An international multi-center serum protein electrophoresis accuracy and M-protein isotyping study.

Part I: Factors impacting LoQ
Part II: LoD and follow-up of small M-proteins

SKML nabespreking sectie Humorale Immunologie
13 Februari 2020

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ifcc International Federation of Clinical Chemistry and Laboratory Medicine

MAYO CLINIC

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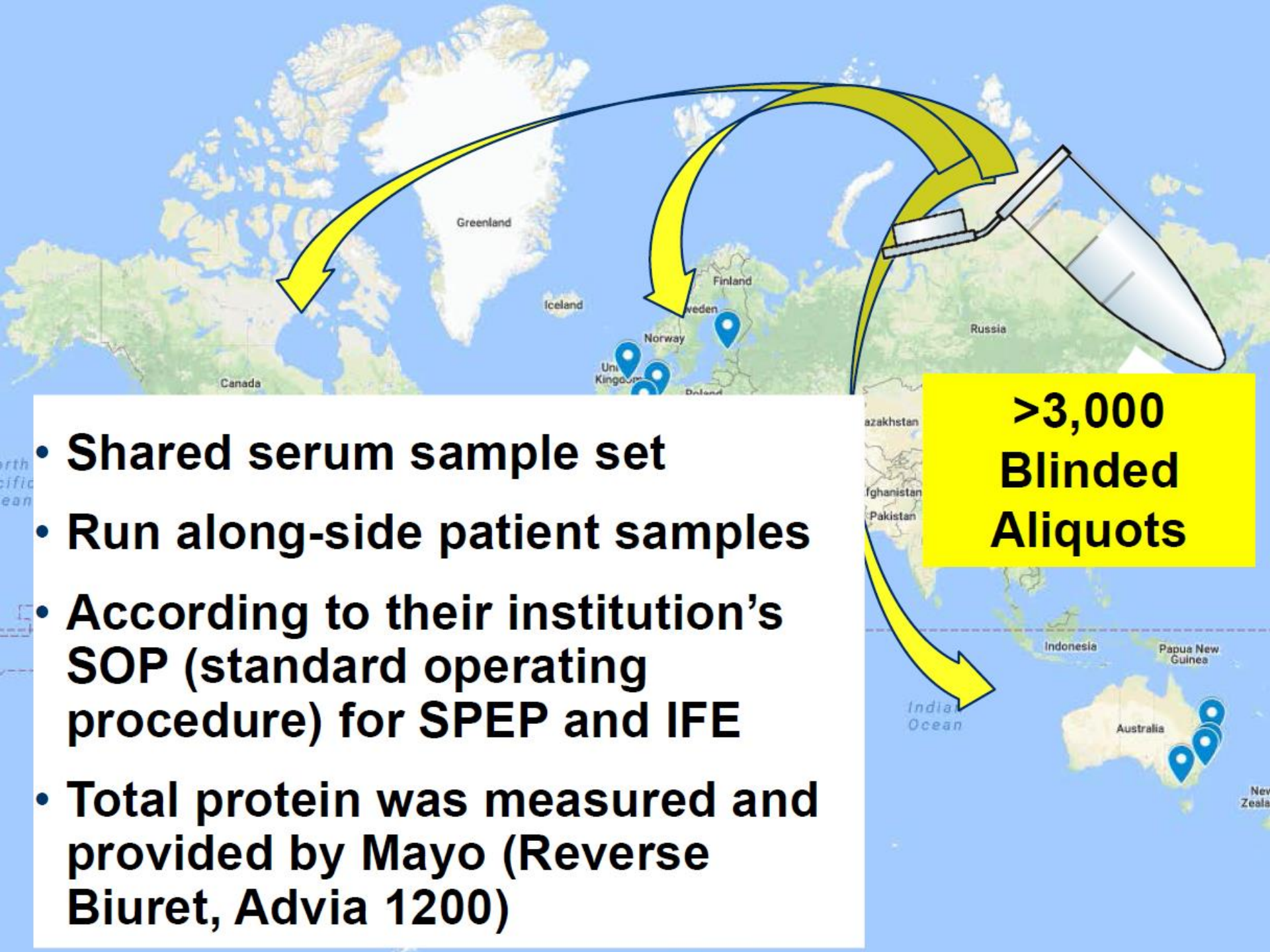
IFCC* Harmonization of Reporting Strategies Working Group

Institution
Mayo Clinic
Pathology Queensland
Hunter Area Pathology
Royal Prince Alfred Hospital
Royal Melbourne Hospital
University Hospital of Padova
University Hospital of Verona
Radboud University Medical Center
University Hospital of Firenze
Polclinico S Matteo Pavia
University of Michigan-Ann Arbor
ARUP
North Estonia Medical Centre
Sebia
Helena Biosciences Europe
Ottawa Hospital



