **6 ‘progressors’ zijn IgG**

Murray et al. American J Clin Pathol 2012

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Reproduceerbaarheid (tussen laboratoria)

Zelfde sample zonder M-proteine 3x rondgestuurd (serumpool patiënten met nierfalen)

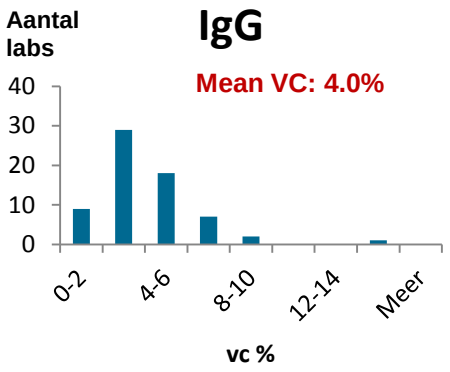
	2011.3A		2012.3A		2013.4B		2015.3A	
Bepaling	Mean	V.C. (%)	Mean	V.C. (%)	Mean	V.C. (%)	Mean	V.C. (%)
IgG (g/L)	9,41	5,9	9,34	7,2	9,61	5,7	9,6	5,6
IgA (g/L)	1,81	4,5	1,80	4,5	1,77	5,6	1,77	4,0
IgM (g/L)	0,73	7,9	0,71	6,7	0,73	8,3	0,71	5,1
Totaal eiwit (g/L)	63,8	3,2	63,9	3,3	64,4	3,1	64,3	3,6
sVLK kappa (mg/L)	79,3	15,6	91,3	15,6	96	12,5	102	30,1
sVLK lambda (mg/L)	77,8	19,5	76,7	13,4	83	8,4	77	16,1
FLC-ratio (kappa/lambda)	1,003	12,6	1,135	12,9	1,175	17,7	1,3	30,5

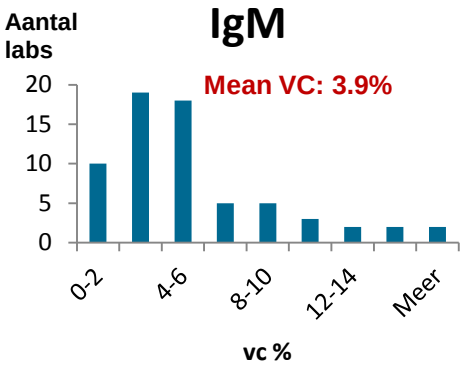
- Ig's stabiel en homogeen
- Relatief hoge VC% bij VLK's

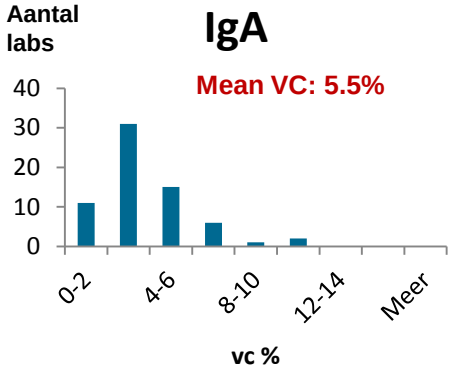
Pattenden et al. Ann Clin Biochem 2007
Jacobs et al. Clin Chem Lab Med 2014

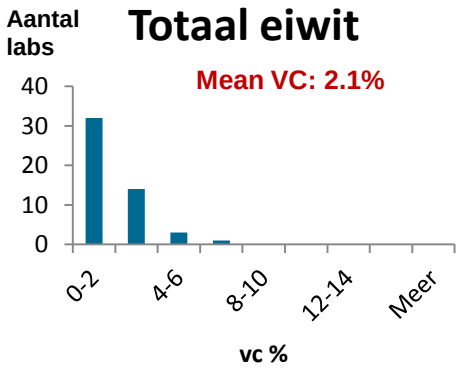
Reproduceerbaarheid (binnen laboratoria)

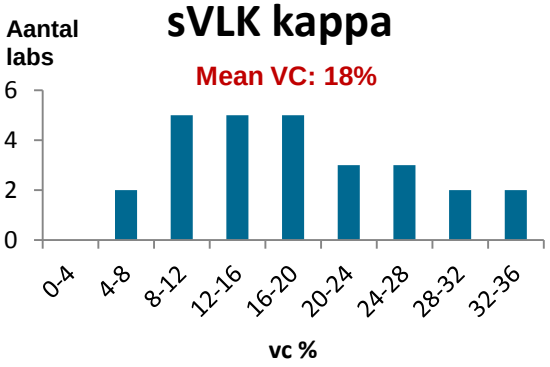
Alleen analyses gedaan indien lab ≥3 resultaten rapporteerde

