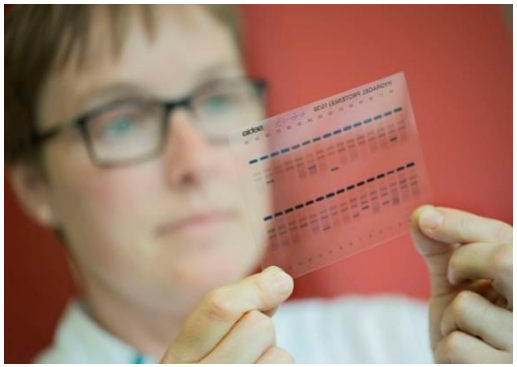
Rondzending M-proteïne diagnostiek

SKML nabespreking, sectie HIM



13 Februari 2020
De Reehorst, Ede

J.F.M. (Hans) Jacobs, Ph.D. M.D.
Radboud University Medical Center
Department of Laboratory Medicine
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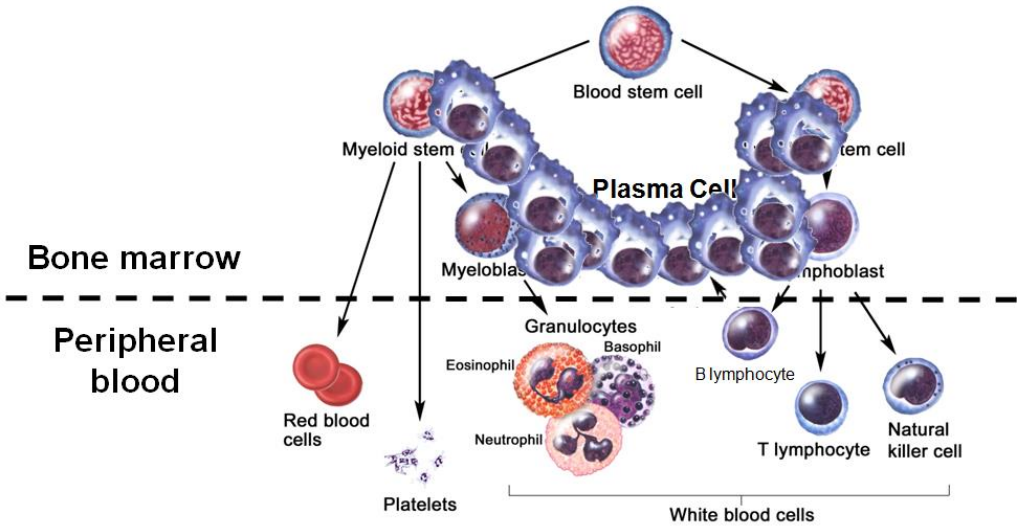
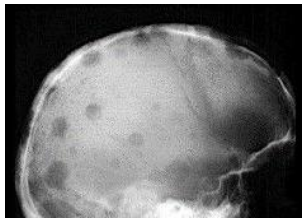
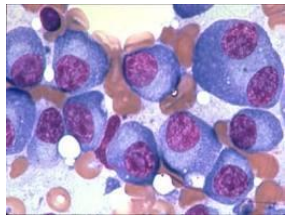
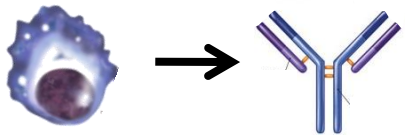
Monoclonal gammopathy; multiple myeloma

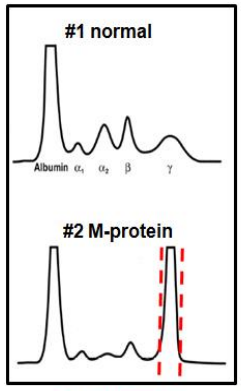




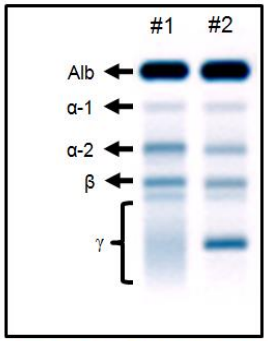




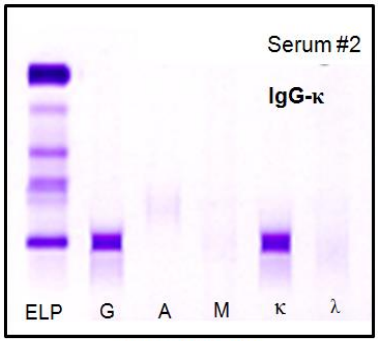
Gel electrophoresis



Densitometry

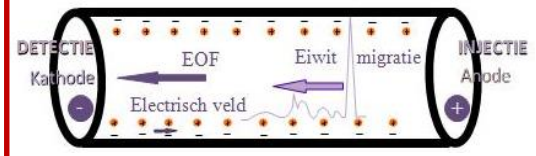


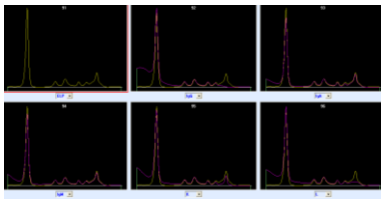
Serum protein electrophoresis



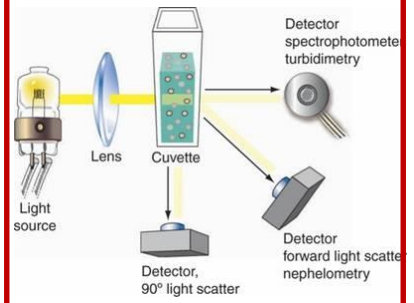
Immunofixation

Capillary electrophoresis



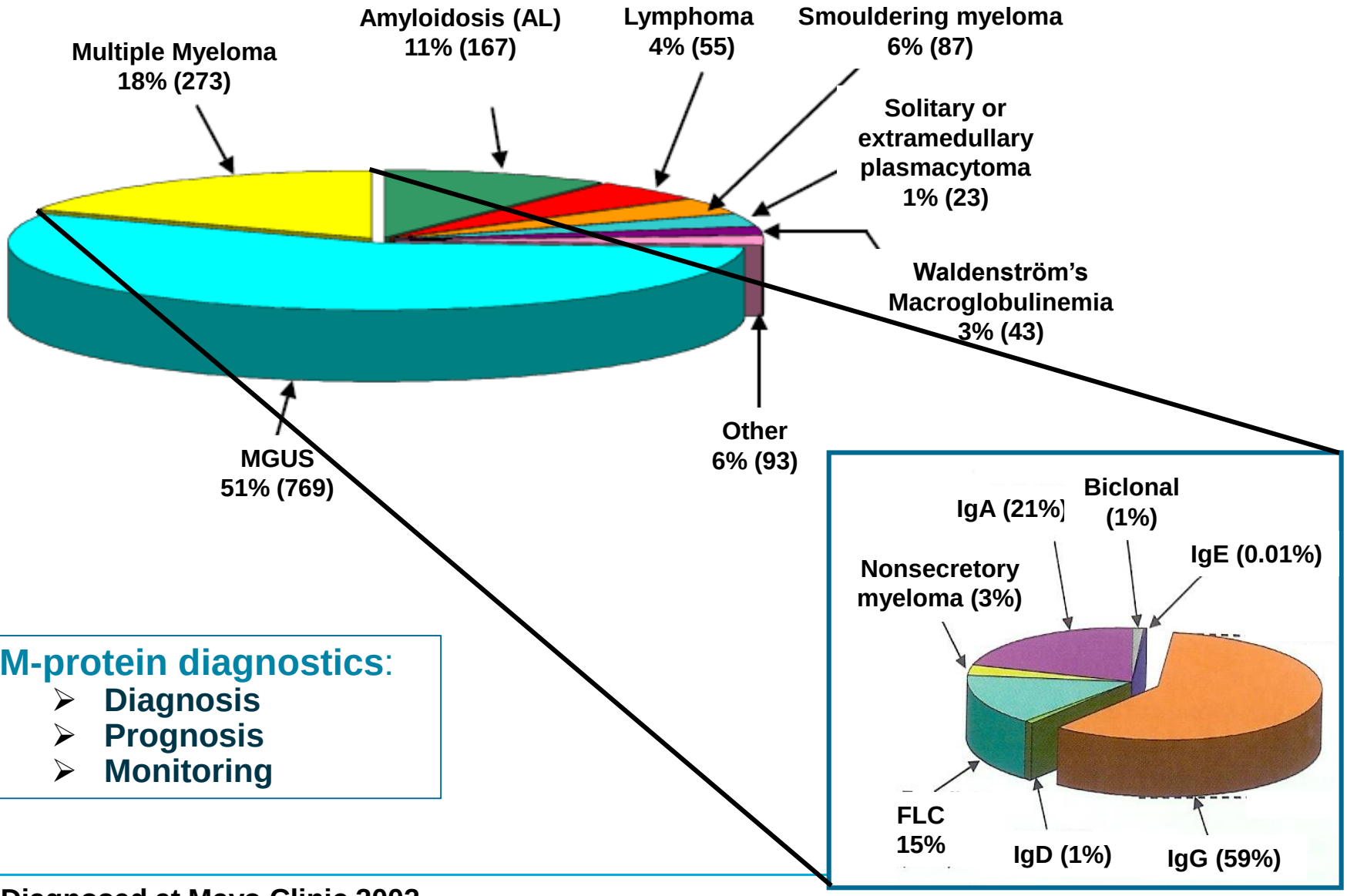


Immunochemistry



Nephelometry / Turbidimetry

Monoclonal gammopathies



M-protein diagnostics:

- Diagnosis
- Prognosis
- Monitoring

Diagnosed at Mayo Clinic 2002
Dimopoulos et al. Blood 2011

M-proteine rondzendingen

Periode 2017.1 t/m 2019.4

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

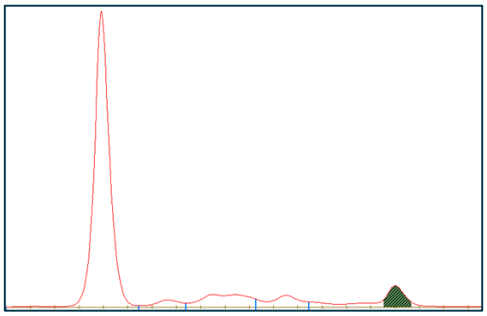
Inventarisatie:

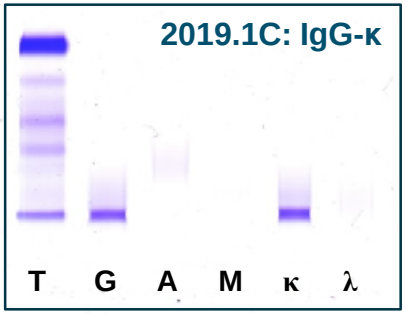
- Typering M proteine
- Kwantificering M proteine
- Kwantificering totaal eiwit, Ig's
- Soms kwantificering Vrije Lichte Ketens (VLK)
- Soms urine of cryobepaling
- Soms additionele vraag

Wat is er rondgestuurd in deze periode:

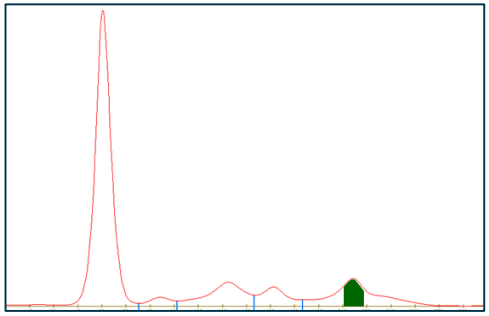
- IgG M-protein 8x
- IgM M-protein 7x
- IgA M-protein 5x
- IgD M-protein 1x
- VLK-K 2x
- VLK-L 1x
- Oligiklonaal 1x
- Geen MPR 9x
- Cryo 1x

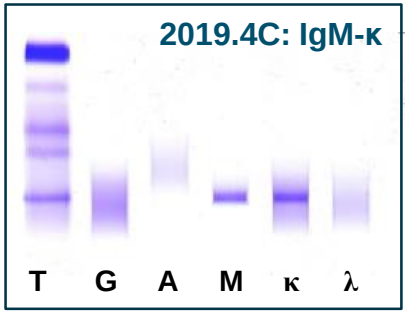
Small M-proteins larger interlab-VC: M-protein dependent…



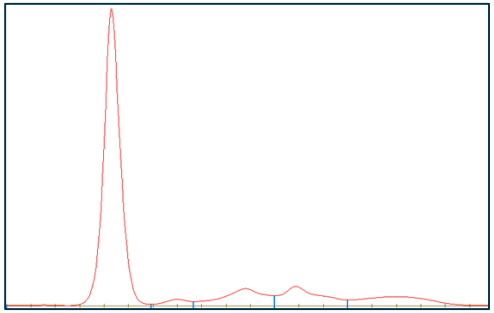


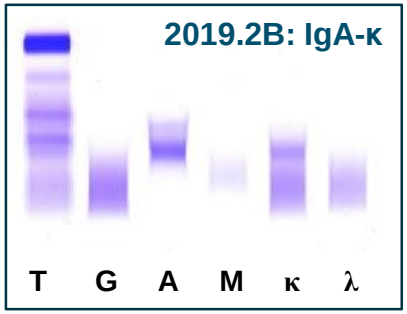
IgG-kappa: 99% goed
Mean conc: 3.3 g/L
Interlab-CV: 19 %





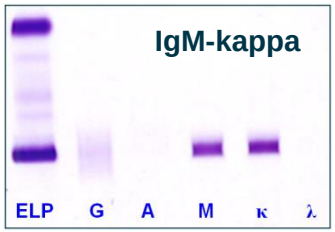
IgG-kappa: 97% goed
Mean conc: 4.2 g/L
Interlab-CV: 36 %

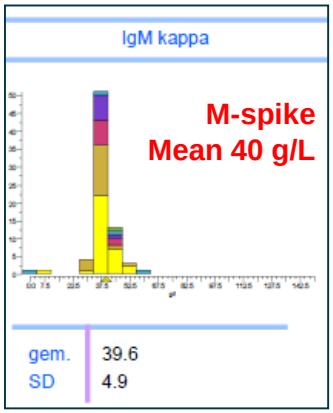


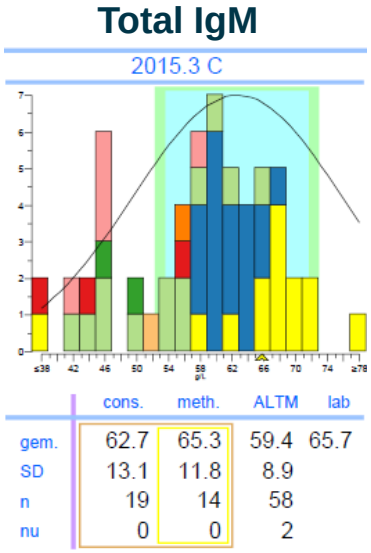


IgA-kappa: 94% goed
Mean conc: 1.9 g/L
Interlab-CV: 69 % (…+NTK)

Quantitative differences M-spike vs Immunochemistry



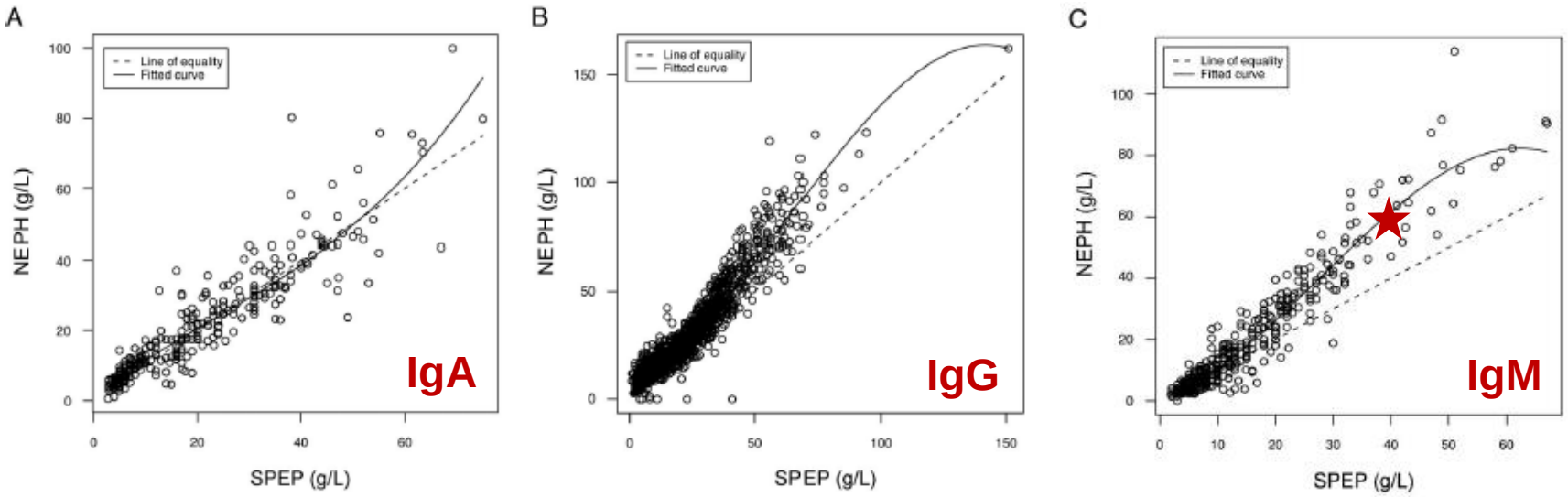




Mean 59.4 g/L

- At high M-protein concentration often poor correlation M-spike vs Immunochemistry
- Follow-up of M-protein with SAME technique…

Quantitative differences M-spike vs Immunochemistry



Adapted from Murray et al. Clin Chem 2009

Response to therapy quantification: Linearity

Standard IMWG response criteria‖	
Stringent complete response	Complete response as defined below plus normal FLC ratio** and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells)††
Complete response	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h
Partial response	≥50% reduction of serum M-protein plus reduction in 24 h urinary M-protein by ≥90% or to <200 mg per 24 h; If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria; If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50–89%. In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size (SPD)§§ of soft tissue plasmacytomas is also required
Stable disease	Not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates. Not meeting criteria for complete response, very good partial response, partial response, minimal response, or progressive disease

'Partial response: ≥50% reduction of serum M-protein…'

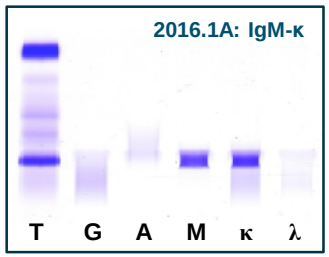
SKML-ID	IgM-K M-protein mean g/L (CV%)	Mean Total IgM
2015.3C	39.6 g/L (CV=8.3%)	59.4 g/L (CV=15%)