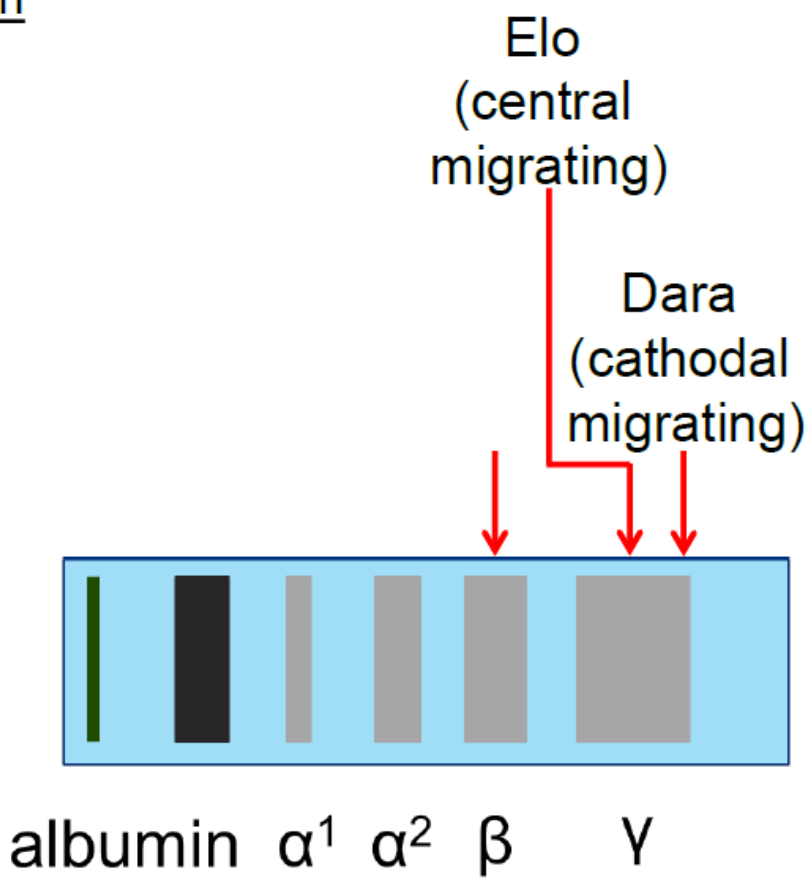
- Shared serum sample set
- Run along-side patient samples
- According to their institution's SOP (standard operating procedure) for SPEP and IFE
- Total protein was measured and provided by Mayo (Reverse Biuret, Advia 1200)

>3,000 Blinded Aliquots

Blinded Shared Sample Set

Artificial "patient" samples containing a M-protein

- Pooled patient samples (~120 mL each)
- Gamma Fraction Background
  - Hypogamma background
    - < 0.5 g/dL
  - Normal gamma background
    - 0.6-1.5 g/dL
  - Hypergamma background
    - > 1.7 g/dL
- Monoclonal antibody (mAb)-spiked samples
  - 1.0-0.0125 g/dL
  - Elotuzumab (Elo)
  - Daratumumab (Dara)
  - IgG1κ isotype
- Beta-migrating-pooled patient samples



SPEP/IFE LOQ Studies

Recycle Bin

EDIT LINKS

Group Announcements

new announcement or edit this list

Title	Body
Open in Internet Explorer	Just a reminder that SharePoint will work best if opened in Internet Explorer. We've encountered problems when trying to open SharePoint and submit using Chrome.

Milestones

Testing Site Information	
Institution Name	RBWH Chem Path
Contact Person Name	Anfernee Tseng
Contact Person E-mail	anfernee.tseng@health.qld.gov.au
Analysis Method	Capillary Electrophoresis
Product Manufacturer & Commercial Name	Sebia CAPI3
Unit of Measure	g/L

