Analysis of urinary GAGs: Evaluation of results from the MPS-scheme

Is it time to evaluate your GAG-methodology?

Berthil Prinsen, SSIEM september 2024

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UMC Utrecht

ERNDiM
Quality Assurance in Laboratory Testing for IEM

Celebrating
30 years of ERNDiM
1994–2024

Conflict of interest

Celebrating
30 years of ERNDiM
1994–2024

I declare to have no conflict of interest.

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EQA schemes ERNDIM

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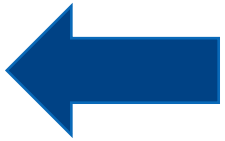
Quantitative schemes
Amino acids (serum)
Acylcarnitines (serum)
Organic acids (urine)
Purines-Pyrimidines (urine)
Special Assays Serum
Special Assays Urine
Special Assays DBS

Qualitative schemes
Amino Acids Interpretation
Diagnostic Proficiency Testing (urine)
Organic acids (urine)
Acylcarnitines (DBS)
Mucopolysaccharides (urine)
CDG (serum)



Hybrid schemes	Interpretation scoring
Lysosomal enzymes (Fib)	Yes
Cystine (WBC)	Yes
Pterins (urine)	Yes
Neurotransmitters (CSF)	Yes

2018: 15 EQA schemes, 1788 scheme registrations

2023: 17 EQA schemes, 2165 scheme registrations (+21%)

Website reporting

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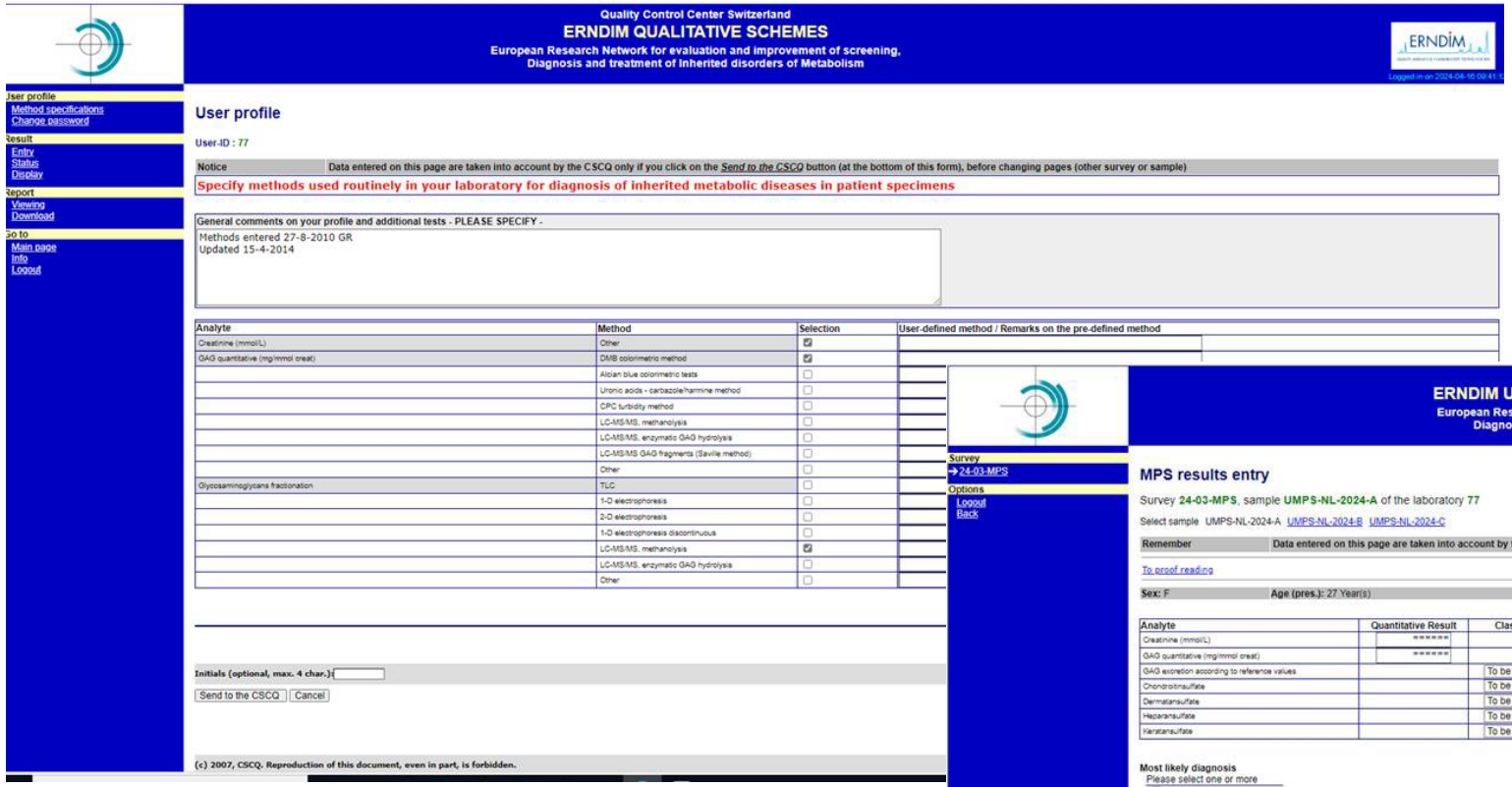
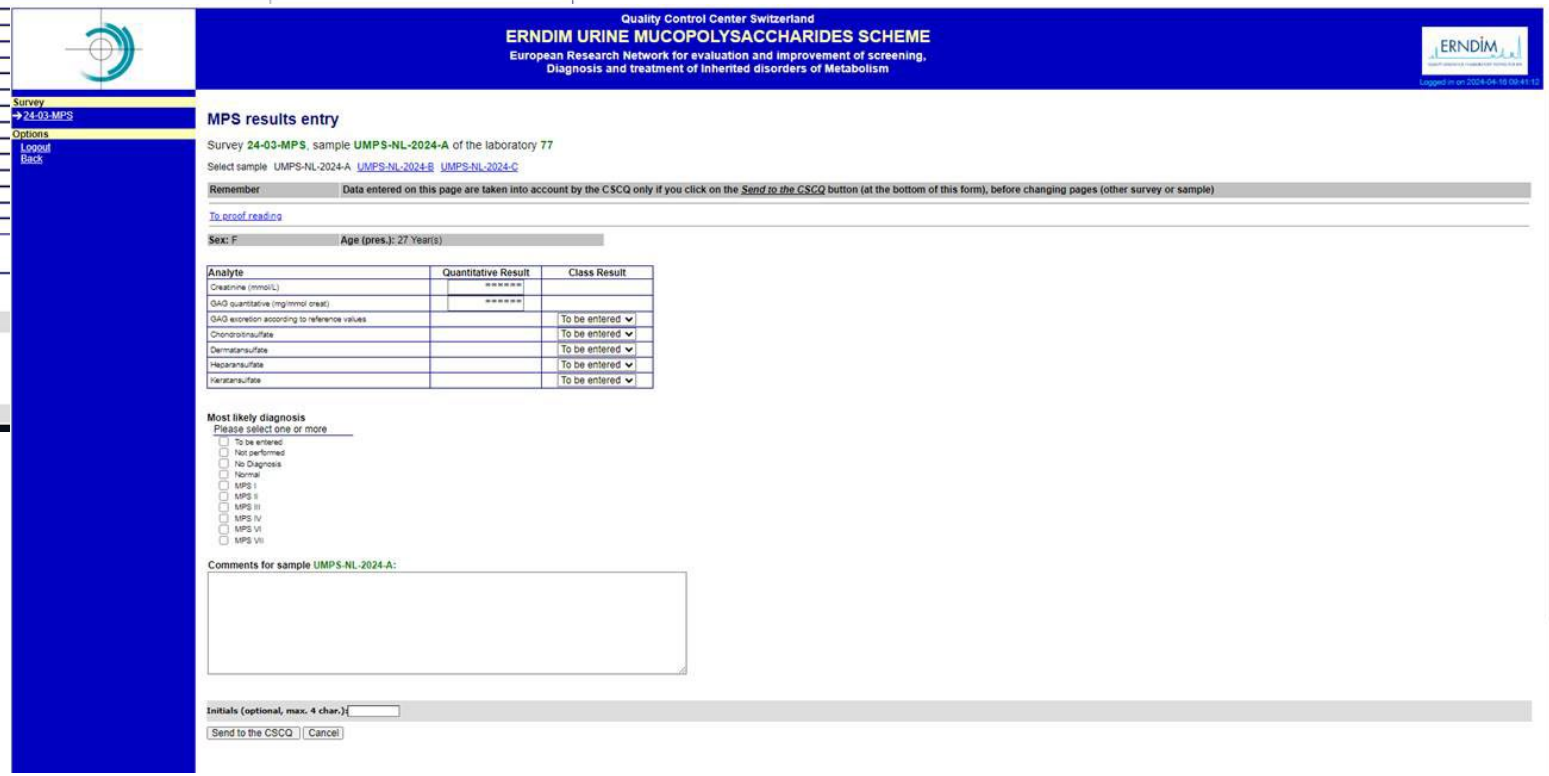
1994–2024