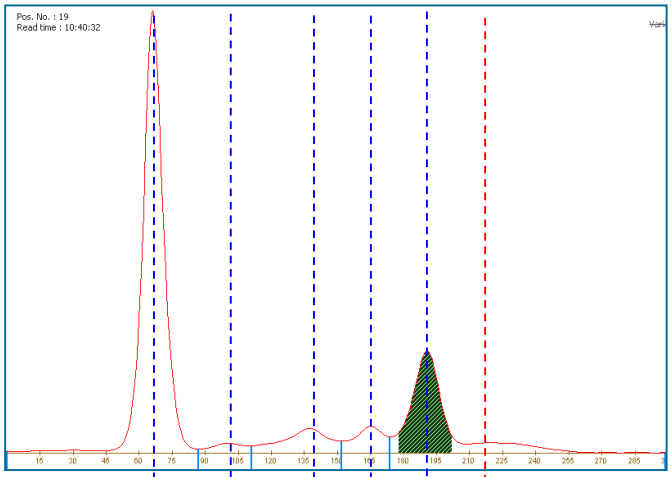
1:1 diluted in normal serum

2017.3A	20.6 g/L (CV=9.5%)	30.8 g/L (CV=10.4%)
DIL SERUM (=2017.3C)	No M-protein	0.6 g/L (CV=7.4%)

Kumar et al. IMWG response criteria. Lancet Oncology 2016

Follow-up of an M-protein



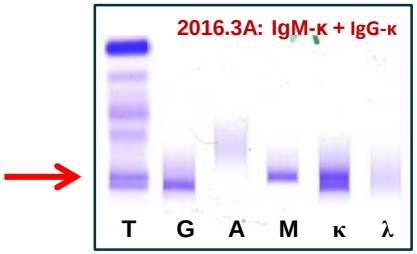


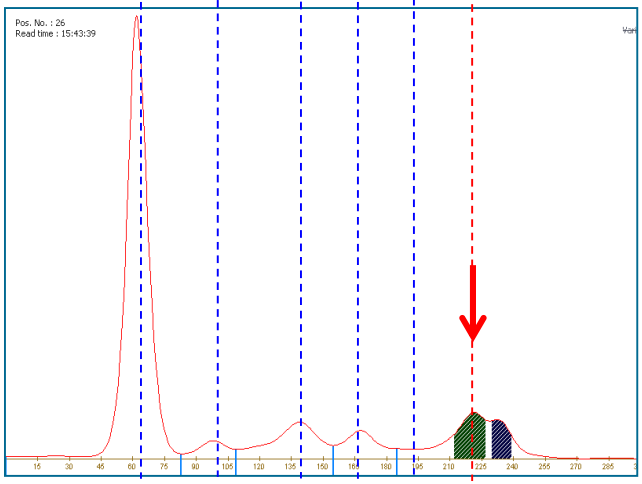
Casus

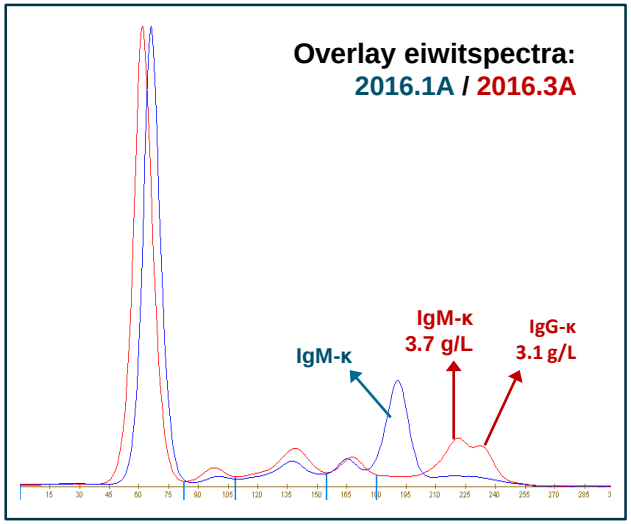
- Man, 65 jaar oud
- Aanhoudende vermoeidheid
- Lymphadenopathie
- Splenomegalie

➢ Diagnose M. Waldenström

- Gecombineerde chemotherapie dexa., rituximab, cyclophos.



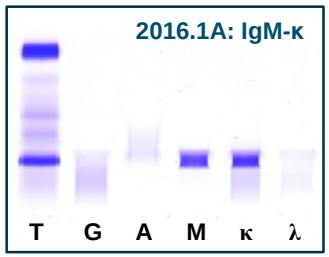


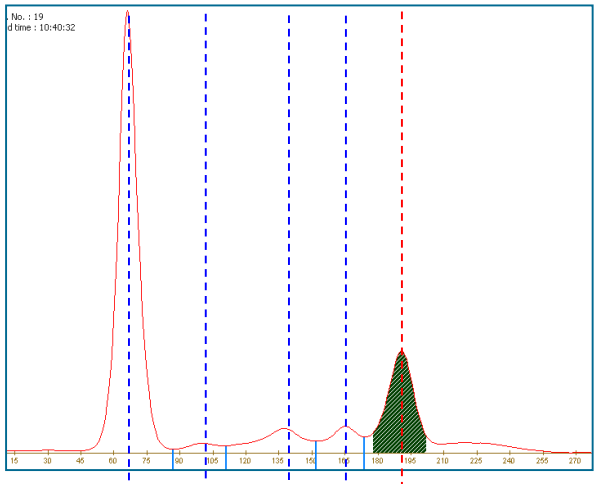


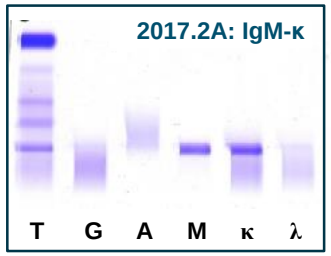
➢ Aan of afwezigheid van oorspronkelijke M-proteine prognostisch belangrijk

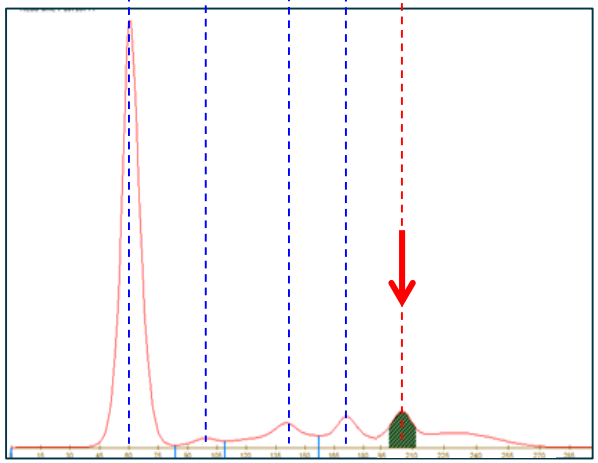
➢ Goed archief is belangrijk voor vervolgen van een bekend M-proteine

Follow-up of an M-protein_part 2









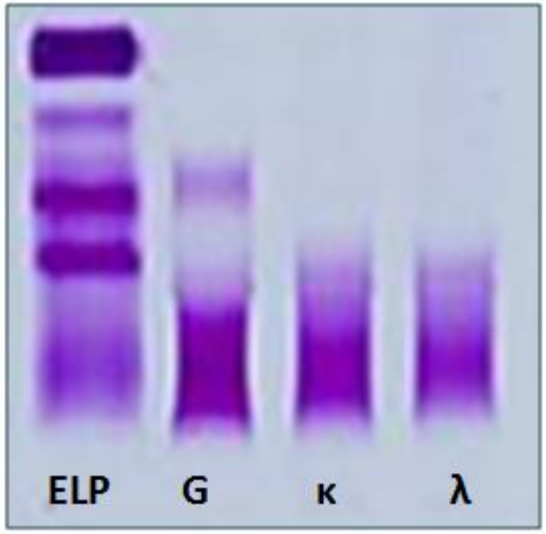
Casus

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

➢ **Diagnose M. Waldenström**

- **Gecombineerde chemotherapie dexa., rituximab, cyclophos.**

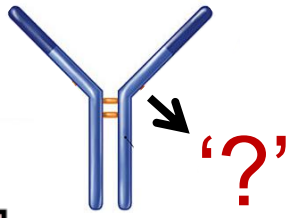
Heavy Chain Disease

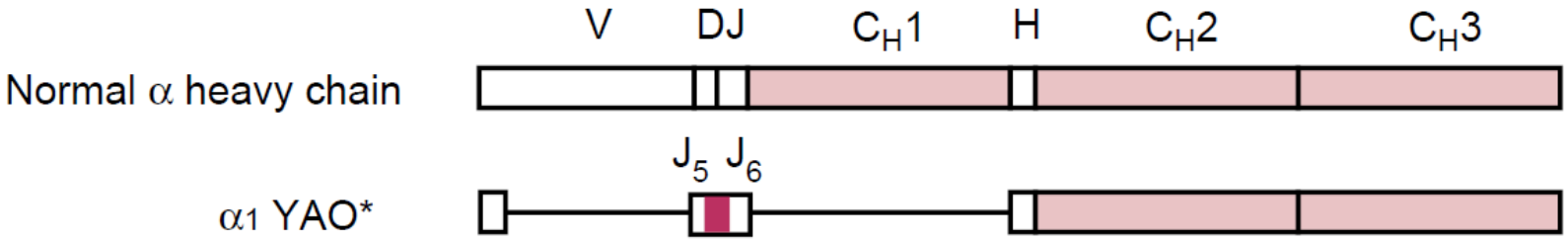


Detection/Quantification of HCD is challenging in most cases:

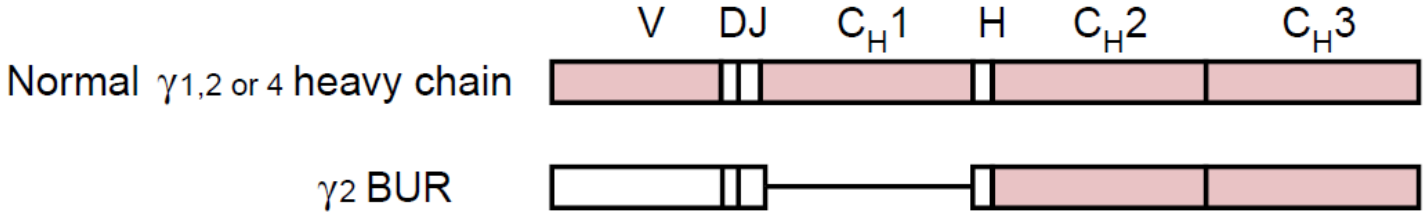
- **All HCD diseases (γ/α/μ) are rare**
- **Often small band**
- **Often smear instead of sharp band**
- **Often co-migrating with other protein-bands**

Heavy Chain Disease: α-HCD, γ-HCD, μ-HCD

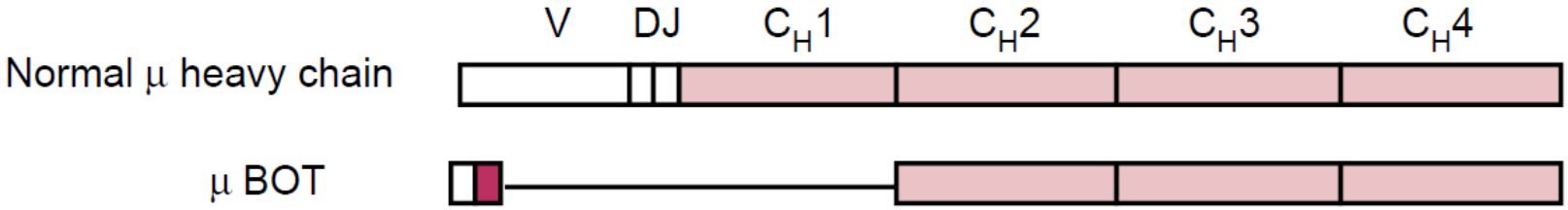




α-HCD



γ-HCD



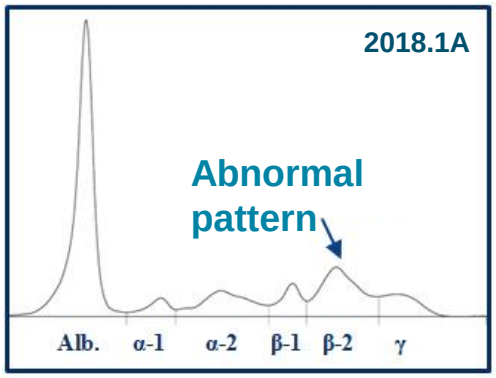
μ-HCD

Adadpted from Wahner-Roedler and Kyle. Best Pract Res Clin Haematol 2005

Radboudumc

Rare patterns…. IgG Heavy Chain

…with courtesy to dr. Dirk Bakkeren and dr. Matthieu Bosman (MMC)



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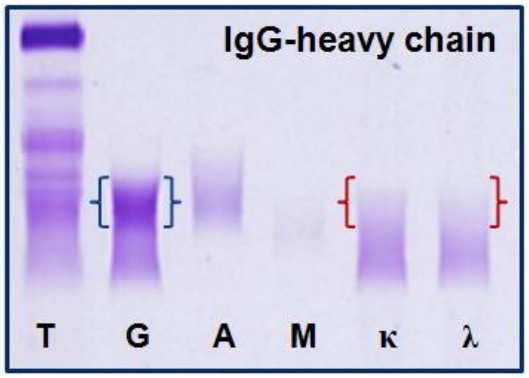

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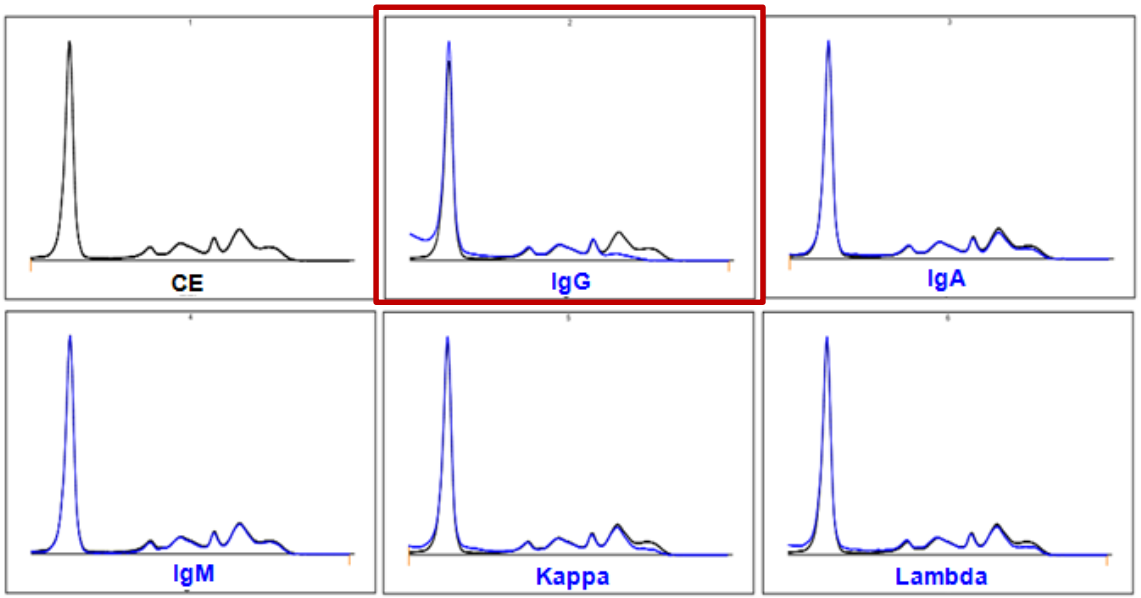
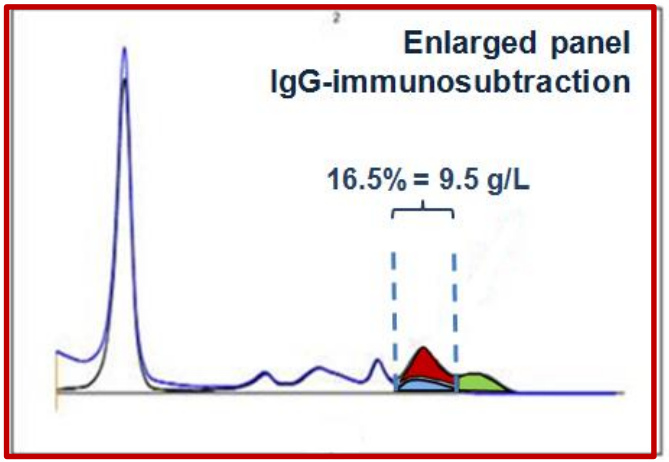
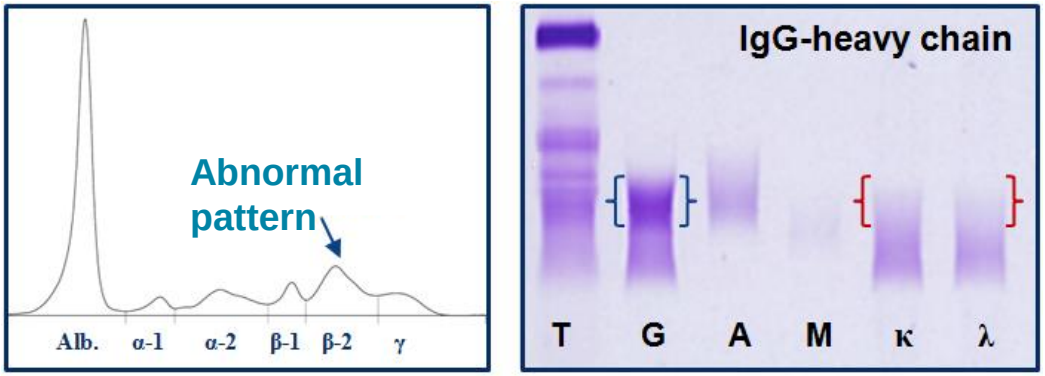




Do we recognize rare patterns…. IgG Heavy Chain



Reported M-protein	Number of labs	Percentage %
IgG heavy chain	29	45
Abnormal pattern*	7	11
IgG-kappa M-protein	1	2
IgG-lambda M-protein	1	2
No M-protein detected	26	41
Total	**64**	**100%**

*Mostly described as IgG M-protein with unknown light chain

Bosman et al. Clin Chem 2019

Improved treatment regimes for MM patients

Parameters

Patient specific

Age	Consider ASCT in 'younger patients'
Neuropathy	Avoid bortezomib and thalidomide if feasible, if not, use weekly and subcutaneous bortezomib
Light chain-induced nephropathy	Use preferentially bortezomib combinations, adaptation of dose of lenalidomide according to GFR mandatory