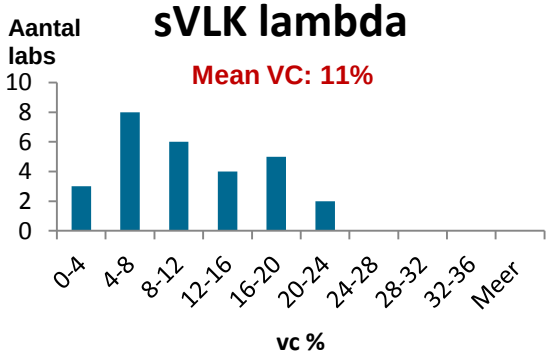


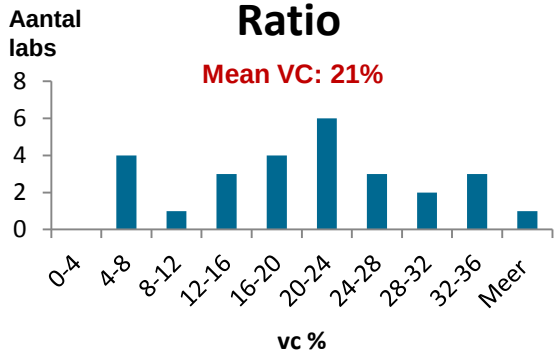






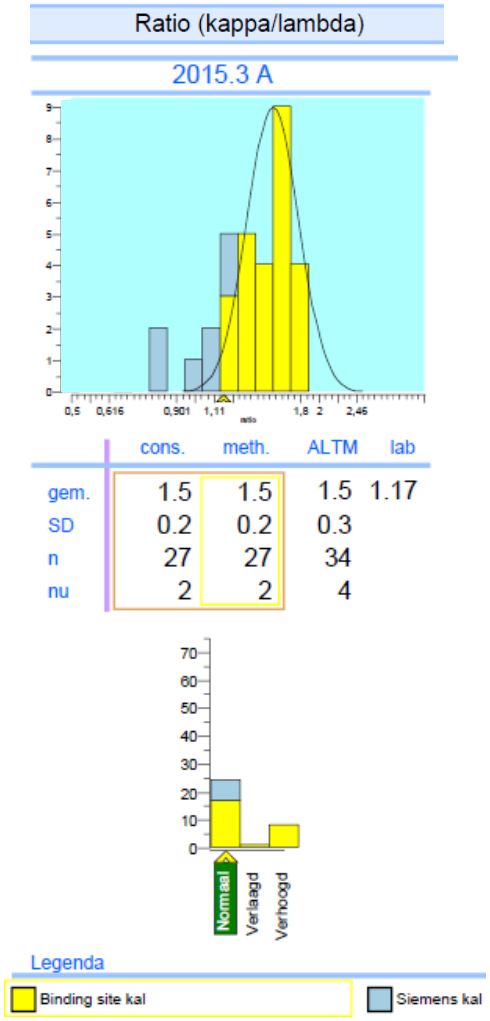






Tate et al. Clin Biochem Rev 2009

VLK verschil tussen Freelite (TBS) en N Latex (Siemens) bij CKD pt

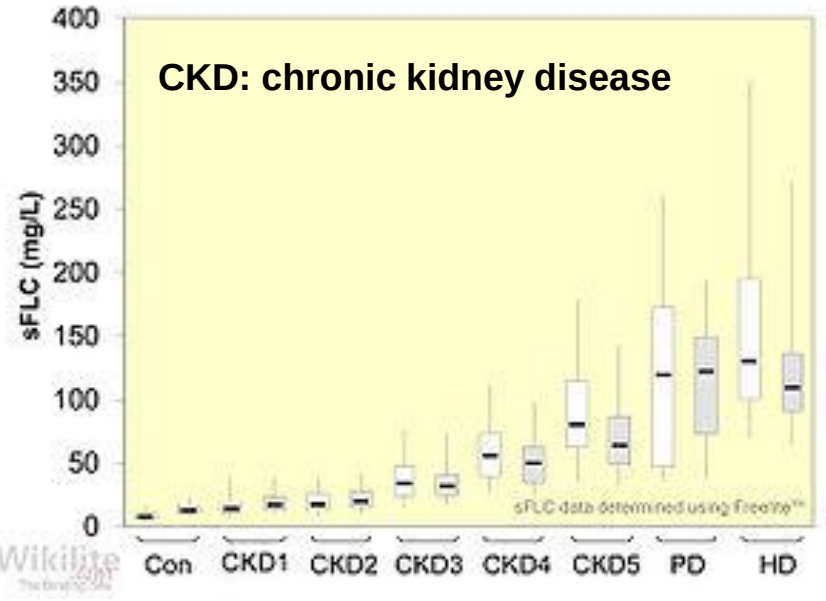


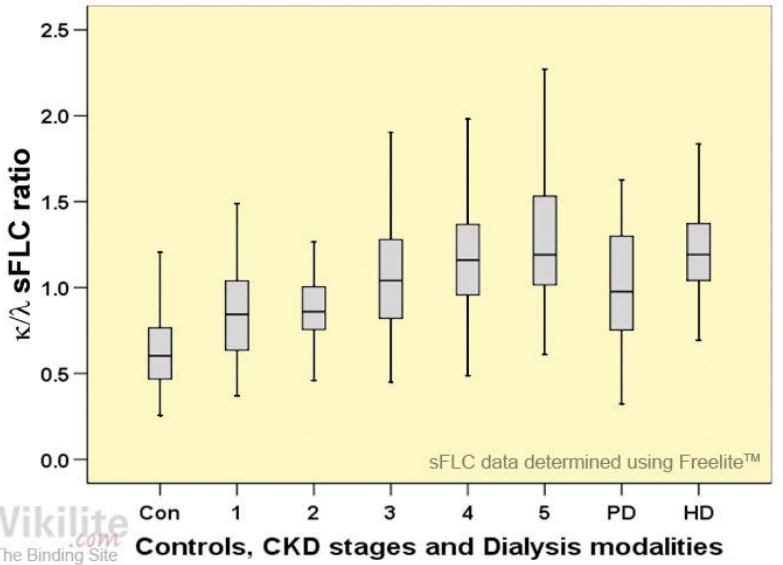
Serum FLC reference ranges

Freelite Reference Range

Normal Adult Serum	Mean Concentration	Median Concentration	95 Percentile Range
Free Kappa	8.36 (mg/L)	7.30 (mg/L)	3.30-19.40 (mg/L)
Free Lambda	13.43 (mg/L)	12.40 (mg/L)	5.71-26.30 (mg/L)
	Mean	**Median**	**Total range**
Kappa/Lambda ratio	0.63	0.60	0.26-1.65

'usual' RR 0.26 – 1.65
Renal RR 0.37 – 3.1





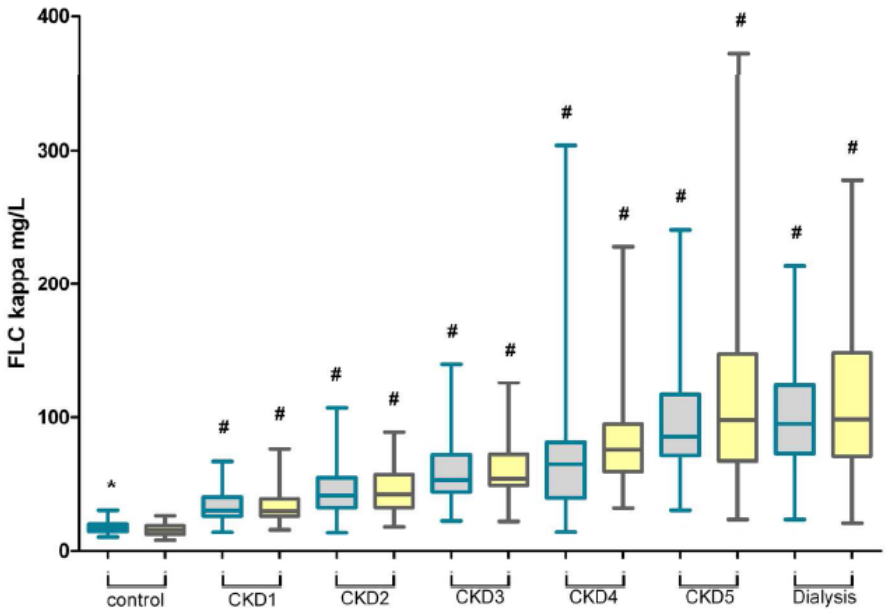
Hutchison et al. Clin J Am Soc Nephrol 2008