Analytical results

Diagnostic interpretation



GAG-analysis (urine)

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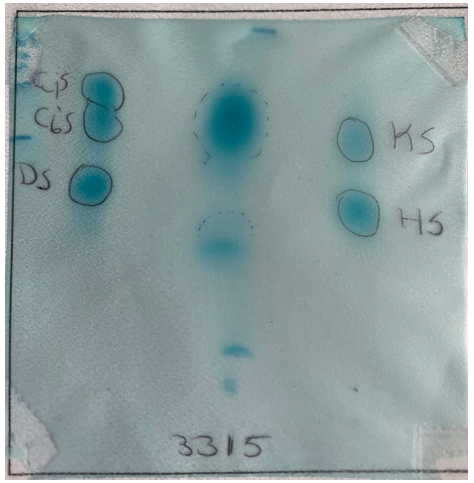
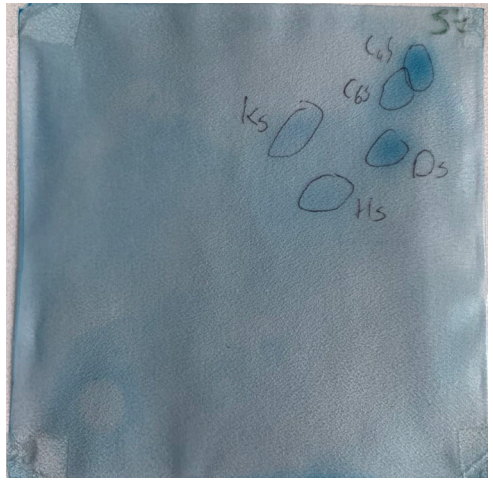
Quantitative test:

- 1,9-dimethylmethylene blue (DMB)
- Alcian Blue
- Carbazole
- Cetylpyridinium chloride (CPC) turbidity
- Harmine testing
- HPLC
- Mass-spectrometry
- …

GAG-subtyping:

- 1D-electrophoresis (discontinuous)
- 2D-electrophoresis
- Capillary Zone Electrophoresis
- HPLC
- TLC
- Mass-spectrometry
- …





ERNDIM UMPS scheme: GAG-analysis

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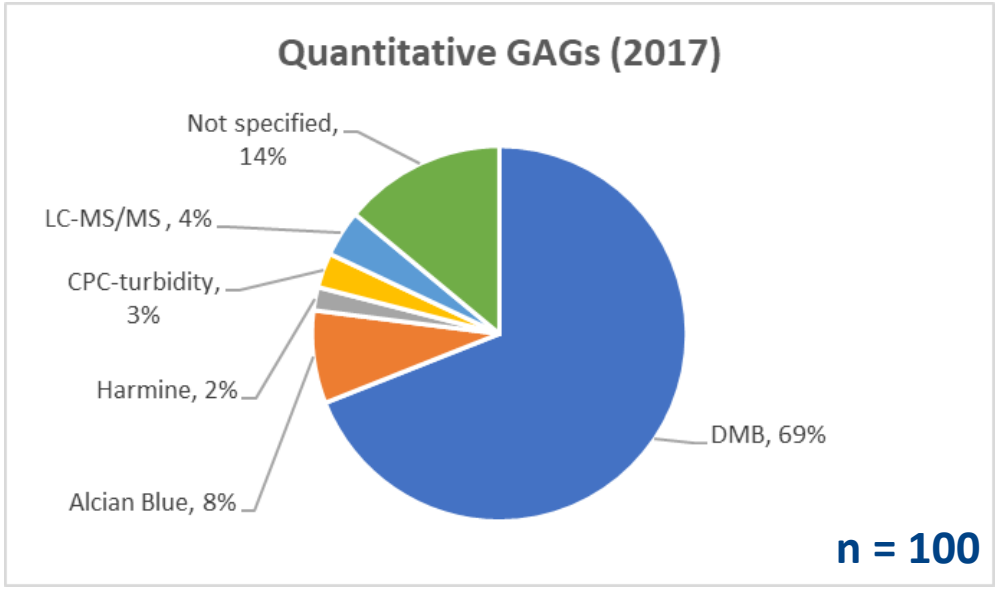
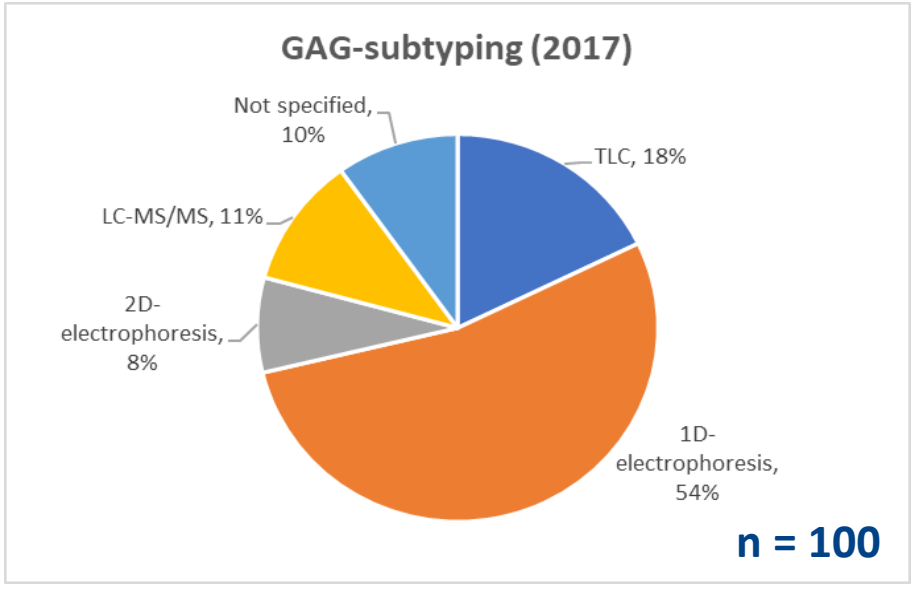
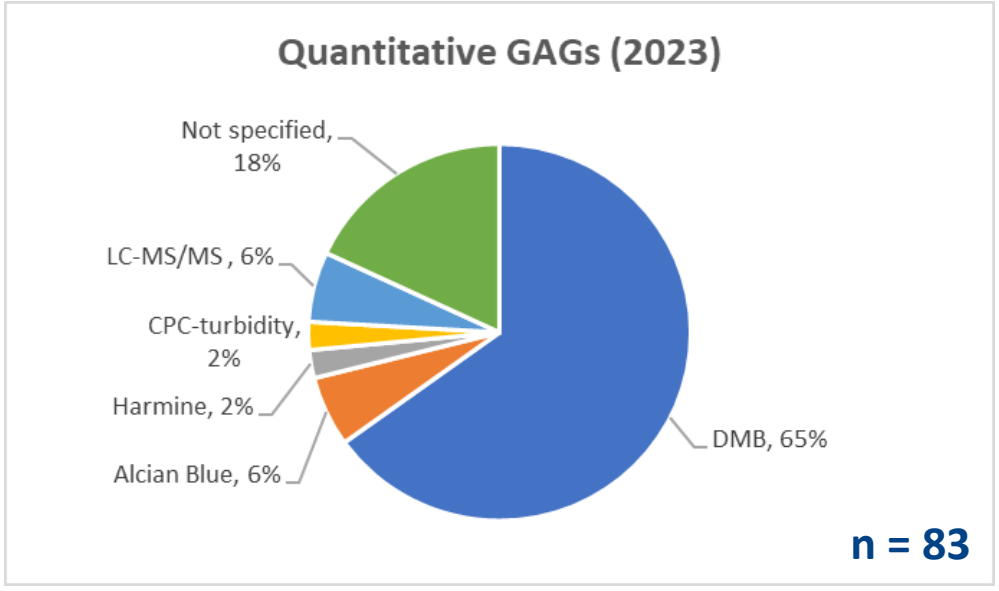
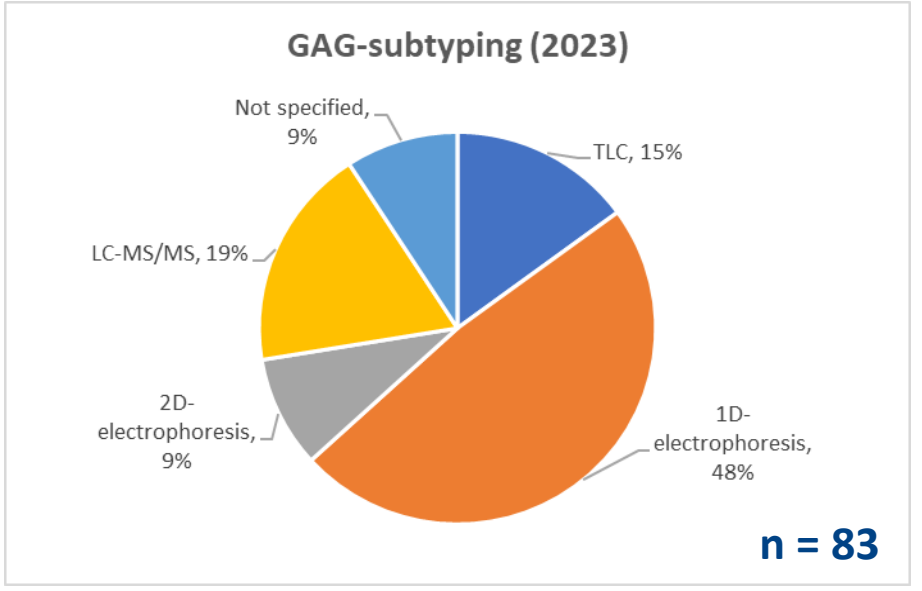