Disease specific

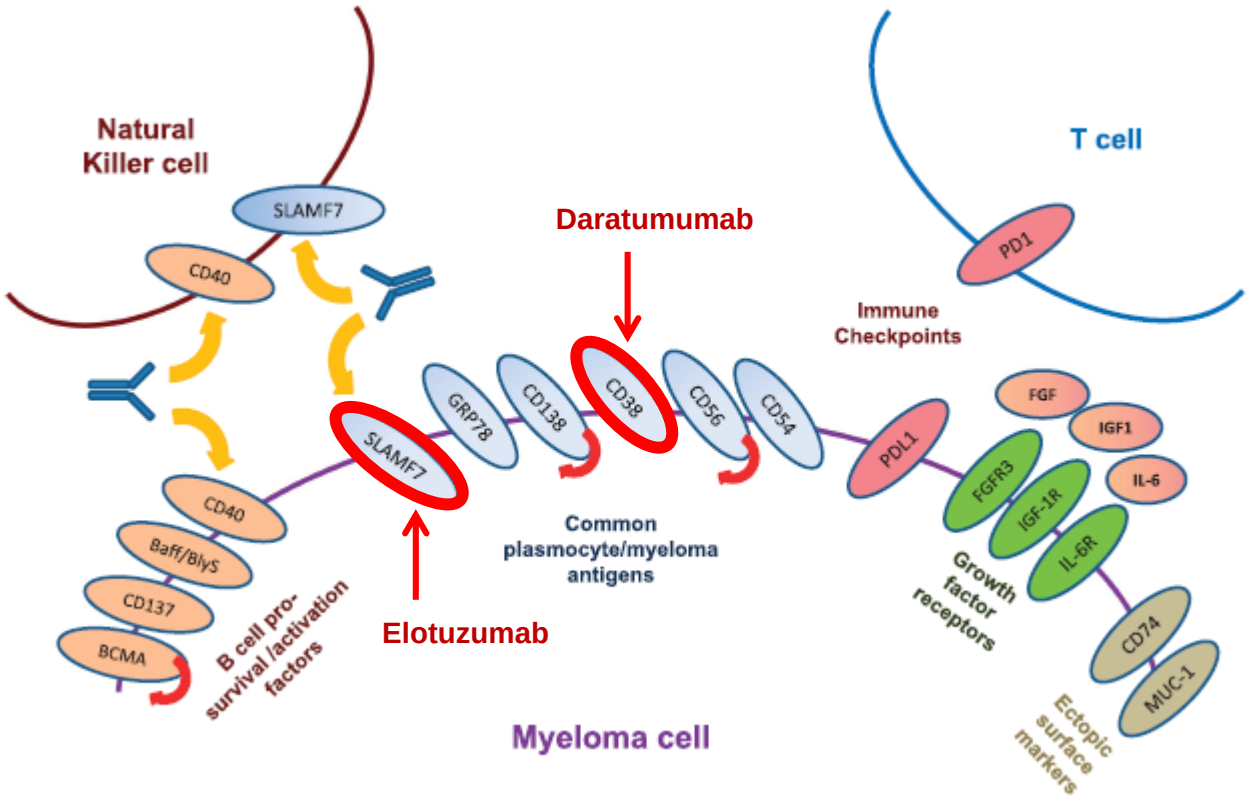
Good prognosis	Retreatment with effective and well tolerated first line regimen feasible
Symptomatic and rapidly progressive	Switch drug class, incorporate novel agents
High risk cytogenetics	Consider bortezomib combinations ± bendamustine

Treatment specific

Significant response to previous treatment, long PFS, well tolerated	Consider retreatment
Insufficient response to previous treatment	Switch drug class and change regimen
All common regimens exploited	Consider DCEP, DT-PACE, enrollment in clinical trials

IMWG guideline: Ludwig et al. Leukemia 2013

Monoclonal antibody therapy in multiple myeloma



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Daratumumab plus Bortezomib, Melphalan, and Prednisone for Untreated Myeloma

M.-V. Mateos, M.A. Dimopoulos, M. Cavo, K. Suzuki, A. Jakubowiak, S. Knop, C. Doyen, P. Lucio, Z. Nagy, P. Kaplan, L. Pour, M. Cook, S. Grosicki, A. Crepaldi, A.M. Liberati, P. Campbell, T. Shelekhova, S.-S. Yoon, G. Iosava, T. Fujisaki, M. Garg, C. Chiu, J. Wang, R. Carson, W. Crist, W. Deraedt, H. Nguyen, M. Qi, and J. San-Miguel, for the ALCYONE Trial Investigators*

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H.M. Lokhorst, T. Plesner, J.P. Laubach, H. Nahi, P. Gimsing, M. Hansson, M.C. Minnema, U. Lassen, J. Krejcik, A. Palumbo, N.W.C.J. van de Donk, T. Ahmadi, I. Khan, C.M. Uhlar, J. Wang, A.K. Sasser, N. Losic, S. Lisby, L. Basse, N. Brun, and P.G. Richardson

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Elotuzumab Therapy for Relapsed or Refractory Multiple Myeloma

Sagar Lonial, M.D., Meletios Dimopoulos, M.D., Antonio Palumbo, M.D., Darrell White, M.D., Sebastian Grosicki, M.D., Ph.D., Ivan Spicka, M.D., Adam Walter-Croneck, M.D., Philippe Moreau, M.D., Maria-Victoria Mateos, M.D., Ph.D., Hila Magen, M.D., Andrew Belch, M.D., Donna Reece, M.D., Meral Beksac, M.D., Andrew Spencer, M.D., Heather Oakervee. M.D.. Robert Z. Orlowski. M.D.. Masafumi Taniwaki. M.D..

Touzeau et al. Review. Leukemia 2017

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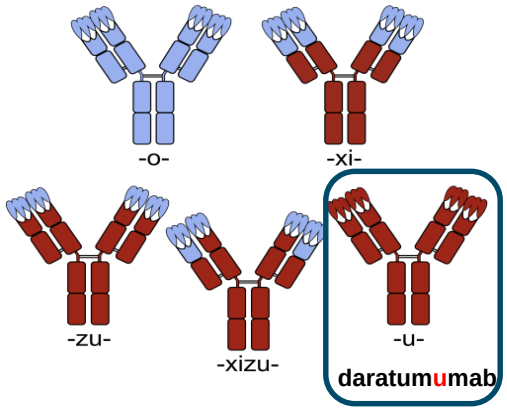
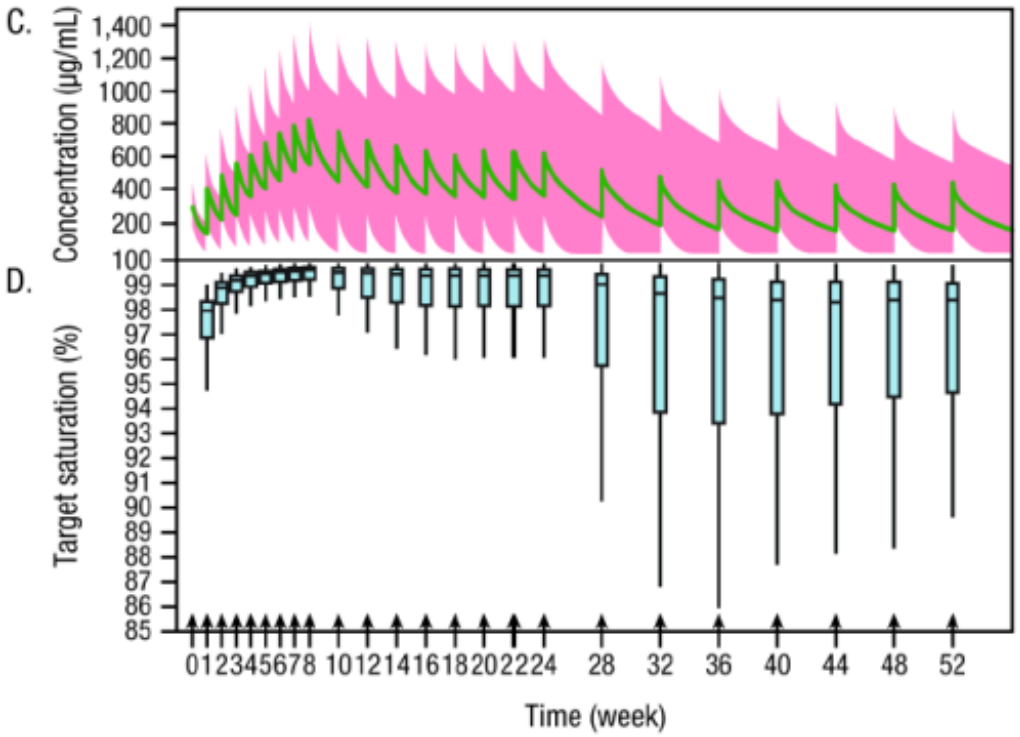
Daratumumab pharmacokinetics