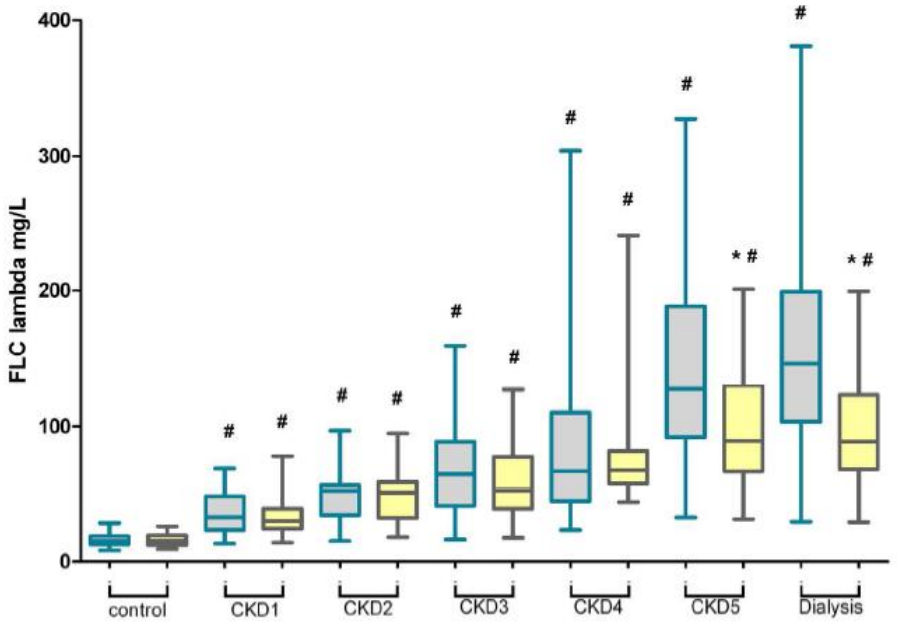
FLC reference ranges

N Latex FLC assay (Siemens)

Freelite FLC assay (The Binding Site)

N=441





Jacobs et al. Clin Chem Lab Med. 2014

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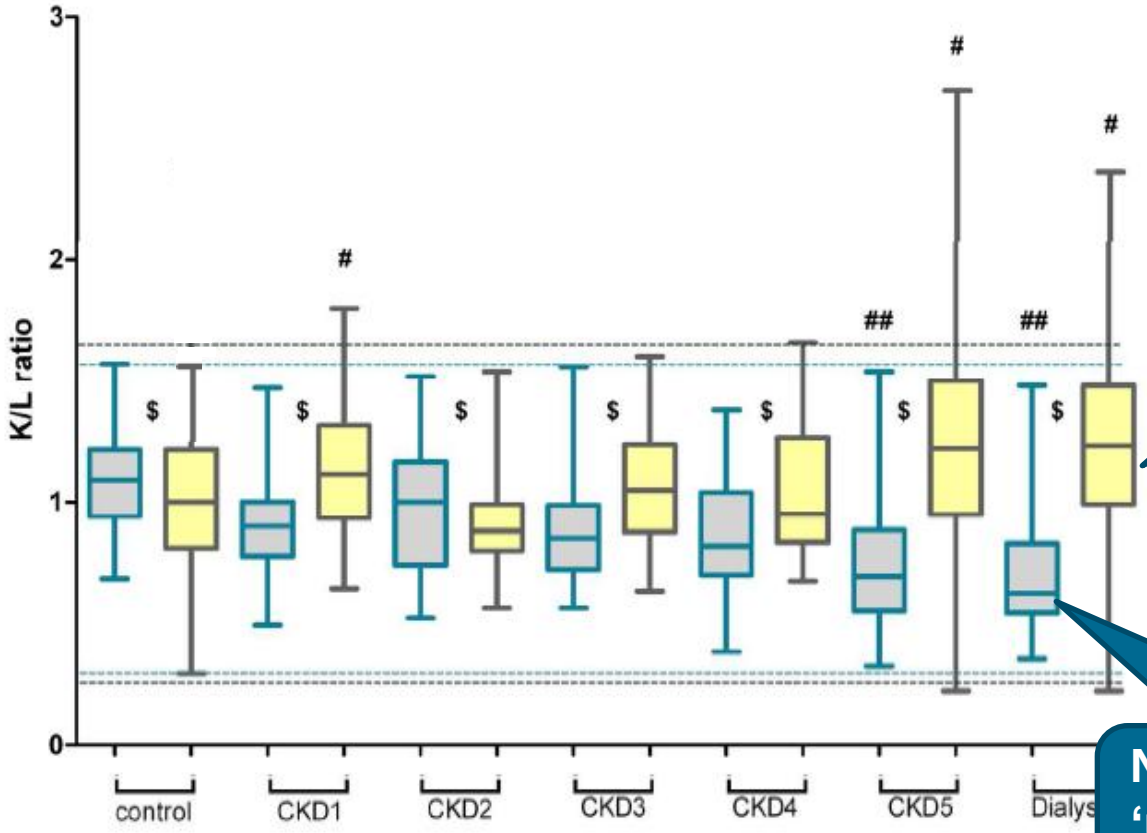
FLC Kappa/Lambda ratios

N Latex FLC assay (Siemens)

Freelite FLC assay (The Binding Site)

N=441



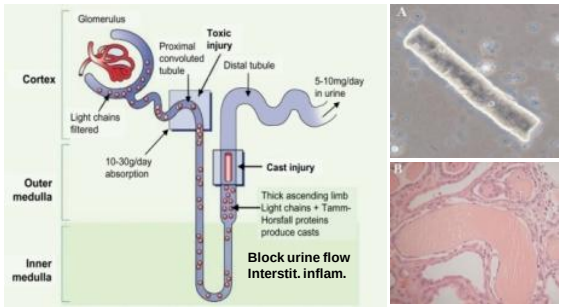
Freelite FLC
'usual' RR 0.26 – 1.65
Renal RR 0.37 – 3.1

N Latex FLC
'usual' RR 0.32 – 1.54
Renal RR 0.32 – 1.54

Jacobs et al. Clin Chem Lab Med. 2014

Multipel myeloom en nierschade

hyperCalcemia, Renal impairment, Anemia, Bone disease (CRAB diagnostic criteria MM)