Result Entry

Run ID	Sample ID	Expected Isotype	Run Date (yyyy-mm-dd)	Total Protein (g/L)	T…	…min (%)	Albumin (g/L)	Alpha-1 (% of total)	Alpha-1 (g/L)	Alpha-2 (% of total)	Alpha-2 (g/L)	Beta1 (% of total)	Beta1 (g/L)	Abnormality in beta fraction?	Beta M-spike (% of total)	Beta spik… (g/L)
EXAMPLE	IFCC-0	IgG kappa	2017-05-01	60.0	21.000	53%	31.80	4.70%	2.82	11.20%	6.72	7.20%	4.32	None	0.00%	0.0
Run 1	IFCC-1	IgG kappa		58…	…0											
Run 1	IFCC-2	IgG kappa		58.00	7.570											
Run 1	IFCC-3	IgG kappa		82.00	24.700											
Run 1	IFCC-4	IgG kappa		49.00	10.400											
Run 1	IFCC-5	IgG kappa		79.00	22.900											
Run 1	IFCC-6	IgG kappa		39.00	12.200											
Run 1	IFCC-7	IgG kappa		75.00	13.100											
Run 1	IFCC-8	IgG kappa		47.00	12.100											
Run 1	IFCC-9	IgG kappa		47.00	8.020											
Run 1	IFCC-10	IgG kappa		55.00	5.340											
Run 1	IFCC-11	IgG kappa		57.00	10.500											
Run 1	IFCC-12	IgG kappa		51.00	5.160											
Run 1	IFCC-13	IgG kappa		80.00	22.600											
Run 1	IFCC-14	IgG kappa		53.00	8.610											
Run 1	IFCC-15	IgG kappa		79.00	21.700											
Run 1	IFCC-16	IgG kappa		70.00	20.900											
Run 1	IFCC-17	IgG kappa		57.00	8.570											
Run 1	IFCC-18	IgG kappa		43.00	11.400											
Run 1	IFCC-19	IgG kappa		81.00	25.500											

Methodology Distribution

SPEP Method	Number of Institutions
Helena AGE	4
Sebia AGE	5
Sebia CZE	10

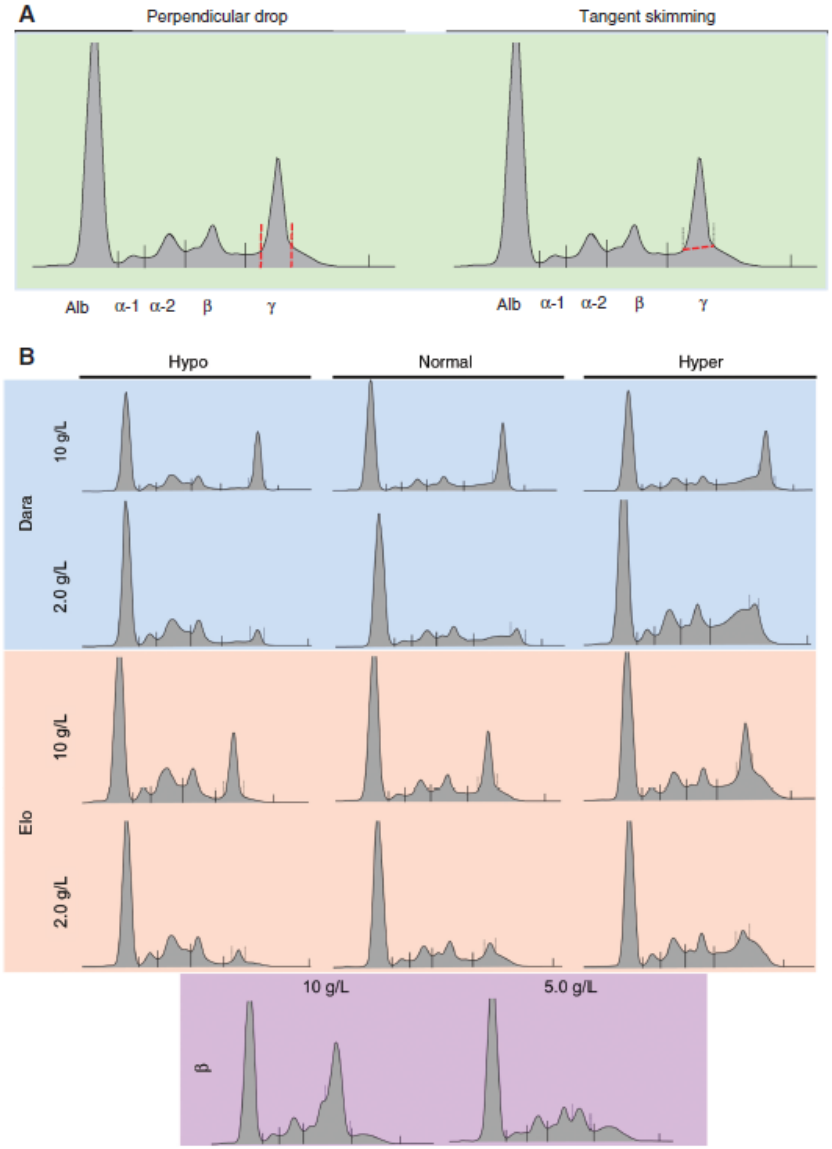
IFE Method	Number of Institutions
Monovalent Antisera	11
Pentavalent Antisera	2
Immunosubtraction	3

*Note: Some institutions provided ≥1 method

Table 1: Institutions' methodology distribution for serum protein electrophoresis and immunofixation/immunosubtraction.