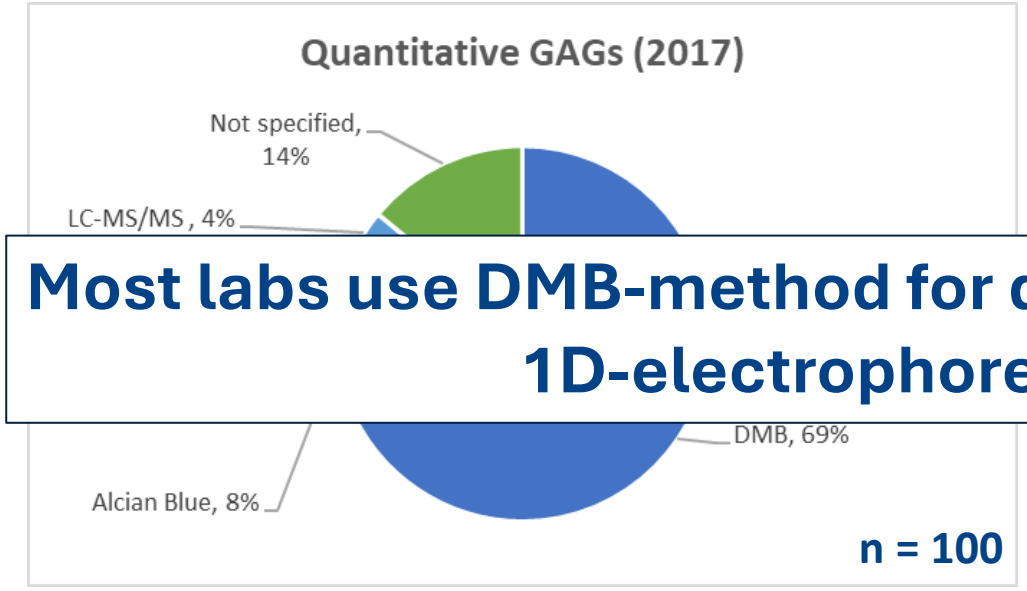


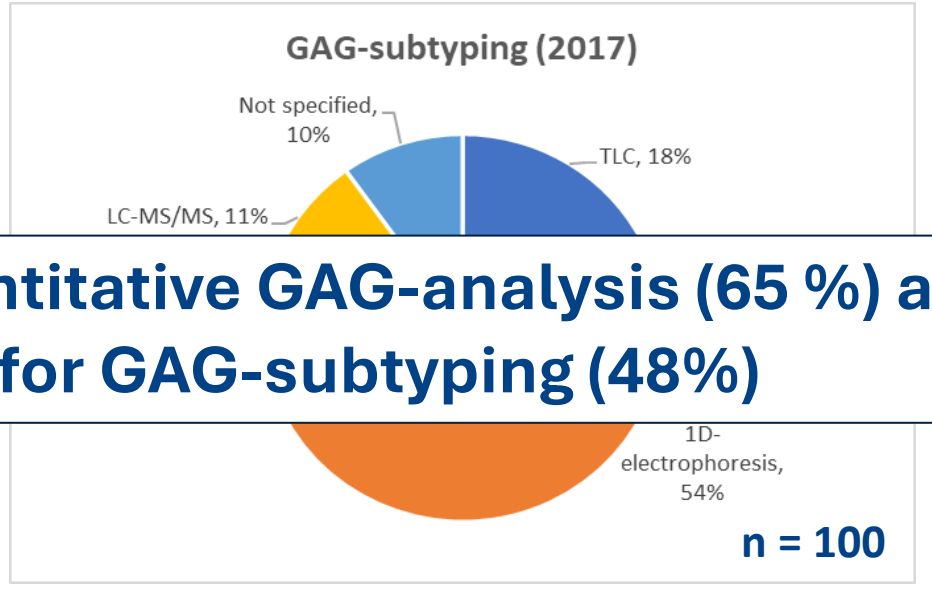




ERNDIM UMPS scheme: GAG-analysis

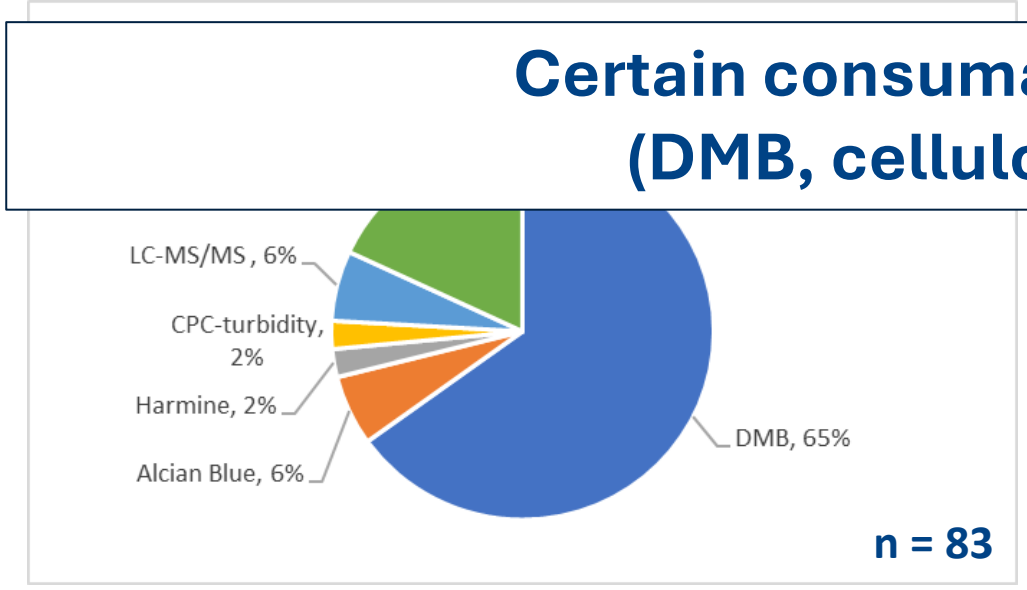
Celebrating 30 years of ERNDiM 1994–2024