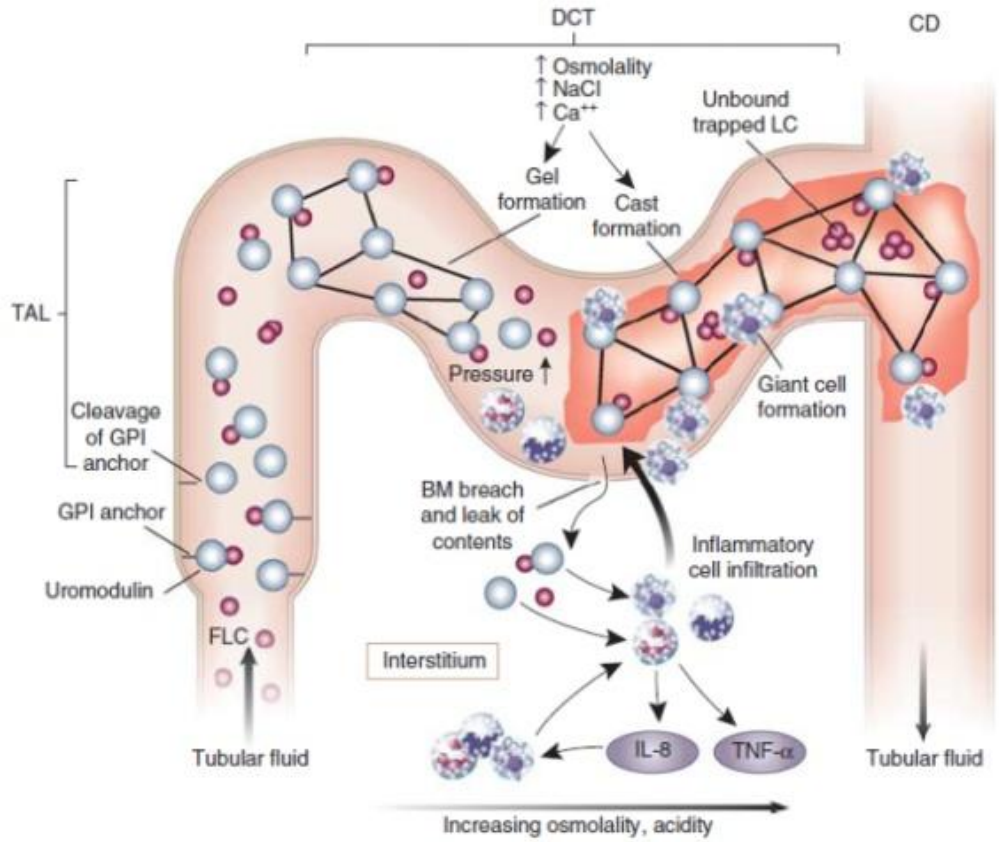
Multiple myeloma at initial presentation

- 18-50% renal impairment (serum creat ↑)
- 12-15% acute renal failure
- 8% become dialysis dependent

Pathology

- Cast nephropathy (myeloma kidney)
- Light chain (AL) amyloidosis
- Light chain deposition disease
- Hypercalcemia
- Nephrotoxic drugs
- Hyperviscosity syndrome
- Monoclonal Ig deposition disease

…



Dimopoulos et al. J Clin Oncol 2010
Basnayake et al. Kidney Int 2011

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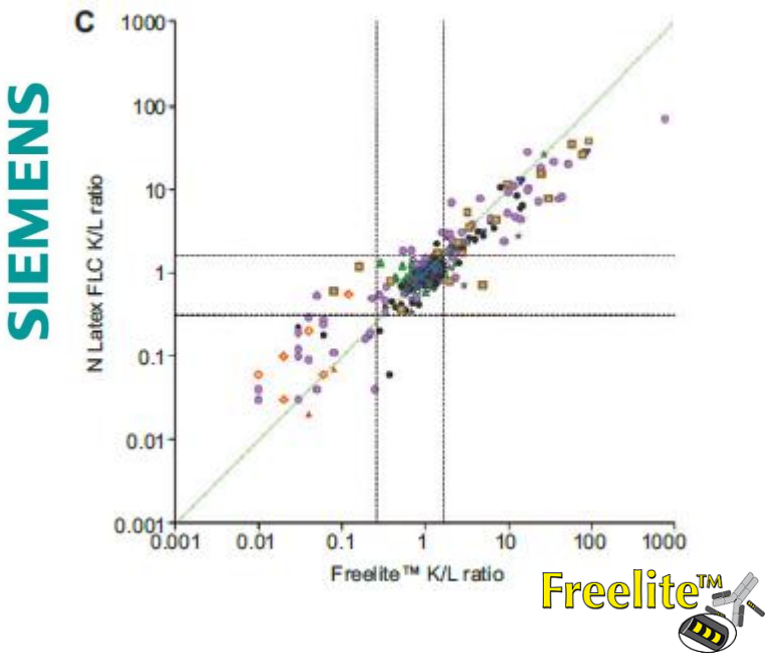
Analytical challenges of monoclonal FLC measurements

- Linearity.
- Interference intact Ig.
- Antigen excess.
- Precision.
- Accuracy.

Hyper variable Fab region

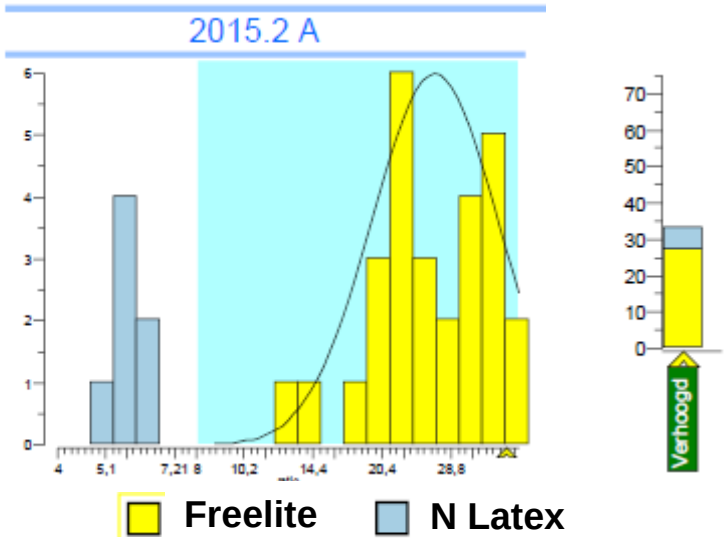
Variation in:
- AA sequence and size
- Charge (pI range 4.5 – 9)
- Glycosylation
- Polymerisation



Tate et al. Clin Biochem Rev 2009
De Kat Angelino et al. Clin Chem 2010
Jacobs et al. Clin Chim Acta 2012