Human IgG1-kappa mAb biologic



16 mg/kg Weekly

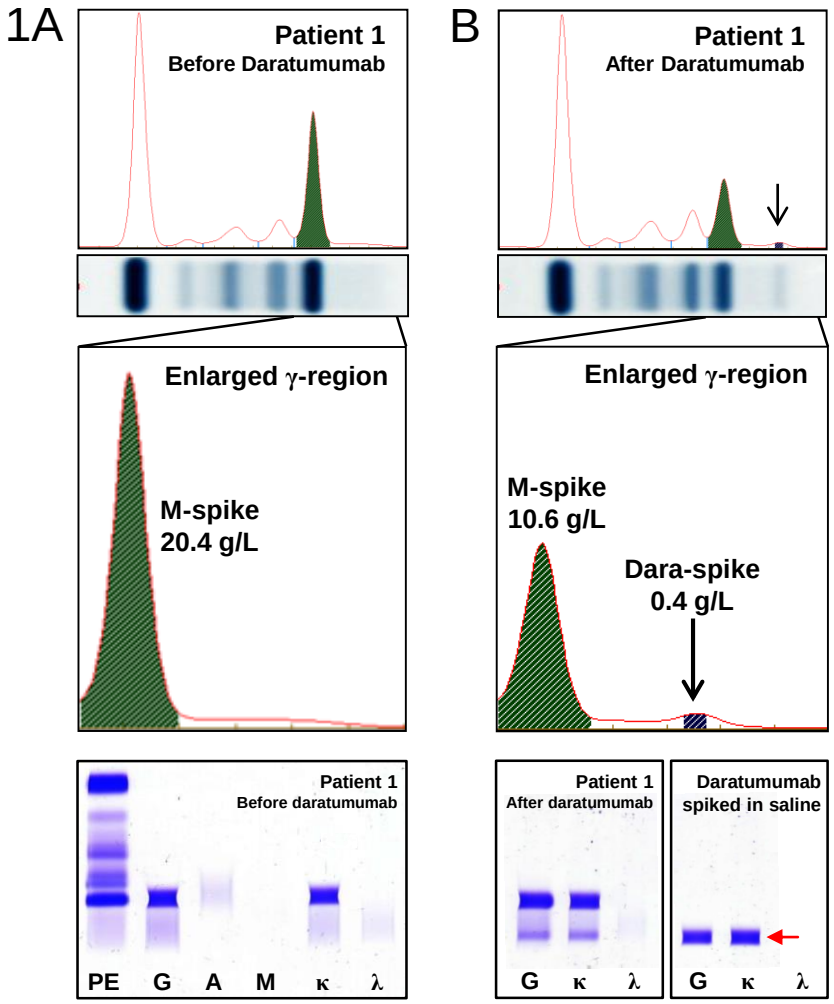
16 mg/kg Every 2 wks

16 mg/kg Every 4 wks

Reaching serum [dara] up to 1 g/L

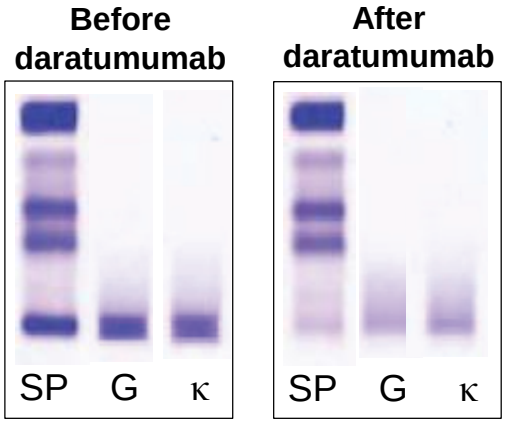
Xu et al. Clin Phar Ther 2017
Clemens et al. Clin Pharmacokinet 2017

Daratumumab and M-protein interference



IMWG response criteria (Durie et al. 2006)

a.o. IFE negative…



Van de Donk et al. Clin Chem Lab Med 2016
McCudden et al. Clin Chem Lab Med 2016

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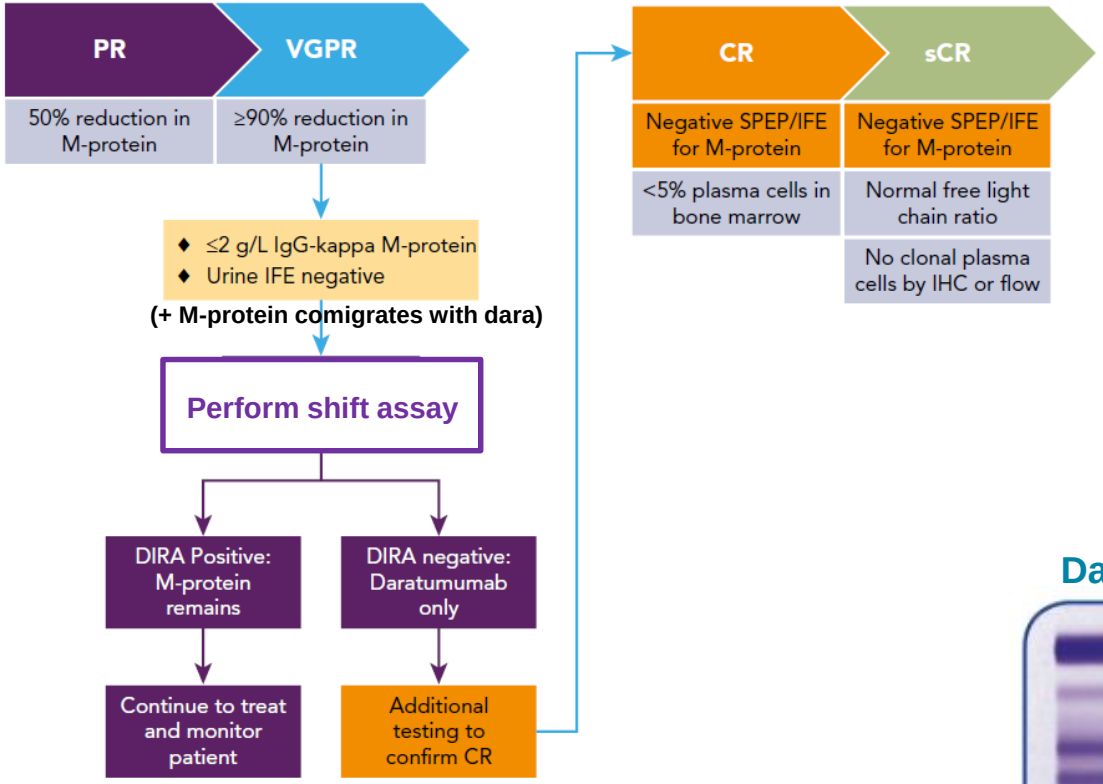
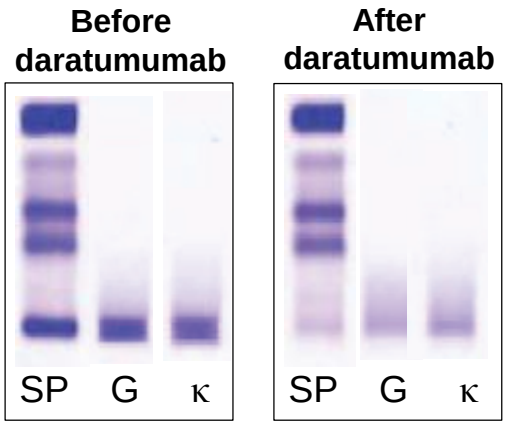
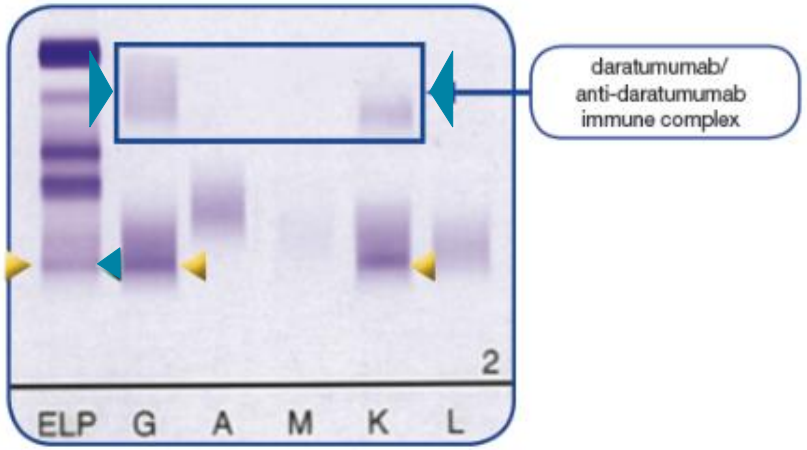
Indication to use DIRA or similar shift-assay





Daratumumab Hydrashift assay (=pos)



Adapted from Van de Donk et al. Blood 2018

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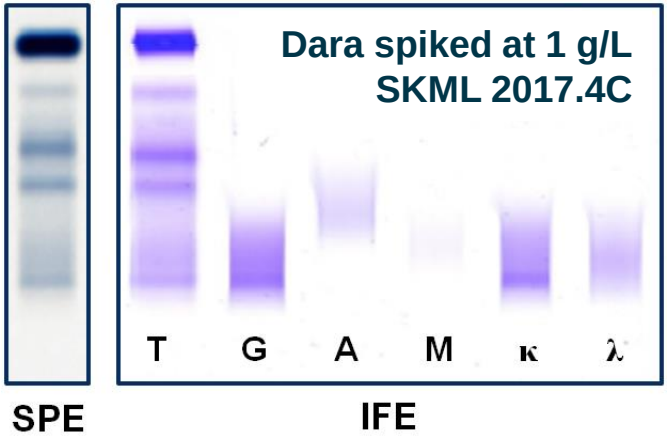
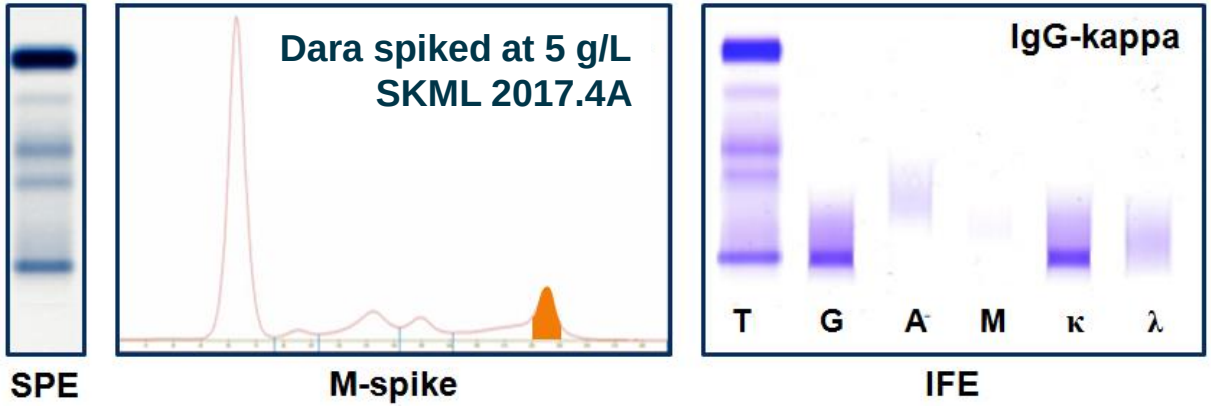
Daratumumab spiked in normal serum



98% IgG-kappa M-protein
Mean M-spike (n=44): 1.7 g/L
Between-lab CV: 46%

Many labs don't spike such small M-proteins and reported <2 g/L



100% IgG-kappa M-protein
Mean M-spike (n=66): 4.9 g/L
Between-lab VC%: 22 %

Normal FLC values (!)