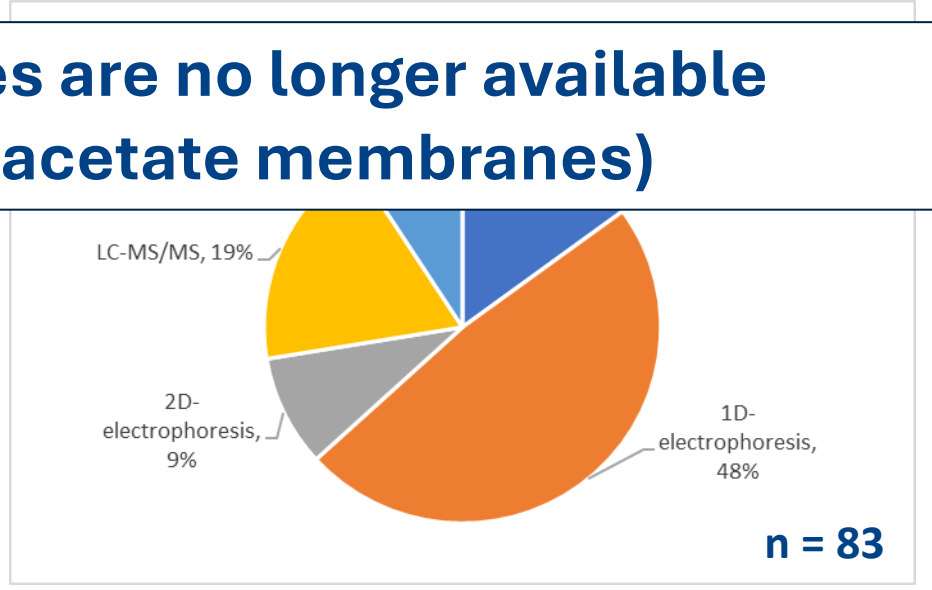




Most labs use DMB-method for quantitative GAG-analysis (65 %) and 1D-electrophoresis for GAG-subtyping (48%)

Certain consumables are no longer available (DMB, cellulose-acetate membranes)





GAG-analysis by mass-spectrometry

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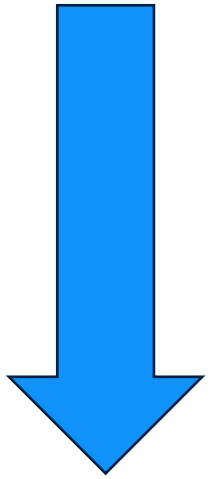
- **Chemical degradation (methanolysis)**
  - *Zhang et al., Clinical Chemistry, 57, 1005-1012, 2011*
  - *Zhang et al., Molecular Genetics and Metabolism, 114: 123-128, 2015*
  - *Auray-Blais et al., Analytica Chimica Acta, 936, 139-148, 2016*

- **Enzymatic degradation**
  - *Langereis et al., Plos One 10, e0138622, 2015*
  - *Kubashi et al., Molecular Genetics and Metabolism, 120, 247-254, 2017*

- **Chemical and Enzymatic degradation (combined)**
  - *Mathis et al., JIMD Reports, 65:116-123, 2024*

- **GAG-substrate analysis**
  - *Lawrence et al., Nature Chemical Biology, 8:197-204, 2012*
  - *Saville et al. Genetics in Medicine, 21:753-757, 2019*

Complex GAGs



Mono/disaccharides

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Participants that use MS-technology for GAG-analysis

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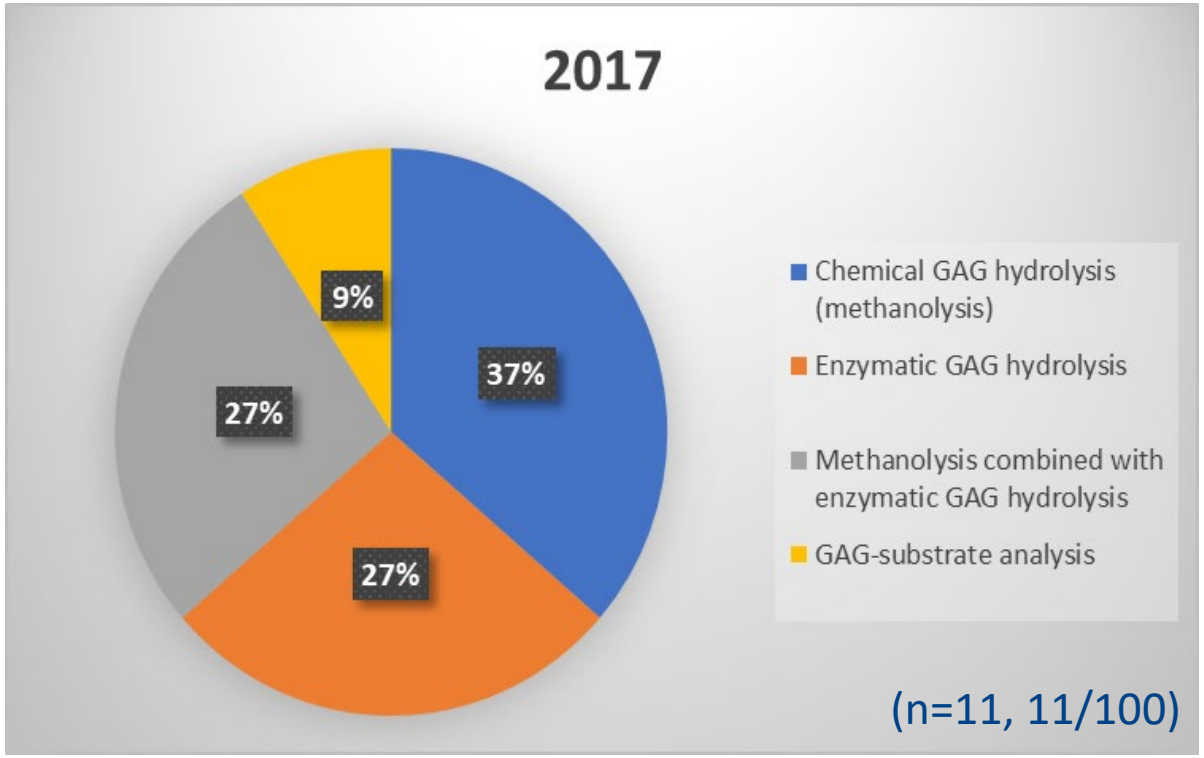