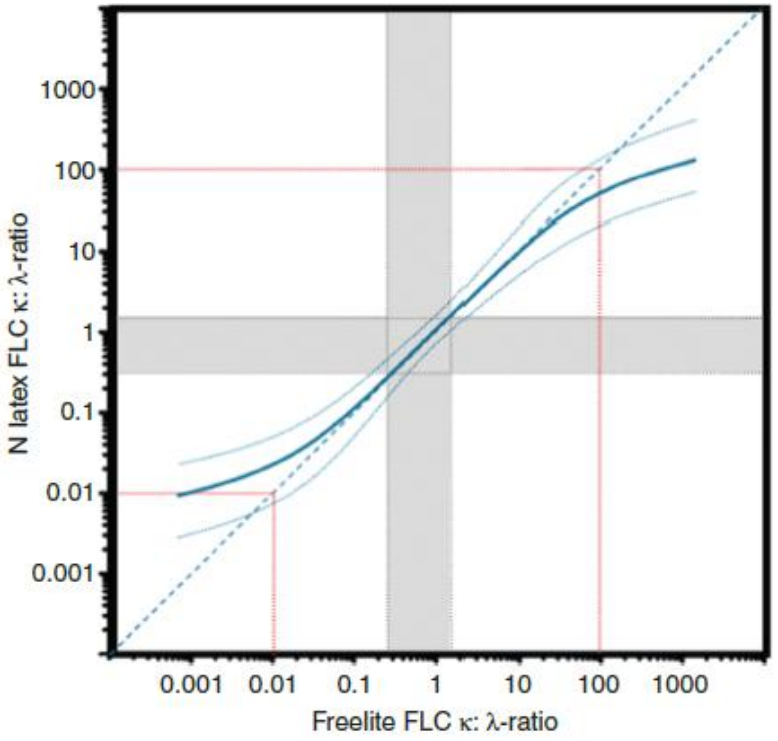
Hoedemakers et al. Clin Chem Lab Med 2011
Jacobs et al. Clin Chem Lab Med 2014
Jacobs et al. Clin Chem Lab Med 2016

The importance of FLC standardisation/harmonisation



Newly Added Criteria To Diagnose MM

Clonal bone marrow plasma cells ≥10% or plasmacytoma plus one of these:	
	2-y Incidence of Organ Damage, %
Clonal marrow plasma cells ≥60%	95
Ratio of involved to uninvolved serum free light chain ≥100	80[a]
≥2 focal bone lesions ≥5 mm on MRI	70-80



Rajkumar et al. Lancet Oncology 2014
Jacobs, Tate & Merlini. Clin Chem Lab Med 2016

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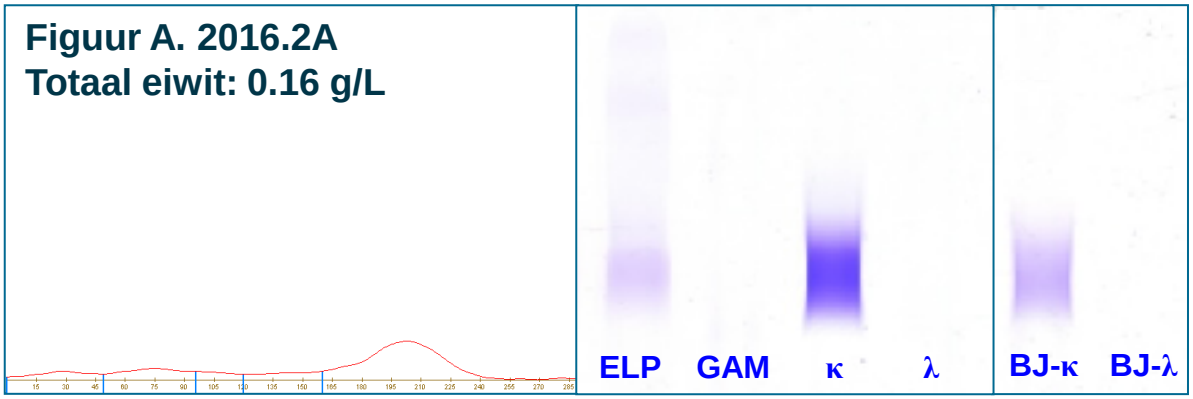
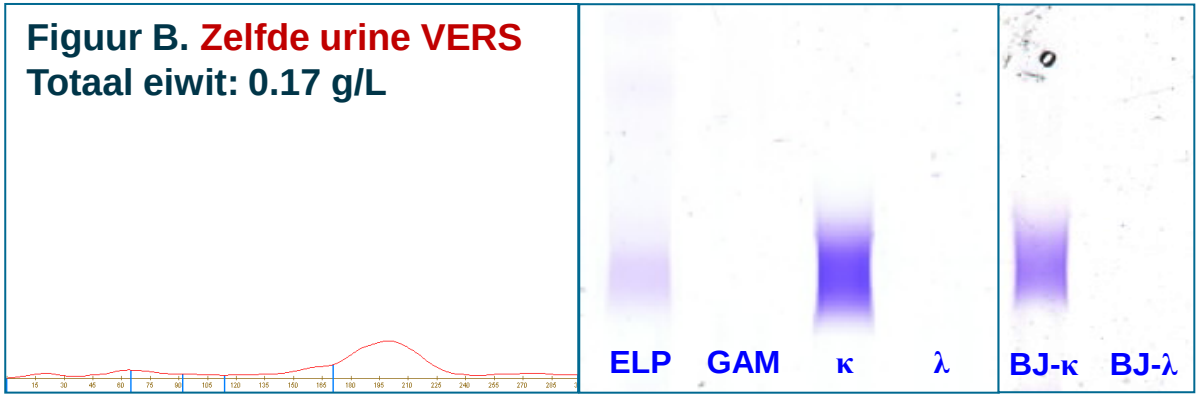
Casus 2016.2A: Bence Jones eiwitten.