	North America	Europe	Australia
SPEP			
Helena AGE			
PD			
Primary	1	1	1
Supplemental	–	–	1
TS			
Primary	–	–	–
Supplemental	–	–	1
Sebia AGE			
PD			
Primary	1	1	1
Supplemental	–	–	1
TS			
Primary	–	–	1
Supplemental	–	1	–
Sebia CZE			
PD			
Primary	1	5	2
Supplemental	–	–	–
TS			
Primary	1	1	–
Supplemental	–	4	1
IFE/ISUB			
Helena			
Mono	–	1	1
Penta	–	–	1
Sebia			
Mono	2	5	2
Penta	–	1	–
ISUB	1	2	–

Serum protein electrophoresis



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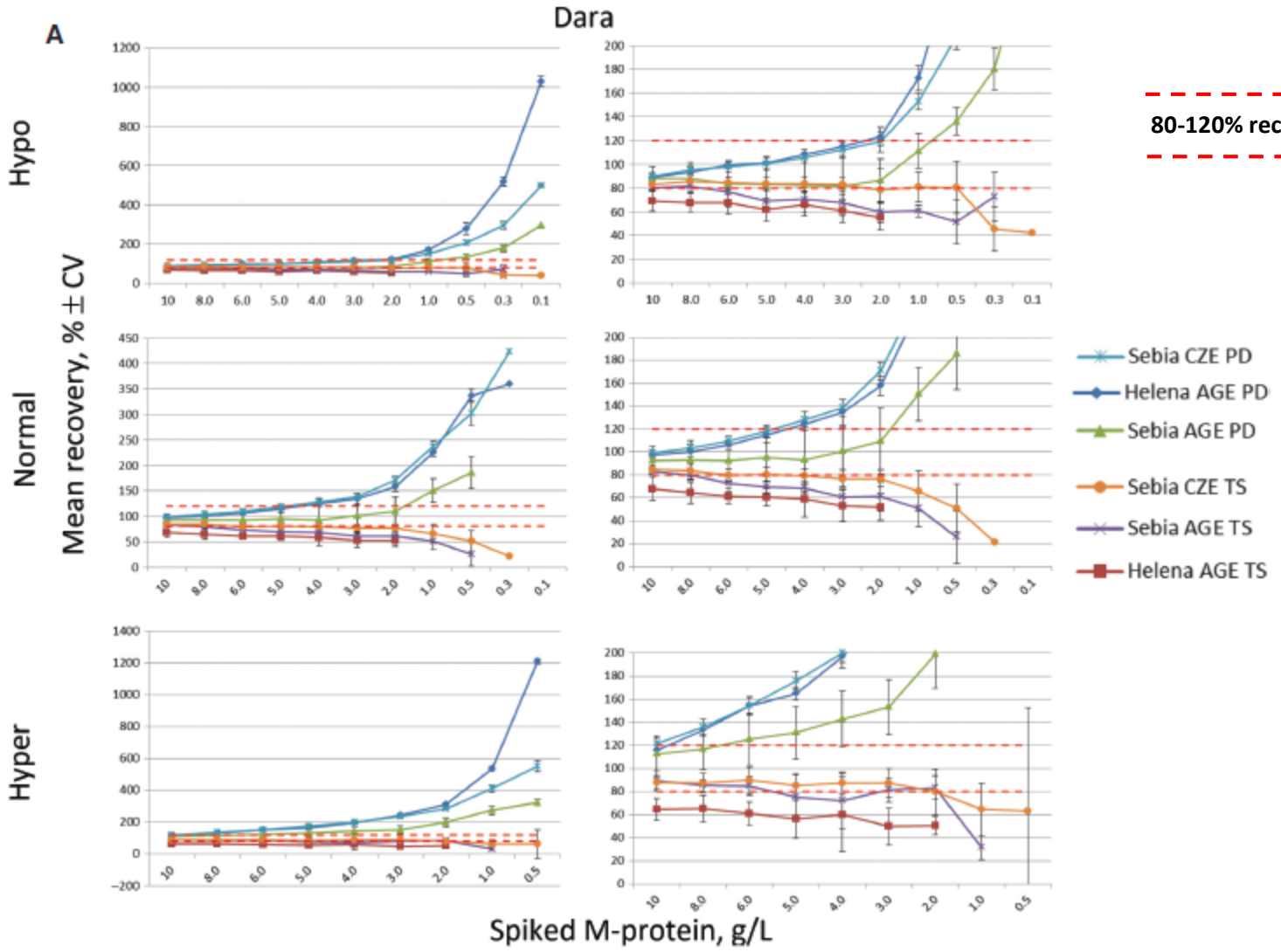
SPE: Accuracy of M-protein quantification (Dara)