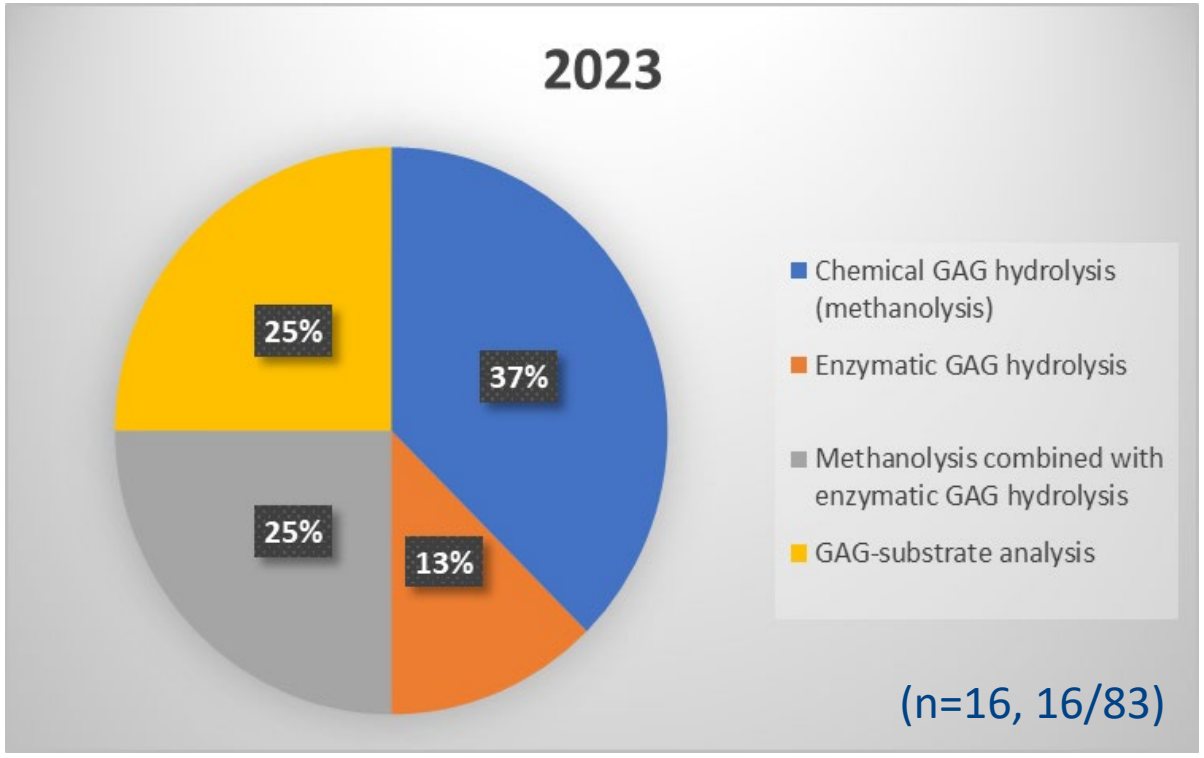

Labs that use MS-technology for GAG-analysis (19.7%, 2023)

Diagnosis of defects in GAG-metabolism: Added value for mass-spectrometry

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Comparative Study > Hum Mol Genet. 2017 Jan 1;26(1):173-183. doi: 10.1093/hmg/ddw377.

Mutation in VPS33A affects metabolism of glycosaminoglycans: a new type of mucopolysaccharidosis with severe systemic symptoms

Hidehito Kondo [1], Nadezda Maksimova [2], Takanobu Otomo [1 3 4], Hisakazu Kato [5], Atsuko Imai [6 7], Yoshihiro Asano [6], Kaori Kobayashi [7 8], Satoshi Nojima [9], Akihiro Nakaya [7], Yusuke Hamada [1], Kaori Irahara [1], Elizaveta Gurinova [10], Aitalina Sukhomyasova [2 10], Anna Nogovicina [10], Mira Savvina [2], Tamotsu Yoshimori [3 4], Keiichi Ozono [1], Norio Sakai [1 11]

Affiliations + expand

PMID: 28013294 DOI: 10.1093/hmg/ddw377

Case Reports > Clin Genet. 2021 Sep;100(3):308-317. doi: 10.1111/cge.14002. Epub 2021 Jun 4.

Homozygous missense VPS16 variant is associated with a novel disease, resembling mucopolysaccharidosis-plus syndrome in two siblings

Yılmaz Yıldız [1 2], Can Koşukcu [1 3], Damla Aygün [1], Meltem Akçaboy [4], Fatma Zehra Öztek Çelebi [4], Yasemin Taşcı Yıldız [5], Gülseren Şahin [6], Caner Aytekin [7], Deniz Yüksel [8], İncilay Lay [9], Rıza Köksal Özgül [1 10], Ali Dursun [1]

Affiliations + expand

PMID: 34013567 DOI: 10.1111/cge.14002

> J Med Genet. 2022 Oct;59(10):957-964. doi: 10.1136/jmedgenet-2021-108061. Epub 2021 Dec 16.

Novel subtype of mucopolysaccharidosis caused by arylsulfatase K (ARSK) deficiency

Sarah Verheyen [1], Jasmin Blatterer [1], Michael R Speicher [1], Gandham SriLakshmi Bhavani [2], Geert-Jan Boons [3 4], Mai-Britt Ilse [5], Dominik Andrae [5], Jens Sproß [6], Frédéric Maxime Vaz [7], Susanne G Kircher [8], Laura Posch-Pertl [9], Daniela Baumgartner [10], Torben Lübke [5], Hitesh Shah [11], Ali Al Kaissi [12], Katta M Girisha [13], Barbara Plecko [14]

Affiliations + expand

PMID: 34916232 PMCID: PMC9554054 DOI: 10.1136/jmedgenet-2021-108061

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Biomarkers for disease and therapy?

Celebrating 30 years of ERNDiM 1994–2024

Metab Brain Dis (2017) 32:1403–1415
DOI 10.1007/s11011-017-0009-1

CrossMark

ORIGINAL ARTICLE

Serum global metabolomics profiling reveals profound metabolic impairments in patients with MPS IIIA and MPS IIIB

Haiyan Fu[1,2] · Aaron S. Meadows[1] · Ricardo J. Pineda[1] · Robert P. Mohney[3] · Steve Stirdivant[3] · Douglas M. McCarty[1,2]

Clinica Chimica Acta 541 (2023) 117250

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

ELSEVIER

Untargeted LC-HRMS metabolomics reveals candidate biomarkers for mucopolysaccharidoses

Clarisse L. Torres[a], Fernanda B. Scalco[b], Maria Lúcia C. de Oliveira[b], Roy W.A. Peake[c], Rafael Garrett[a,c,*]

Human Molecular Genetics, 2017, Vol. 26, No. 19 3837–3849

doi: 10.1093/hmg/ddx277
Advance Access Publication Date: 19 July 2017
Original Article

OXFORD

ORIGINAL ARTICLE

Abnormal polyamine metabolism is unique to the neuropathic forms of MPS: potential for biomarker development and insight into pathogenesis

Christian Hinderer[1,†], Nathan Katz[1,†], Jean-Pierre Louboutin[2], Peter Bell[1], Jakub Tolar[3], Paul J. Orchard[3], Troy C. Lund[3], Mohamad Nayal[1], Liwei Weng[4], Clementina Mesaros[4], Carolina F.M. de Souza[5], Amauri Dalla Corte[5,7], Roberto Giugliani[6,7] and James M. Wilson[1,*]

International Journal of *Molecular Sciences*

MDPI

Review

Biomarkers for Lysosomal Storage Disorders with an Emphasis on Mass Spectrometry

Ryuichi Mashima *, Torayuki Okuyama and Mari Ohira

Department of Clinical Laboratory Medicine, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan; okuyama-t@ncchd.go.jp (T.O.); ohira-m@ncchd.go.jp (M.O.)
* Correspondence: mashima-r@ncchd.go.jp

Received: 28 February 2020; Accepted: 4 April 2020; Published: 14 April 2020

check for updates

Advantages of mass-spectrometry based techniques for GAG-analysis

Celebrating 30 years of ERNDiM 1994–2024

- Mass-spectrometry can perform both quantitative GAGs and GAG-subtyping in a single run
- Mass-spectrometry is a sensitive and specific method for detecting metabolites → less urine is needed for routine diagnostics
- Different GAG-species or GAG-related metabolites can be quantified
- Follow-up of high risk screening (NBS, e.g. MPS-I)
- …

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ERNDIM UMPS scheme

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1994–2024

1. Detection of metabolites

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GAG-subtyping: MPS-I(H) (M, 5 years old, circulated in 2022)