Figuur A. 2016.2A
Totaal eiwit: 0.16 g/L



- **Circa 65% eiwitten = BJE**
- **BJ-kappa is circa 0.1 g/L**
- **Niet gerapporteerd door 17 van de 60 deelnemers**

Figuur B. Zelfde urine VERS
Totaal eiwit: 0.17 g/L



- **Invriezen geen invloed op <u>deze</u> BJ analyse**

SERUM
VLK-κ = 1620 mg/L
VLK-λ = 2.8 mg/L
VLK-ratio = 579

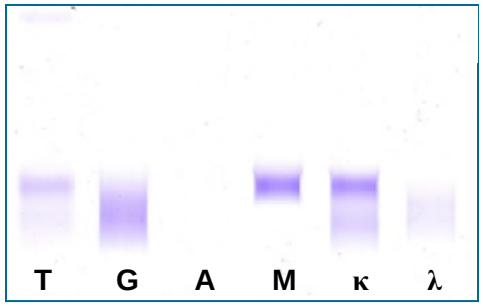
- **BJE kunnen behoorlijk polymeriseren, ook in direct geanalyseerde urine.**
- **De elektroforese data kunnen daardoor ogenschijnlijk polyklonaal profiel geven.**
- **Echter de abnormale verhouding tussen VLK-kappa en VLK-lambda is dan suggestief voor klonale karakter.**
- **Kwantificeren middels totaal eiwit in urine en spike in elektroforese.**

Casus 2016_4A, cryoglobuline bepaling

Casus

- Man, 63 jaar oud. Bekend met SLE.
- 1 a 2x per maand: gedurende 1 week licht verhoogd temp, moeheid, pijn in gewrichten
- Sinds kort daarbij ook kleine, licht verheven, rode vlekjes op de benen (purpura)

Bevindingen:



- 34/46 deelnemers rapporteerden terecht pos.
- Meerderheid type 2, enkele labs type 1
- Forse spreiding in kwantificering (0,2-1.273 mg/L)

Type 2: Monoclonaal IgM-kappa met polyclonaal IgG

Achtergrond informatie bij deze patient:

- Betreft SLE patient met secundaire cryoglobulinemie met glomerulonefritis
- Sterk verlaagd C4 en normaal C3 tijdens cryo-relapse
- CRP verhoogd en normaliseert na behandeling

Vermeesch et al. Analytische aspecten cryo-bepaling. Clin Chem 2008.

Cryoglobuline

'Cryogloblinaemia: systemic inflammatory syndrome that generally involves small-to-medium vessel vasculitis due to CG-containing immune complexes'

Immuunglobulines die precipiteren bij koude

Komen vaak voor bij:

- Lymfoproliferatieve aandoeningen (Waldenström, MM, NHL)
- Systeemziekten (RA, SLE, syst sclerodermie, M. Sjögren)
- Leverziekten (Hepatitis B, C, AIH, cirrhose)
- Chronische infecties (HIV, ziekte v Lyme)
- Zonder aantoonbare onderliggende oorzaak

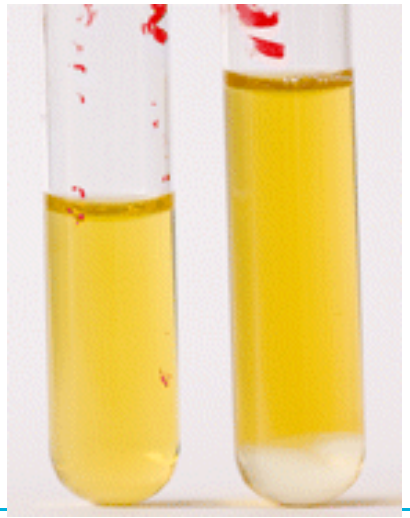
Kwantificering

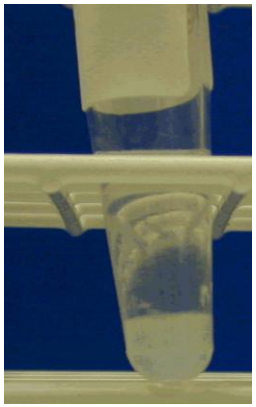
Typering

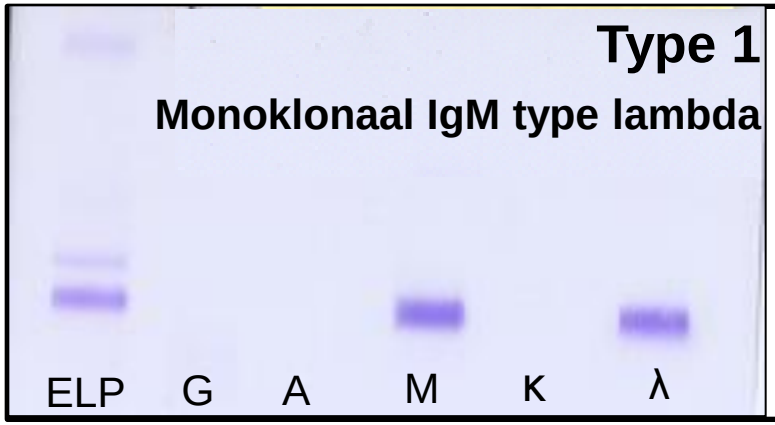
Eenmalig bij verhoogd cryoglobuline

Indeling volgens Brouet:

Type 1: monoklonaal Ig
Type 2: monoklonaal Ig met polyklonaal Ig
Type 3: polyclonaal Ig