Turner et al. Part I. CCLM in press

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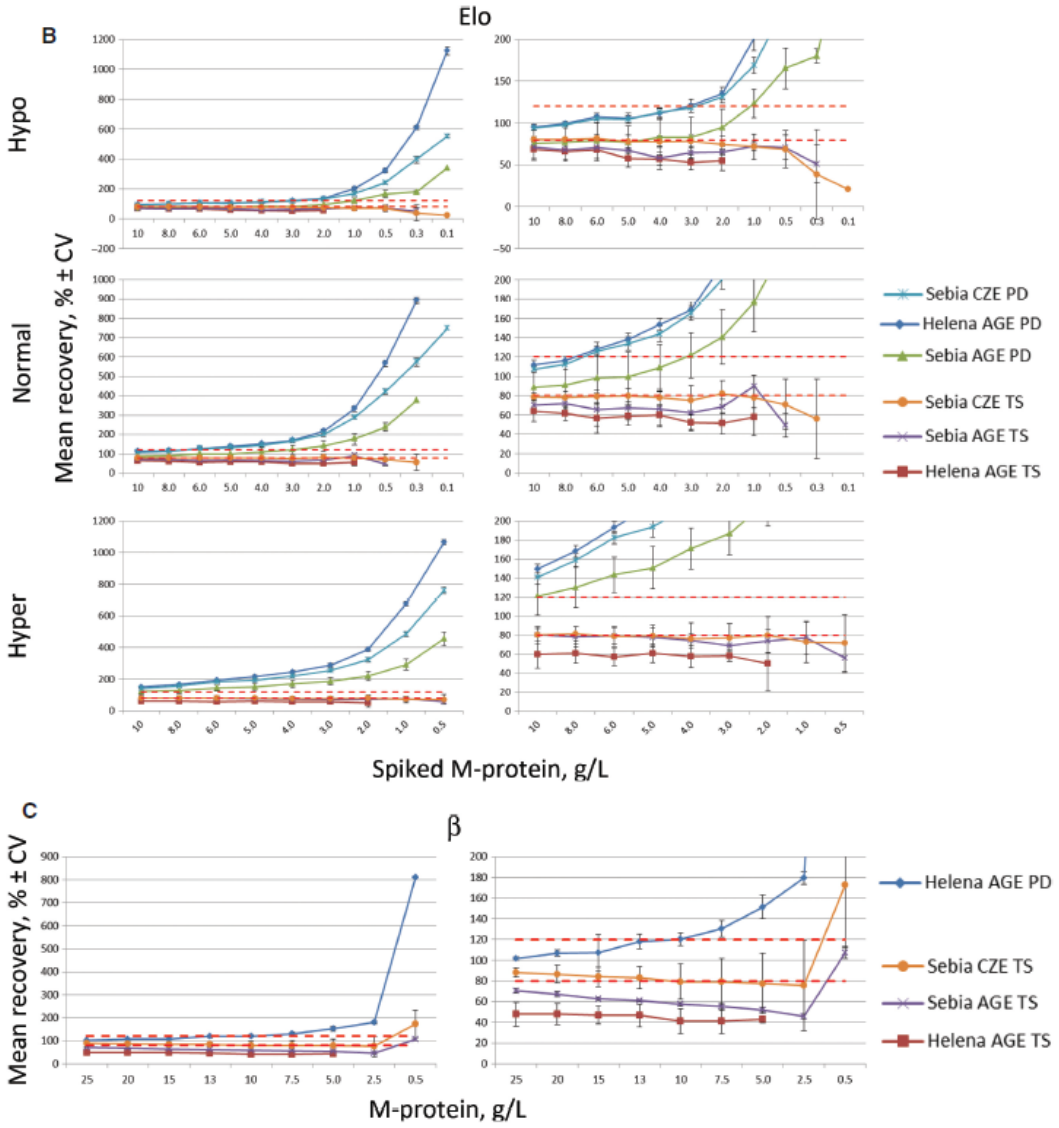
SPE: Accuracy of M-protein quantification (Elo and B-migrating)



80-120% recovery range

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SPE: LoD

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Conclusions Part I

Accuracy of M-protein quantitation is dependent on:

M-protein concentration

- Highest inaccuracy in smal M-proteins

Gamma fraction background

- Highest inaccuracy with hyper-gamma

Migration pattern of M-protein

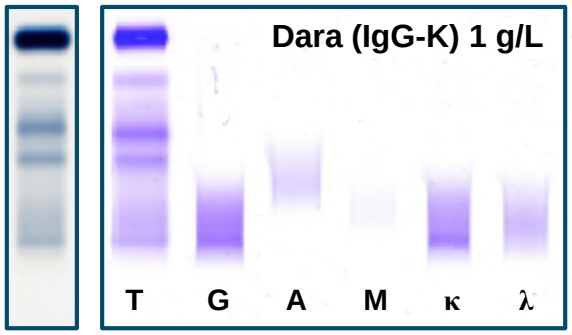
- Highest inaccuracy in central gamma migration