Rosenberg et al. Clin Biochem 2016

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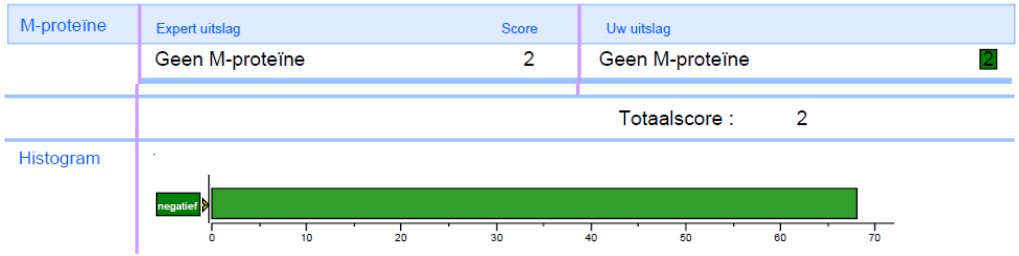
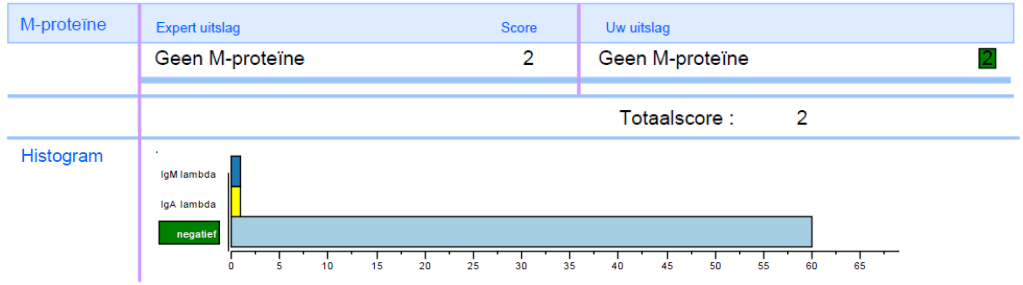
Rapportage bij sera zonder M-proteine (n=9)

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

2017.3C



2019.1B



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

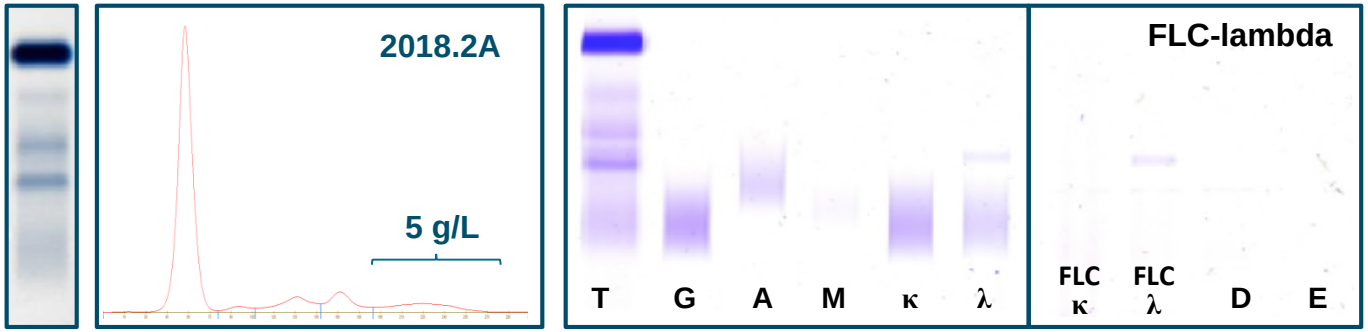
IFE immunofixation electrophoresis; Ig, immunoglobulin; MGUS, monoclonal gammopathy of undetermined significance.

Samenvatting van dubieuze banden (NTK):

- **84% van patienten persisteert 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

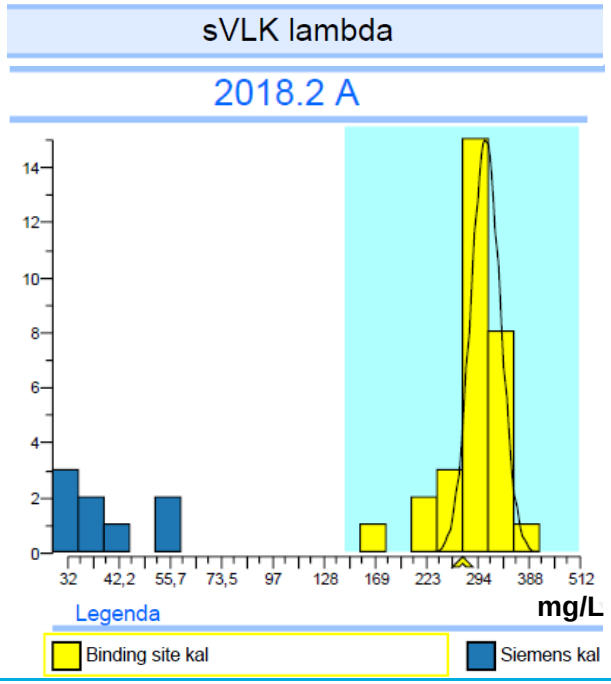
Small M-proteins can be clinically very relevant: monoclonal FLC

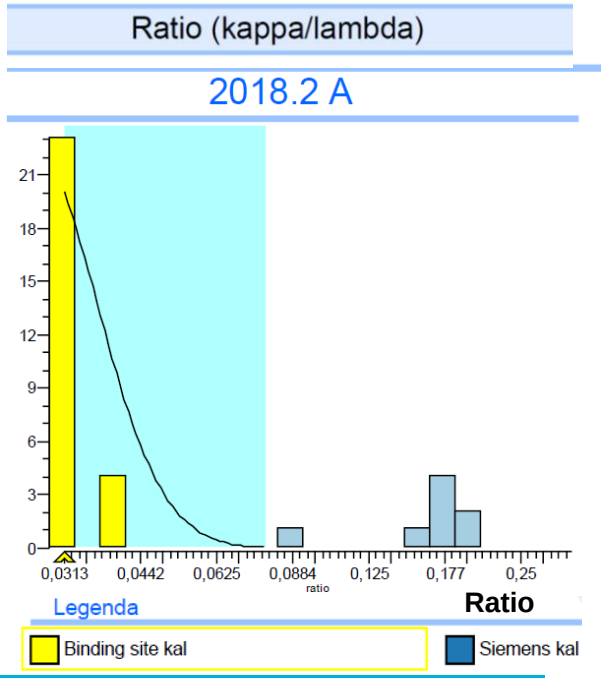


SPE | Densitogram | Immunofixatie elektrophorese (IFE)

Labs report:
60% FLC-Lambda
40% Negative

FLC quantification





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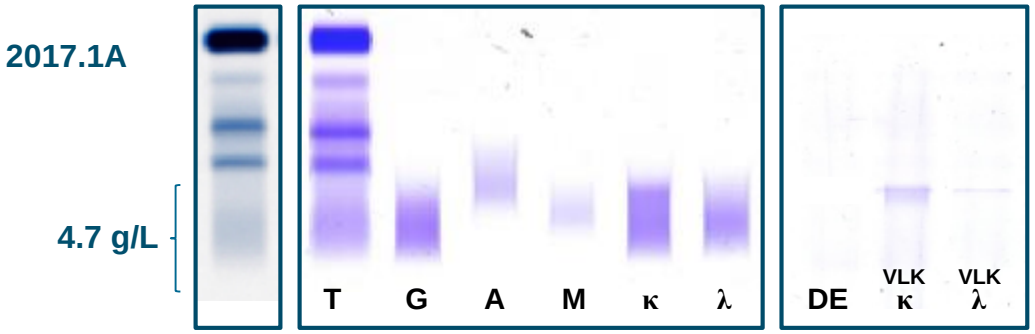
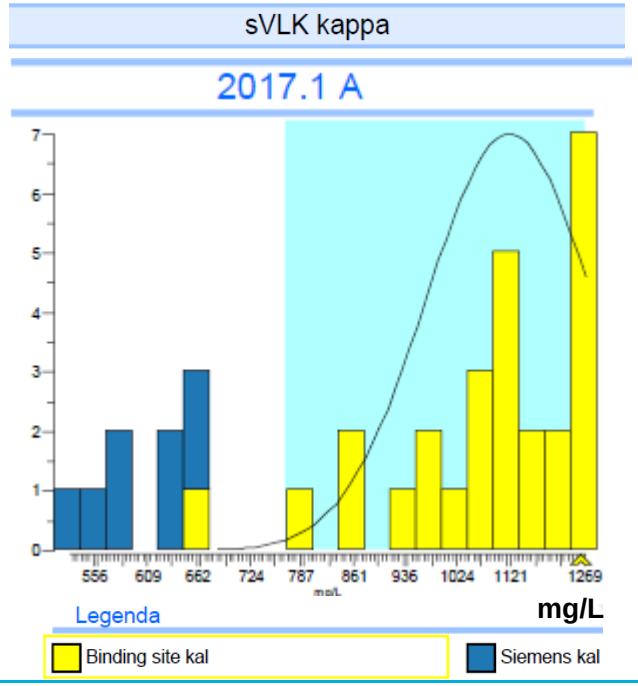
Monoclonal FLC_part 2