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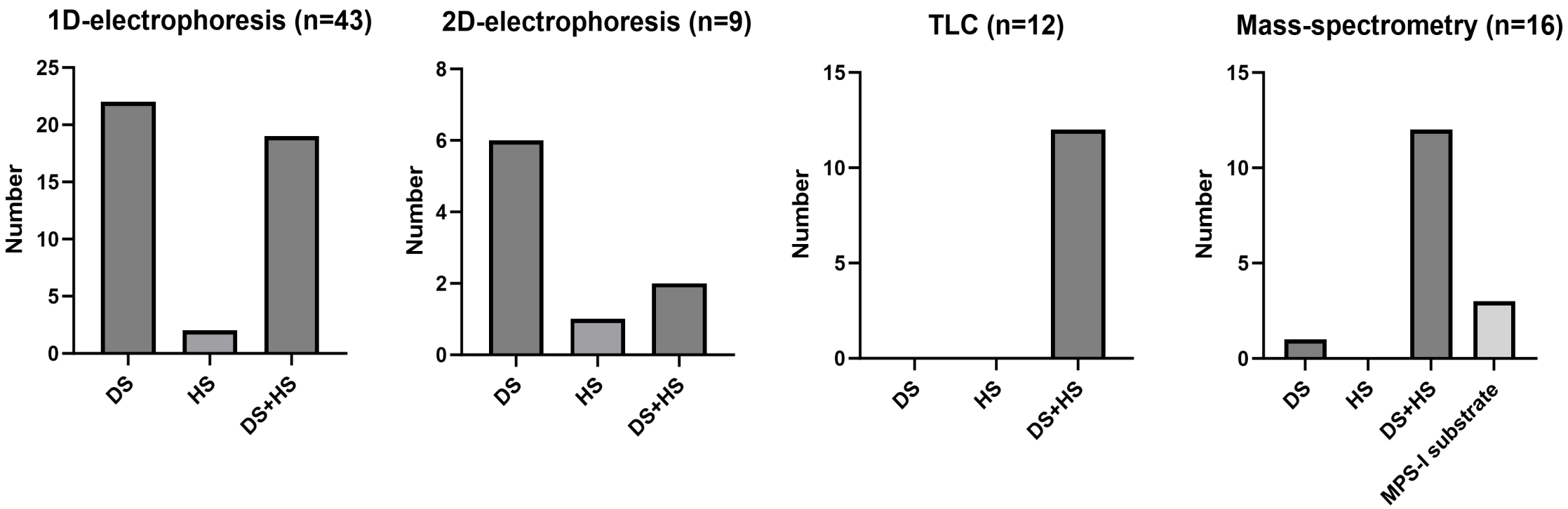
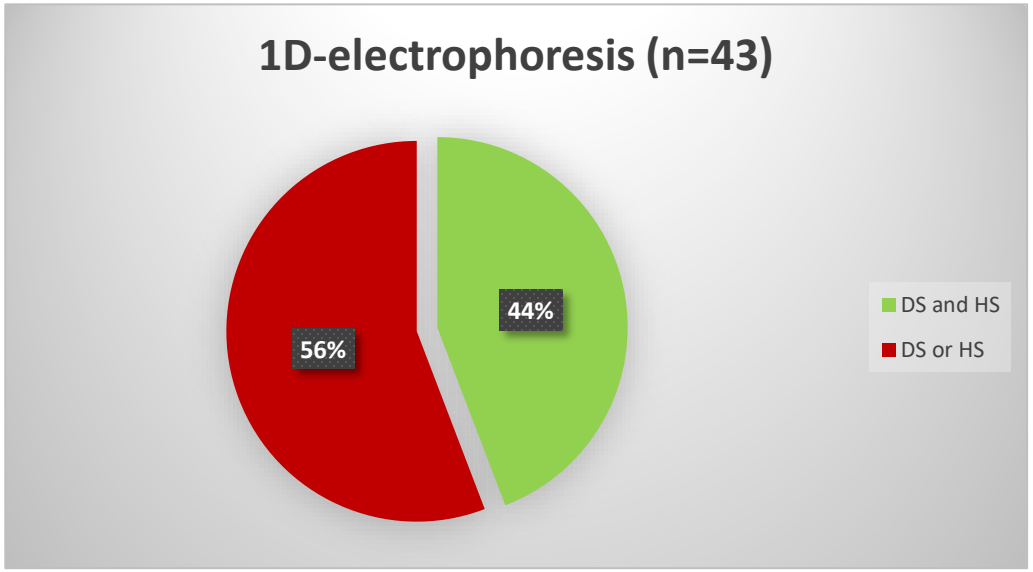
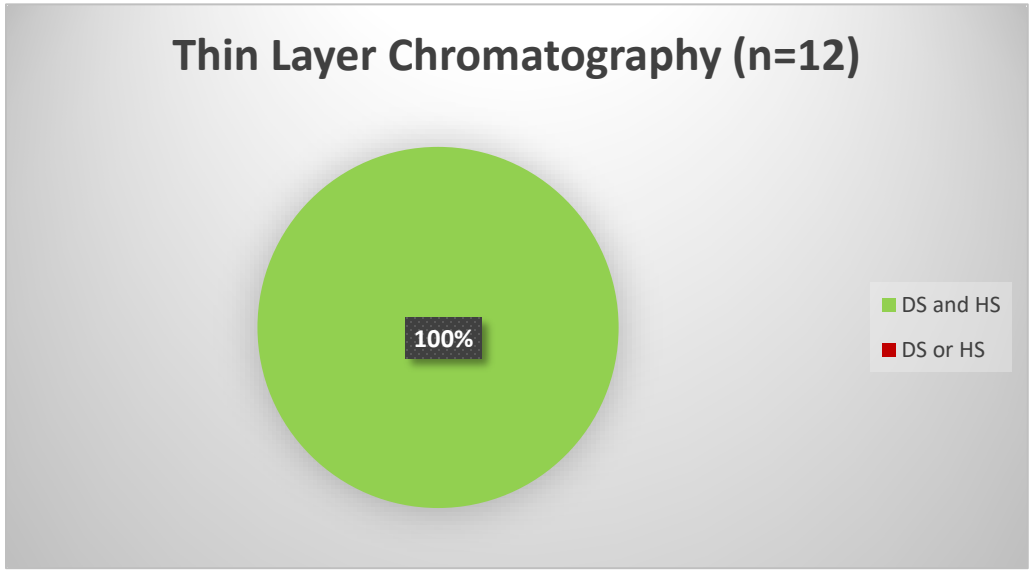


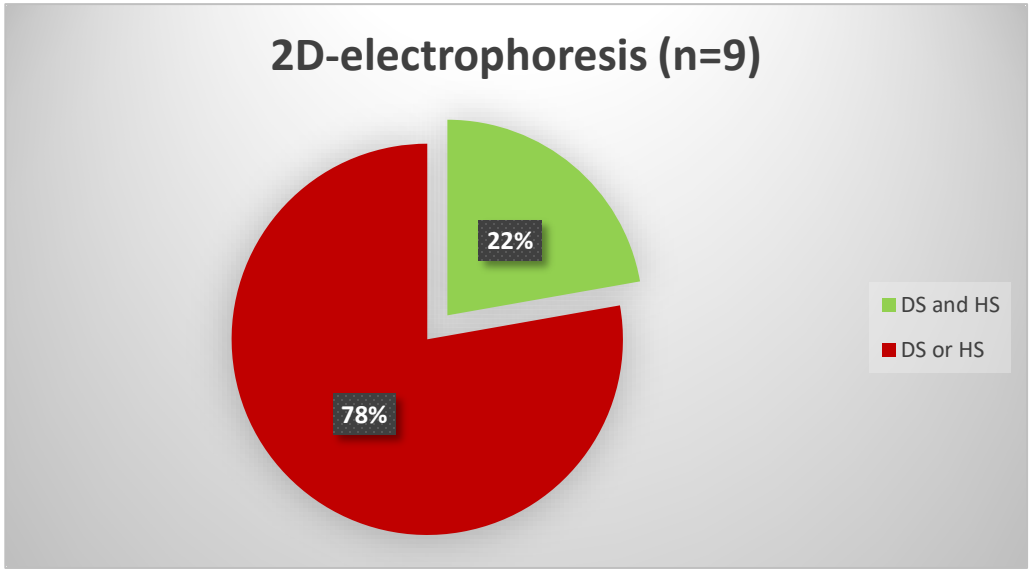
Similar sample for MPS-I was circulated in 2018, 2020 and 2021