Gating strategy

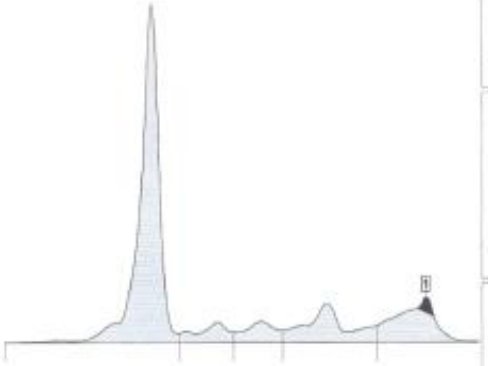
- TS leads to underestimation
- PD leads to overestimation

'The quantitation of small M-proteins especially in high polyclonal background is associated with analytical inaccuracy'

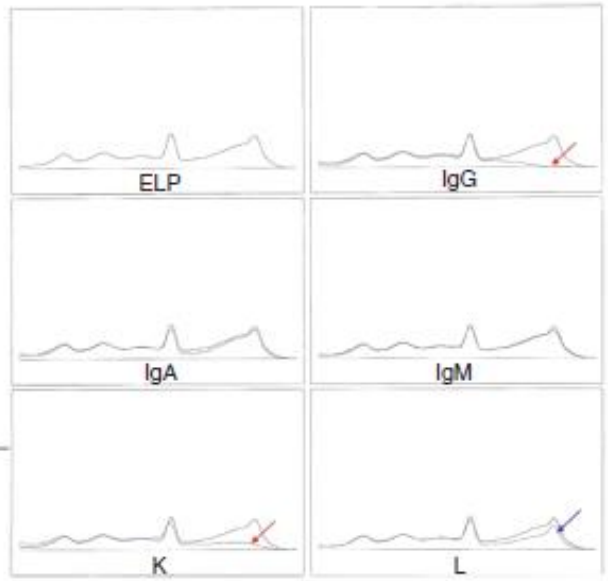
IFE and ISUB (Dara in normal gamma region)



SPE IFE



CZE SPEP



ISUB

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SPE, IFE and ISUB: LoD

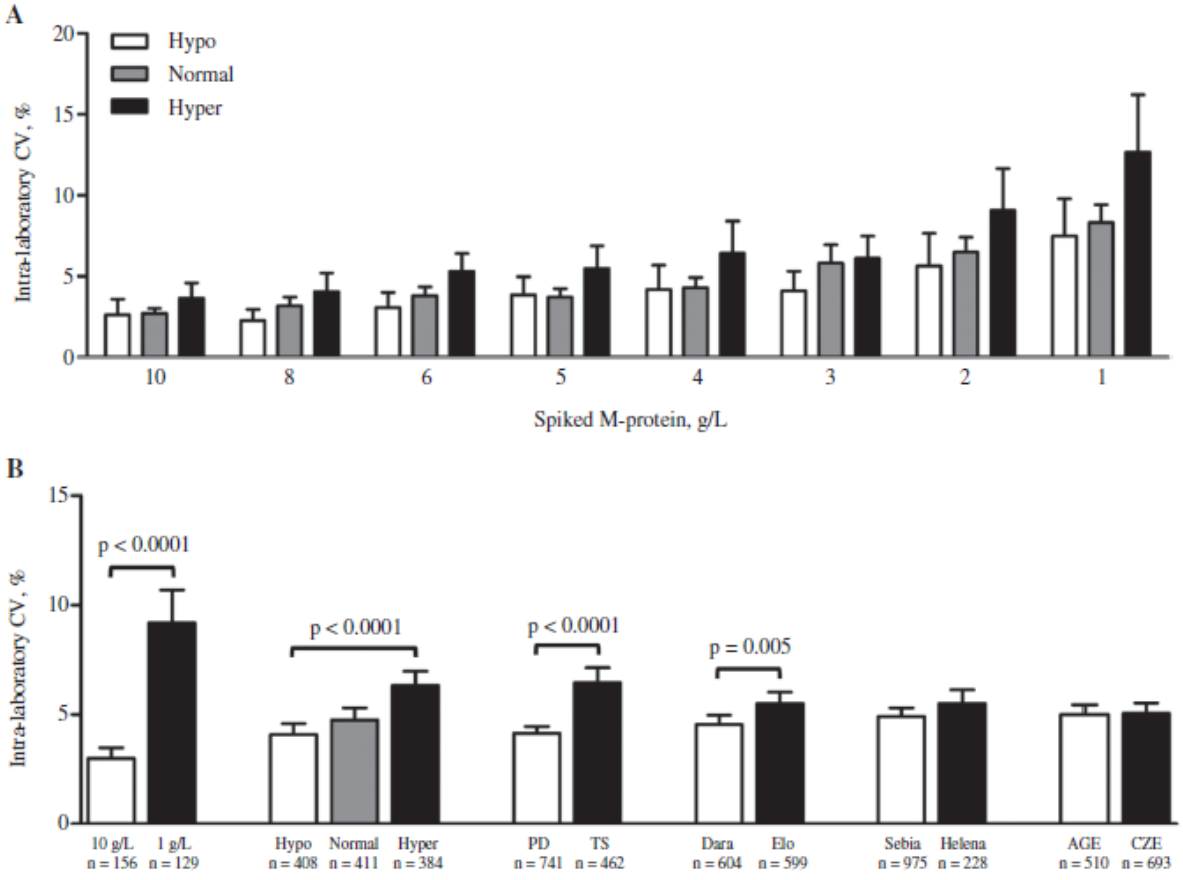
Table 1: Limit of detection for SPEP and immunofixation/immunosubtraction.