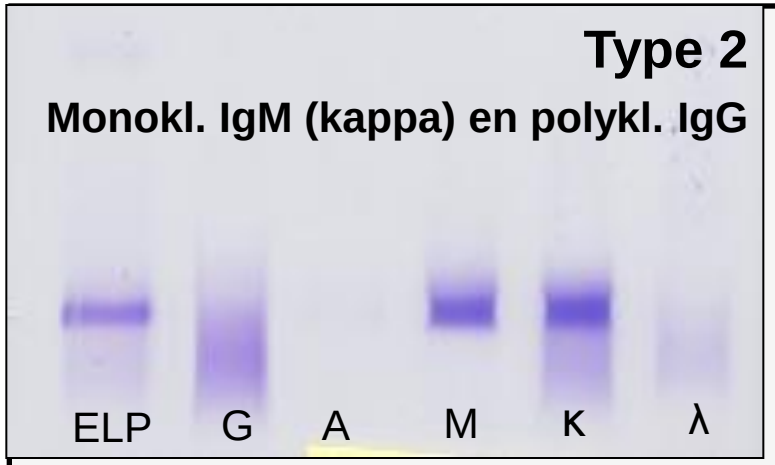
Type 1

Monoklonaal IgM type lambda

Oorzaak:
Immuno/lymfoproliferatieve aandoeningen

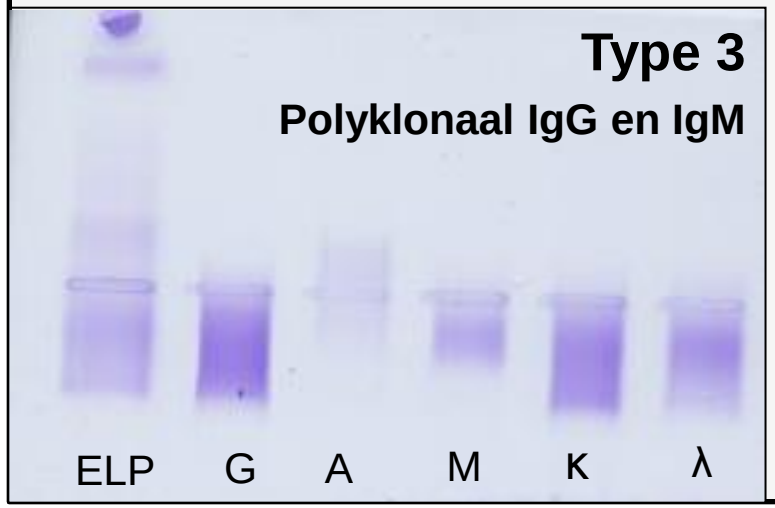
M. Waldenström, Multiple Myeloma, CLL,…

Symptomen m.n. door obstructie van perifere bloedvaten door hyperviscositeit vanwege het monoclonaal Ig + cryoactiviteit.



Type 2

Monokl. IgM (kappa) en polykl. IgG



Type 3

Polyklonaal IgG en IgM

Oorzaak:
Chronische B-cel stimulatie

- **Chronische infecties (HCV), met name type 2**
- **Soms lymfoproliferatieve aandoening (type 2)**
- **Autoimmuniteit (Sjögren, SLE, RA etc), met name type 3**

Gemengde CG slaan intravasculair neer bij koude en induceren een vooral immuuncomplex-gemediëerde inflammatie (leucoclastische vasculitis) en complementactivatie in de betroffen organen:

- **palpabele purpura**
- **arthralgieën**
- **Myalgia**

‘Meltzer’s triad’

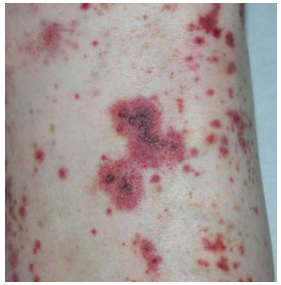
- **Vaak RF positief**
- **Soms mengbeelden…**

Clinical features

Up to date

Contrasting features of types of cryoglobulinemia

	Type I	Type II	Type III
Symptoms and signs			
Purpura	+	+++	+++
Gangrene/acrocyanosis	+++	+ to ++	±
Athralgias >> arthritis	+	++	+++
Renal	+	++	+
Neurologic	+	++	++
Liver	±	++	+++



Palpable purpura

'Clinical features, epidemiology and prognosis of type I CG largely reflect the underlying lymphoproliferative disorder such as M. Waldenstrom or MM'

Type II and III (so called mixed CG)

- **Cutaneous: in nearly all pts; palpable purpura, erythematous macules, hemorrhagic crusts, ulcers.**
- **Musculosceletal: Artralgia and myalgia common, artritis and myositis are uncommon.**
- **Neuopathy: peripheral neuropathy is common**
- **Pulmonary: subclinical pulmonary manifestations (dyspnea, cough, pleurisy)**
- **Renal: membranoproliferative glomerulonephritis, isolated proteinuria or hematuria**
  - **often chronic kidney disease within 3 to 5 years**

'Exacerbation often as a result of cold exposure'

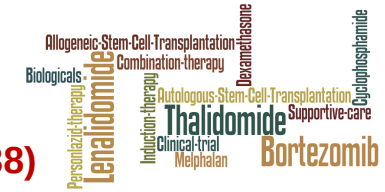
'Treatment of cryoglobulinemia mainly depends on underlying disorder'

Ramos-Casals et al. Cryoglobulinaemias. Lancet 2012.

Radboudumc

Nieuwe ontwikkelingen binnen M-proteine diagnostiek

- **Nieuwe therapieën invloed op M-proteine diagnostiek**



Daratumumab (anti-CD38)

- **Aanpassingen rond M-proteine diagnostiek (sFLC) in richtlijnen**

Table 1. Summary of the main applications of the free light chain assay in plasma cell dyscrasias.

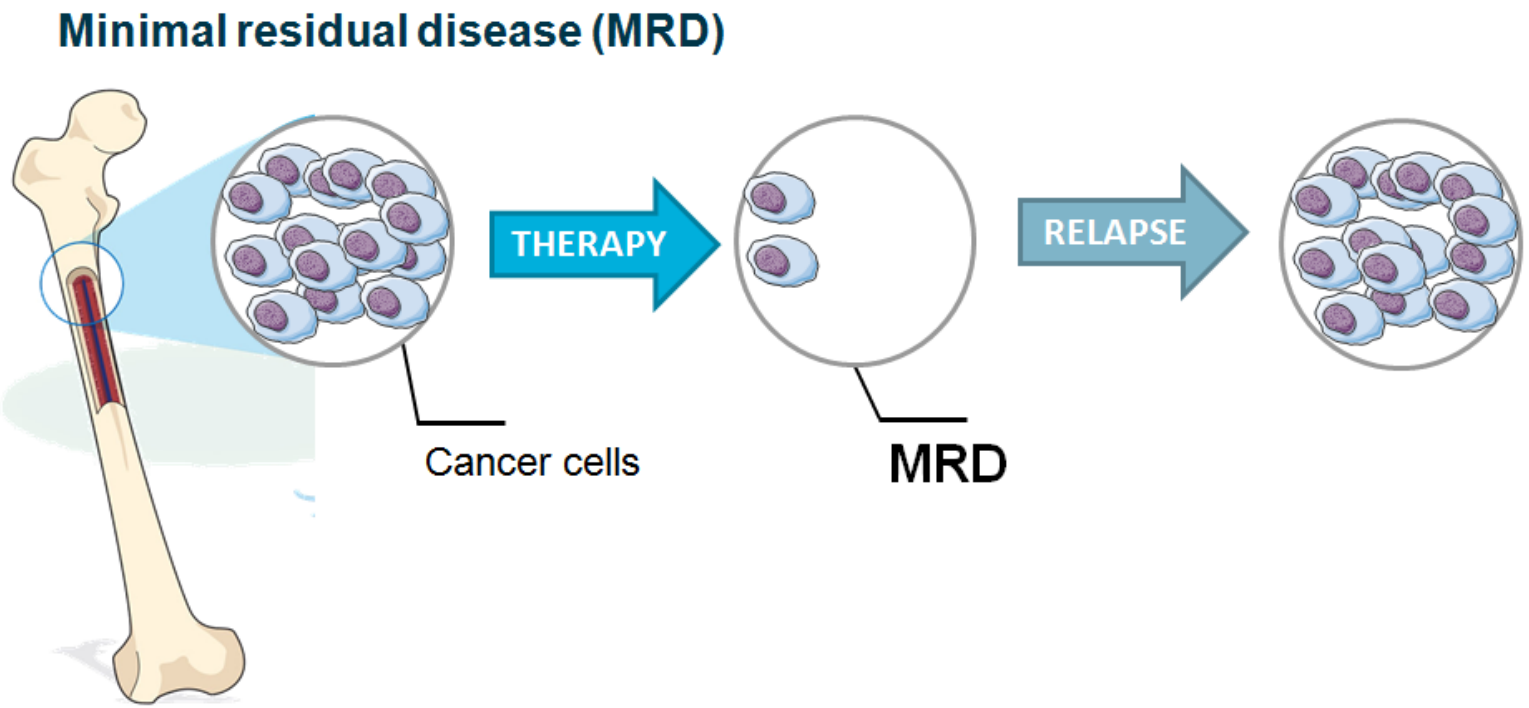
Setting	Recommendations	Notes
Screening	Serum tests s-PE, s-IFE and rFLC, are indicated and sufficient to screen for PCDs other than AL amyloidosis which requires all the serum tests and s-IFE	Once a diagnosis of PCD is made urine studies are necessary in all conditions
Risk stratification	FLC needed for risk stratification in MGUS, SMM, AL amyloidosis and solitary plasmacytoma in association with other parameters depending on the condition	The 2011 IMWG specific guidelines on risk stratification in MM state that although the rFLC for prognostication in MM patients may be useful under some circumstances, the general applicability is unknown
Monitoring	Indicated in AL amyloidosis, oligosecretory MM and MM in advanced stage	FLC is essential in the management of AL amyloidosis. The IMWG experts recommend the use of the FLC assay (as alternative to u-IFE) in MM patients with advanced stage of the disease to screen for 'light chain escape'
Response assessment	FLC necessary for the definition of sCR in MM. Required in AL amyloidosis and oligosecretory MM	In AL amyloidosis the hematologic response is assessed solely on the basis of the FLC concentration