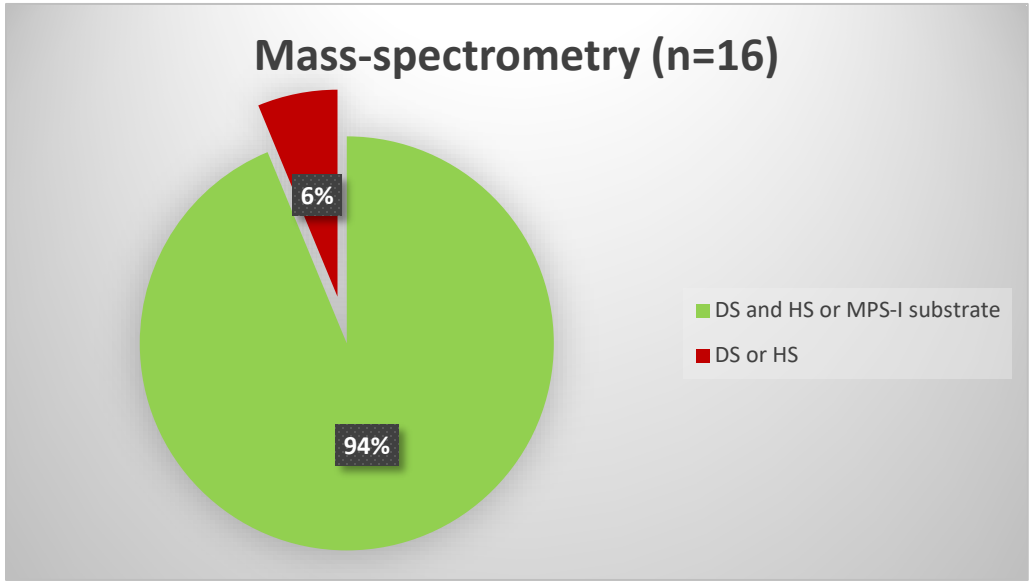
GAG-subtyping: MPS-I(H) (M, 5 years old, circulated in 2022)

Celebrating 30 years of ERNDiM 1994–2024



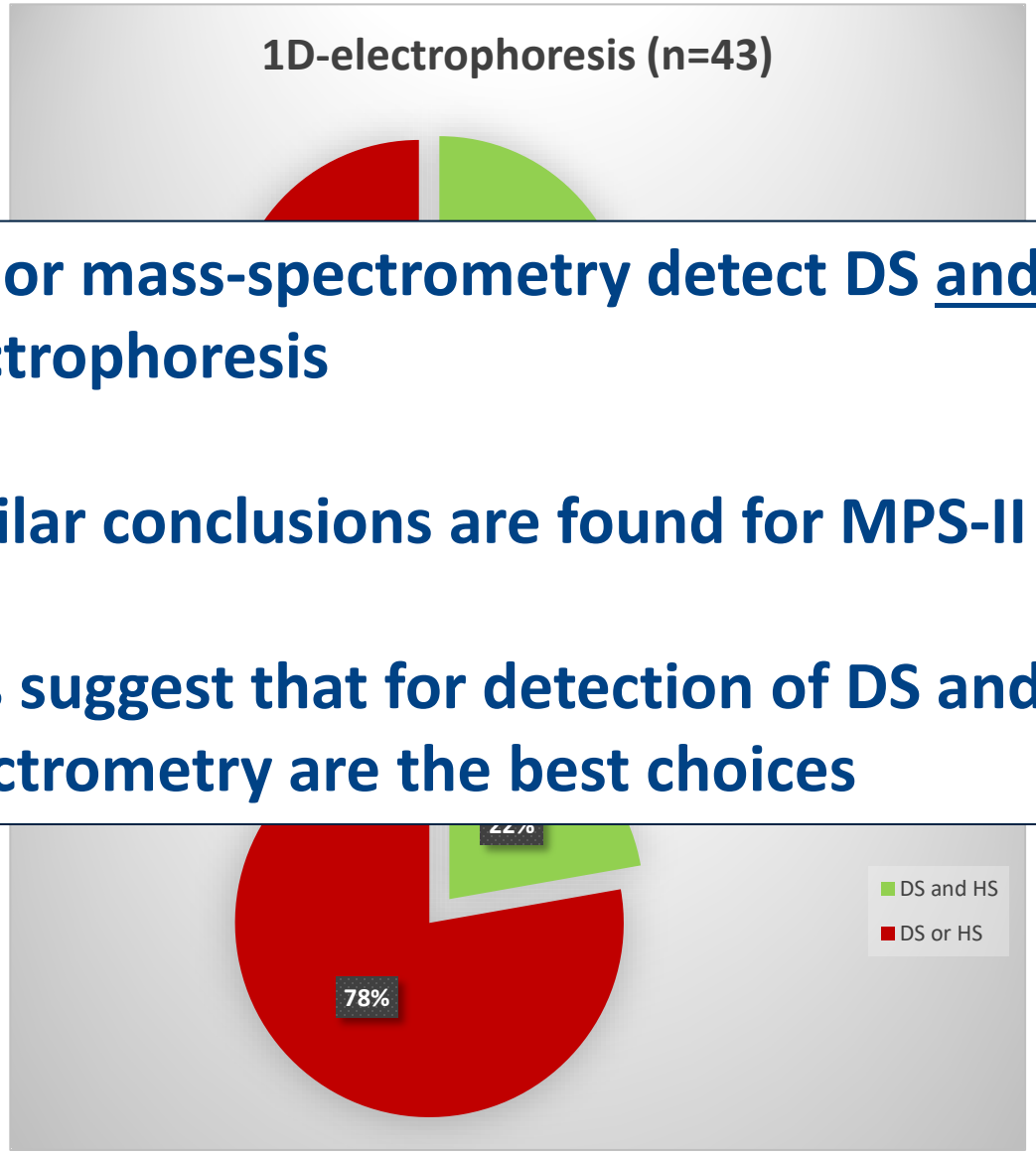


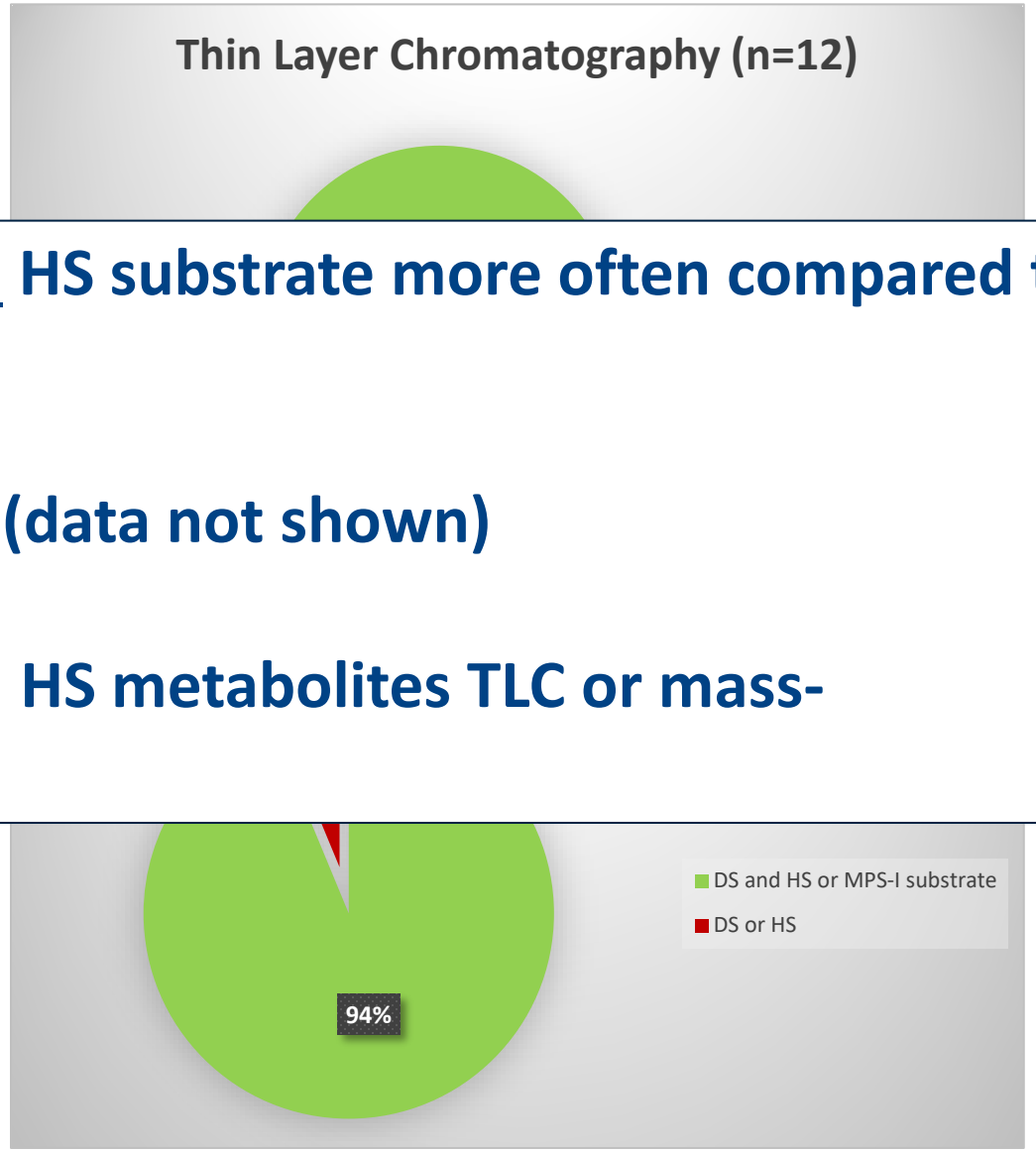




GAG-subtyping: MPS-I(H) (M, 5 years old, circulated in 2022)