	Dara			Elo			Beta
	Hypo	Normal	Hyper	Hypo	Normal	Hyper	
SPEP LOD							
Helena AGE	0.5	0.5	1.0	0.5	0.5	1.0	<0.5
Sebia AGE	0.5	0.5	1.0	0.5	0.5	1.0	<0.5
Sebia CZE	0.5	0.5	0.5	0.5	0.3	0.5	<0.5
IFE/ISUB LOD							
Helena							
Mono	<0.1	0.5	<0.5	<0.1	0.3	<0.5	<0.5
Penta	<0.1	<0.1	<0.5	<0.1	<0.1	<0.5	–
Sebia							
Mono	<0.1	0.5	1	<0.1	0.5	2	<0.5
Penta	<0.1	0.3	<0.5	<0.1	<0.1	<0.5	–
Sebia							
ISUB	0.3	0.5	<0.5	0.3	0.3	0.5	<0.5

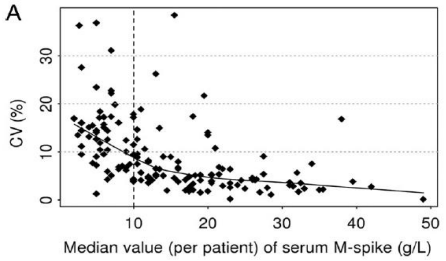
SPEP LOD and IFE/ISUB LOD (g/L) were defined as the lowest M-protein concentration in which an M-protein was detected and qualitatively reported in all samples analyzed. Samples marked with (<) are cases where the LOD was below the tested concentrations.