- **Nieuwe sFLC assays (naast Freelite en N Latex nu ook Seralite)**

Lokhorst et al. NEJM 2015
Rajkumar et al. Lancet Oncology 2014

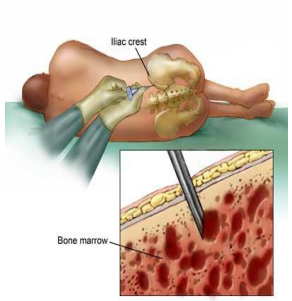
Vd Donk et al. Clin Chem Lab Med 2016
Campbell Clin Chem Lab Med 2017

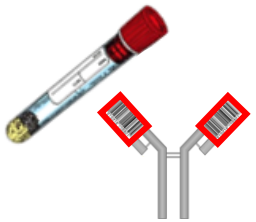
Improved treatment regimes require increased assay sensitivity

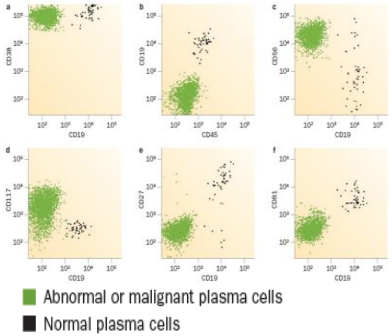


IMWG guideline: Ludwig et al. Leukemia 2013

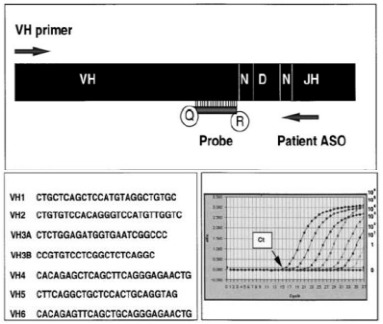
MRD R&D: bone marrow versus blood





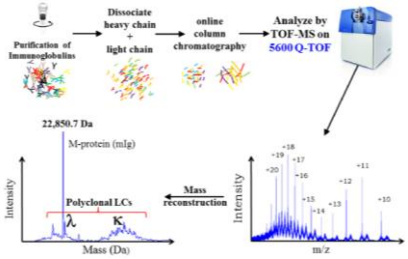


Multicolour flow cytometry



ASO qPCR

Next Generation Sequencing



Mass Spectrometry

Landren et al. Am J Hematol 2014 (flow cytometry)
Puig et al. Leukemia 2014 (ASO q-PCR)
Martinez-Lopez et al. Blood 2014 (next generation sequencing)
Mailankody et al. Nat Reviews 2015 (review on all three methods)

Mills et al. Methods 2015
Van Duijn et al. Anal Chem 2016

Tevens een interessant sample geschikt voor de M-proteine rondzending?

Zeer welkom!

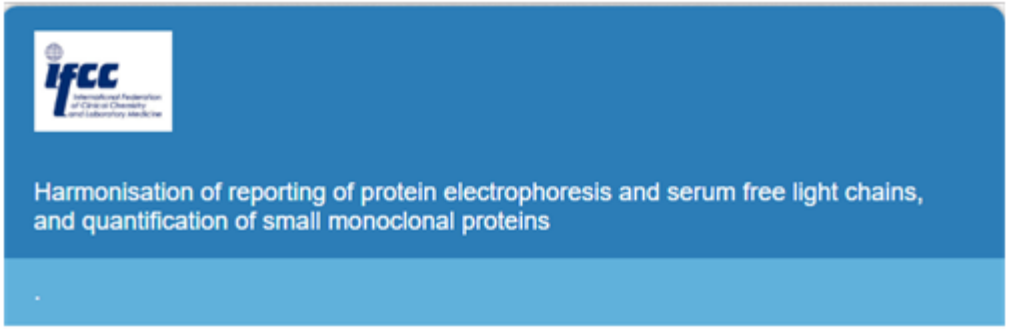
- **100 ml serum nodig per monster (!)**
- **Echter, samples kunnen ook gespiked worden in normaal serum. In dat geval minder serum nodig.**
- **Graag contact:**

H.Jacobs@Radboudumc.nl

IFCC enquete over M-proteine diagnostiek

- **Doel: harmoniseren M-proteine diagnostiek (analyse + rapporteren)**
- **Zeer goed internationaal initiatief**
- **Invullen duurt circa 20 minuten**
- **Deadline 30 april 2017**
- **Direct via IFCC website:**

www.surveymonkey.com/r/IFCC-WG-ICQA



Met dank aan:

Gertrude van der Wiel

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Renate van der Molen

Ondersteuning SKML