Labs report:
40% FLC-Kappa
60% Negative

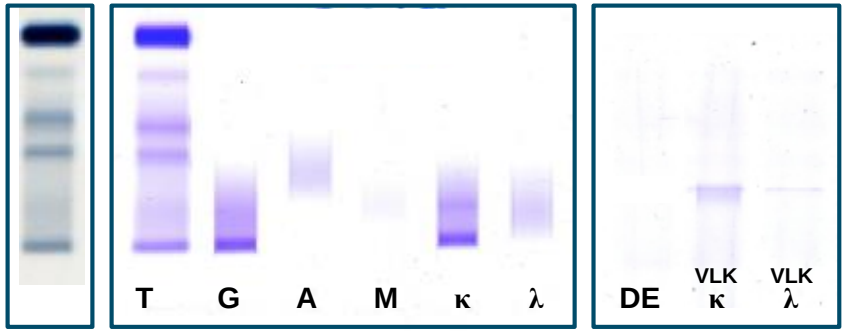
FLC quantification



Monoclonal FLC_part 3

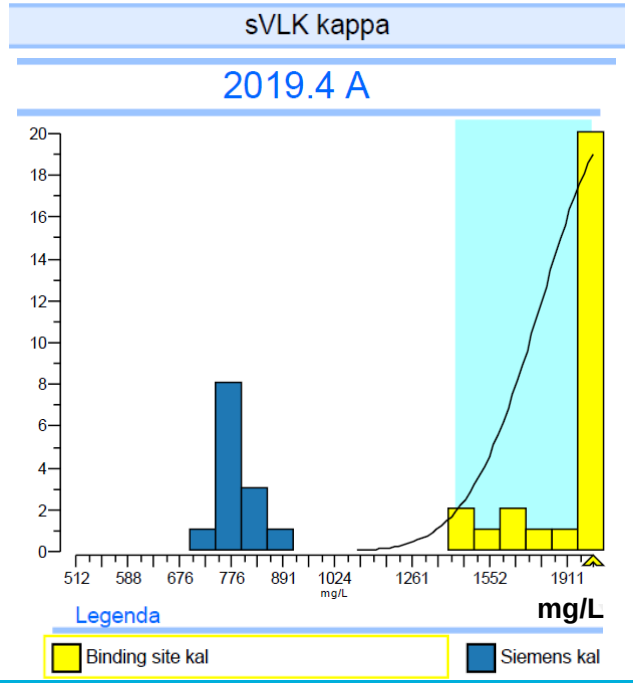
2019.4A

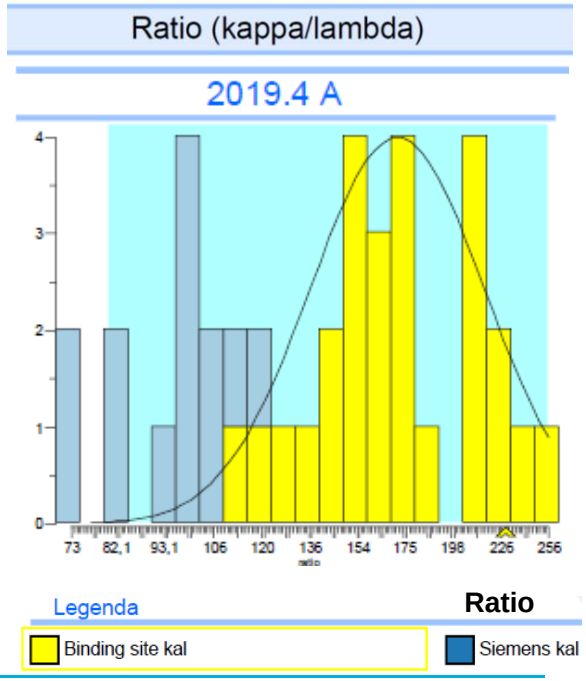
3.5 g/L



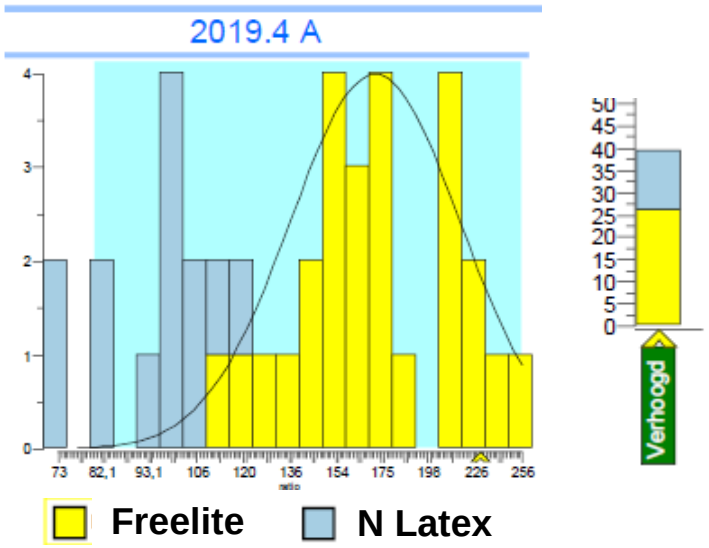
40% FLC-Kappa
60% IgG-K + FLC-K

FLC quantification



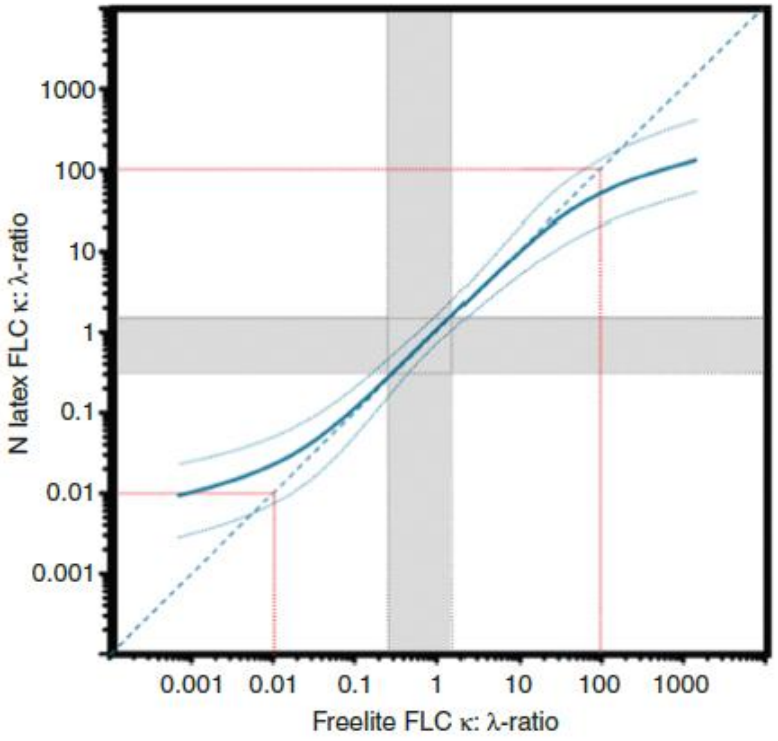


The importance of FLC standardisation/harmonisation



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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The importance of FLC standardisation/harmonisation

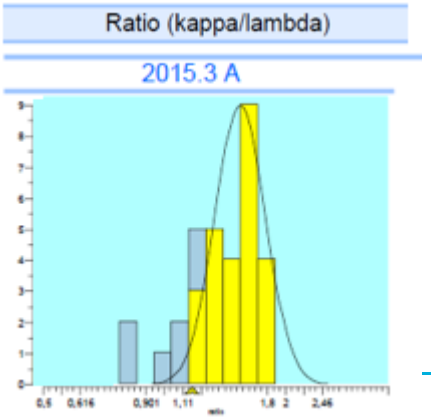
Table 1: Characteristics of FLC assays.

	Freelite [3, 9, 10]	N-Latex FLC [6, 11]	Seralite [7, 12]	Sebia FLC [8, 13]
Assay principle	Nephelometry/turb	Nephelometry	Lateral flow	ELISA
Antibodies	Polyclonal	Monoclonal	Monoclonal	Polyclonal
Calibrator	Polyclonal FLC	Polyclonal FLC	Monoclonal FLC	Polyclonal FLC
Sample volume	20 µL	κ: 90 µL, λ: 40 µL	100 µL	8 µL
Intra-assay VC	0.4–2.2%	0.3–1.2%	2.7–9.2%	5.1–7.6%
Inter-assay VC	0.7–4.1%	0.5–1.9%	2.7–9.6%	1.9–7.6%
Reference values	κ: 3.3–19.4 mg/L	κ: 6.7–22.4 mg/L	κ: 5.2–22.7 mg/L	κ: 5.2–15.3 mg/L
	λ: 5.7–26.3 mg/L	λ: 8.3–27.0 mg/L	λ: 4.0–25.1 mg/L	λ: 8.2–18.1 mg/L
	κ/λ: 0.26–1.65	κ/λ: 0.31–1.56	κ/λ: 0.5–2.5	κ/λ: 0.37–1.44
Adj. FLC-ratio[a]	κ/λ: 0.37–3.1	No	No	κ/λ: 0.46–2.23
Company	The Binding Site	Siemens	Abingdon Health	Sebia

[a]Adjusted κ/λ FLC-ratio reference values for patients with impaired renal function. FLC, free light chain; VC, variation coefficient.

FLC-ratio **>100** **>35** **?** **>20**

Adjusted FLC-ratio in CKD patients…



Fleming et al. CCLM 2020

Erasmus MC
Universitair Medisch Centrum Rotterdam

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

Zeer welkom!

- **70 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

Dank je wel

Collega's Lab Medische Immunologie
Corrie de Kat Angelino

Radboudumc

Ondersteuning SKML bureau
SKML Sectie Humorale Immunologie

skml

M-protein diagnostics = Personalized diagnostics