Jacobs et al. Part II. CCLM in press

Consistency within single institute: intra-laboratory CV%



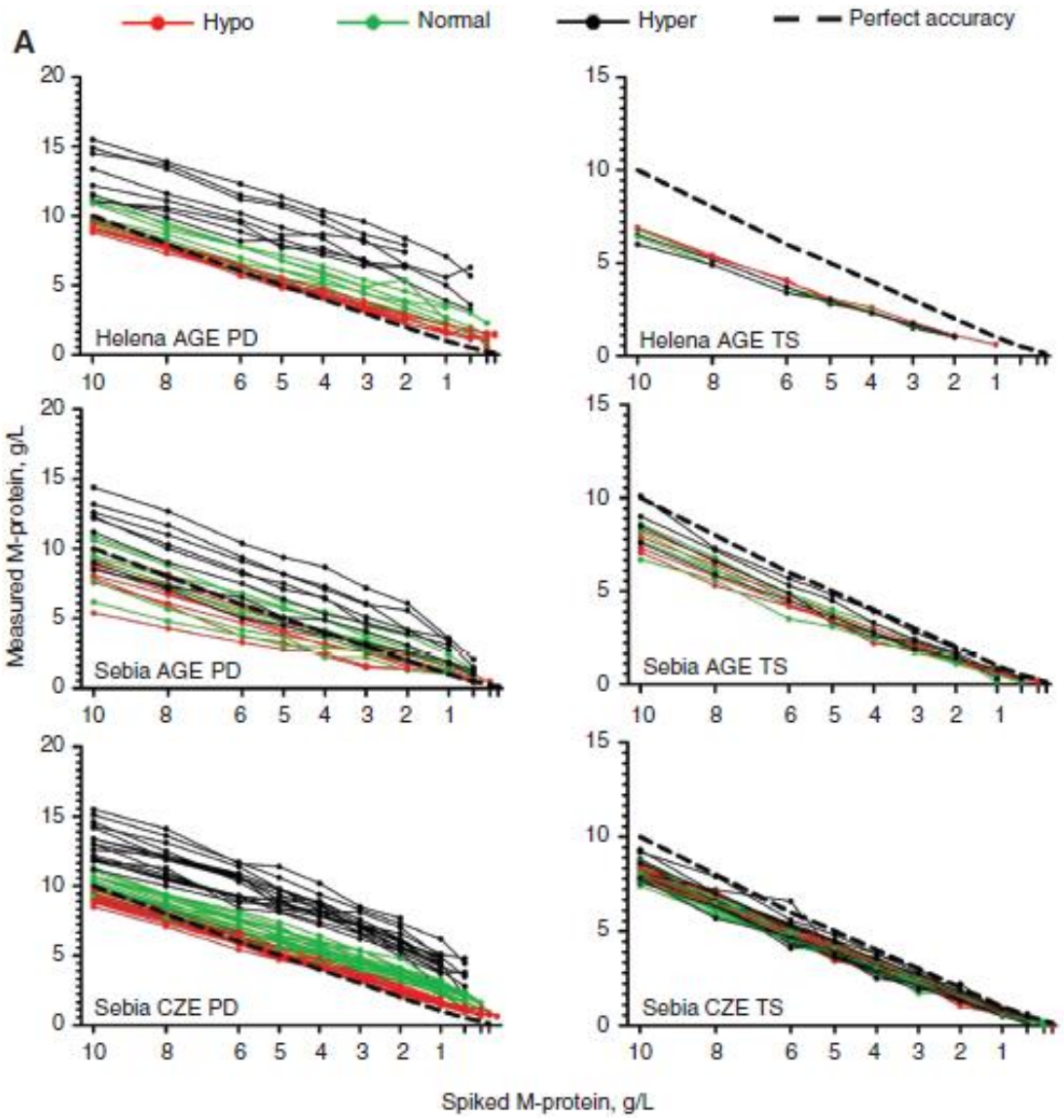
Average CV (all samples)
= 5.0%



Katzmann et al. Clin Chem 2011

Jacobs et al. Part II. CCLM in press

Follow-up within single institute



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Conclusions Part II

Satisfactory intra-laboratory precision was observed (average CV=5%)
Dependent on:

- M-protein concentration (best precision large M-proteins)
- Gamma fraction background (best precision hypo-gamma)
- Gating strategy (best precision PD gating)

'Quantification of small M-proteins to monitor patients over time is appropriate, when subsequent testing is performed within the same laboratory'

Contributions

DE GRUYTER Clin Chem Lab Med 2020; aop

Katherine A. Turner, Jody L. Frinack, Michael W. Ettore, Jillian R. Tate, Maria Stella Graziani, Joannes F.M. Jacobs, Ronald A. Booth, Christopher R. McCudden, David F. Keren, Julio C. Delgado, Galina Zemtsovskaja, Robert O. Fullinfaw, Anna Caldini, Theo de Malmanche, Katina Katakouzinos, Matthew Burke, Giovanni Palladini, Sara Altinier, Martina Zaninotto, Gabriella Righetti, Marie Therese Melki, Stephen Bell and Maria Alice Vieira Willrich*

An international multi-center serum protein electrophoresis accuracy and M-protein isotyping study. Part I: factors impacting limit of quantitation of serum protein electrophoresis

DE GRUYTER Clin Chem Lab Med 2020; aop

Joannes F.M. Jacobs[a,*], Katherine A. Turner[a], Maria Stella Graziani, Jody L. Frinack, Michael W. Ettore, Jillian R. Tate, Ronald A. Booth, Christopher R. McCudden, David F. Keren, Julio C. Delgado, Galina Zemtsovskaja, Robert O. Fullinfaw, Anna Caldini, Theo de Malmanche, Katina Katakouzinos, Matthew Burke, Giovanni Palladini, Sara Altinier, Martina Zaninotto, Gabriella Righetti, Marie Therese Melki, Stephen Bell and Maria Alice Vieira Willrich*

An international multi-center serum protein electrophoresis accuracy and M-protein isotyping study. Part II: limit of detection and follow-up of patients with small M-proteins

Editorial

Mario Plebani

New insights on the analytical performances for detecting and quantifying monoclonal proteins

Mayo Clinic Team – SPEP LOQ Study

Jody Frinack, MT (ASCP)
Development Technologist

- IRB protocol
- Drug purchasing
- Pools preparation
- Sharepoint set-up
- Communications
- Material Transfer Agreements
- Shipping
- Data generation

Michael Ettore
LIS Technical Specialist

- Excel set-up
- Excel macros
- Excel data organization

Katie Turner, Ph.D.
Clinical Chemistry Fellow

- Data analysis
- Data compilation
- Data presentation
- Communications

Maria Willrich, Ph.D.
Protein Immunology
Lab Director

- Principal Investigator

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