Celebrating 30 years of ERNDiM 1994–2024





TLC or mass-spectrometry detect DS and HS substrate more often compared to electrophoresis

Similar conclusions are found for MPS-II (data not shown)

This suggest that for detection of DS and HS metabolites TLC or mass-spectrometry are the best choices

ERNDIM UMPS scheme

Celebrating
30 years of ERNDiM
1994–2024

1. Detection of metabolites
2. **GAG-subtyping in MPS-IV**

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GAG-subtyping: MPS-IVA (F, 22 years old, circulated in 2023)

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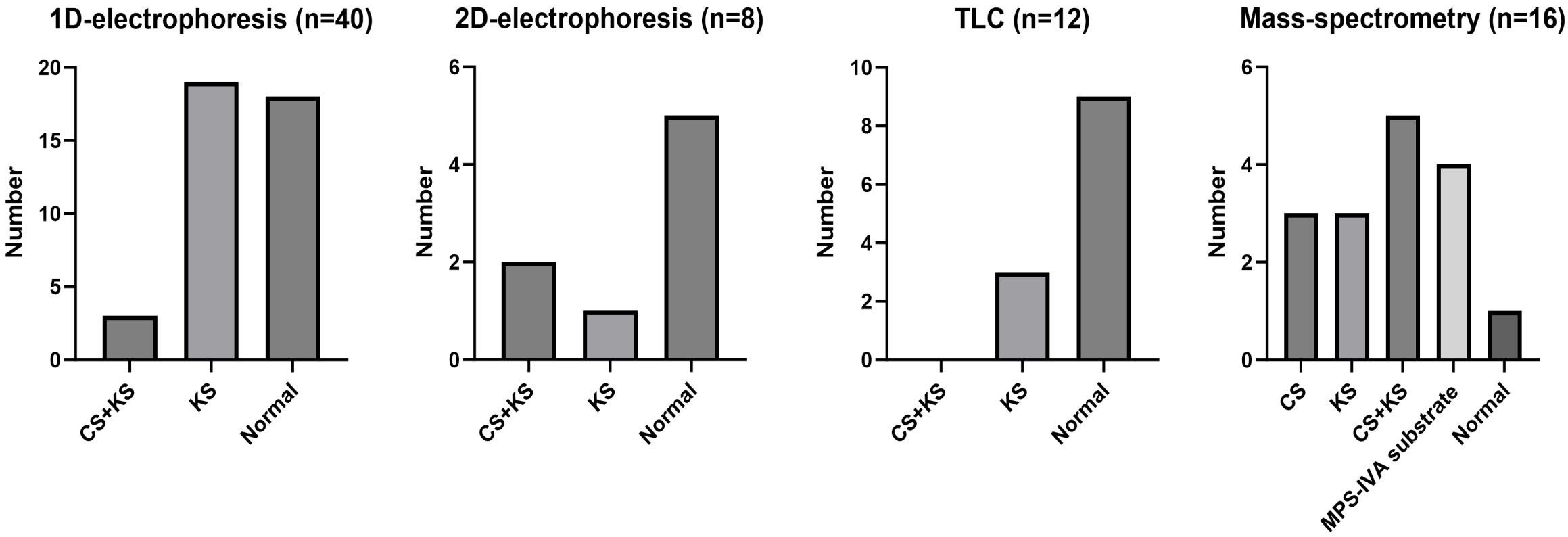
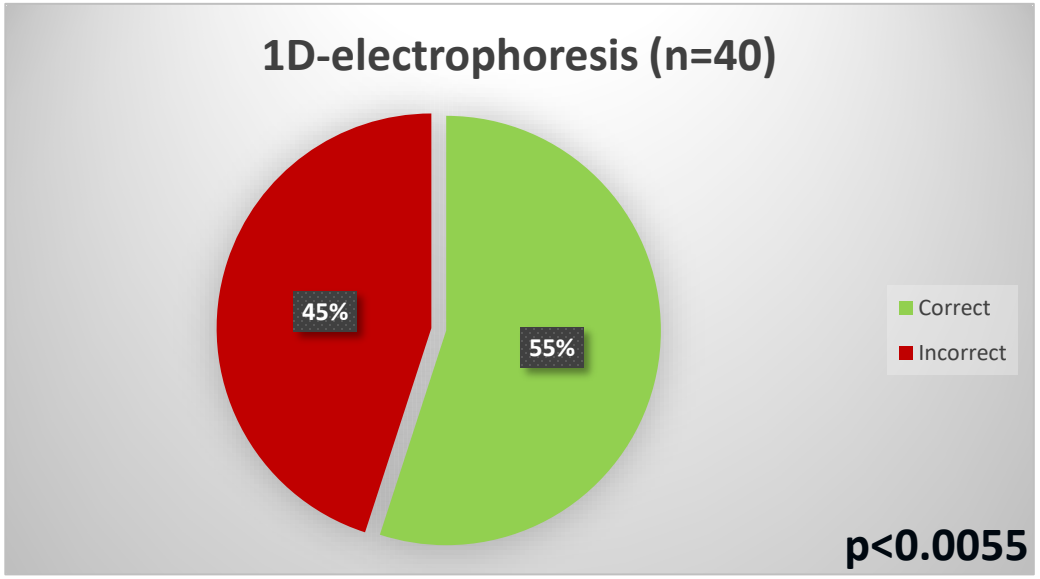


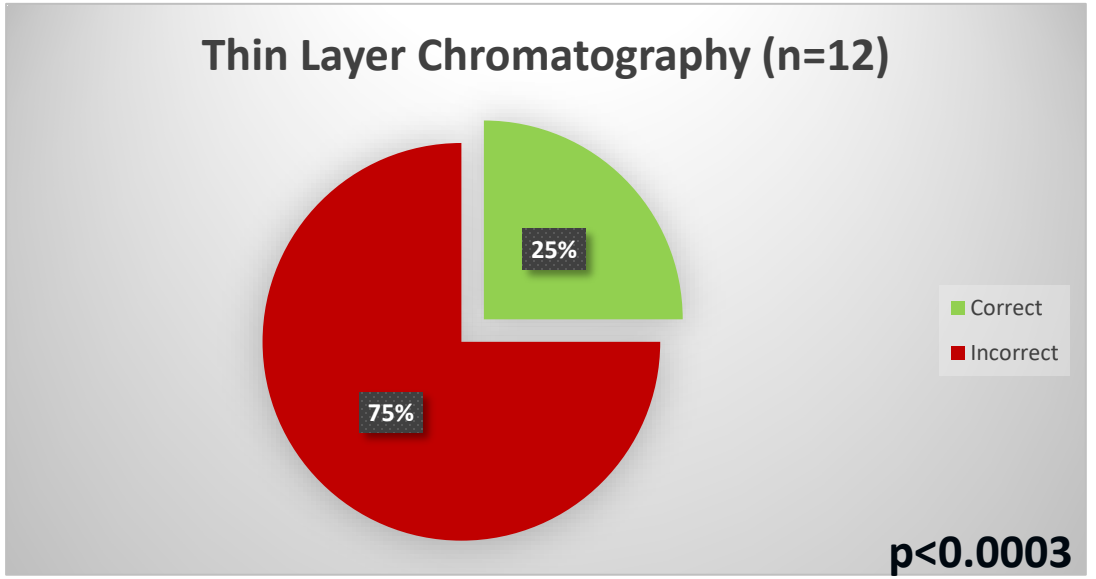
Similar sample for MPS-IVA was circulated in 2018, 2020 and 2021

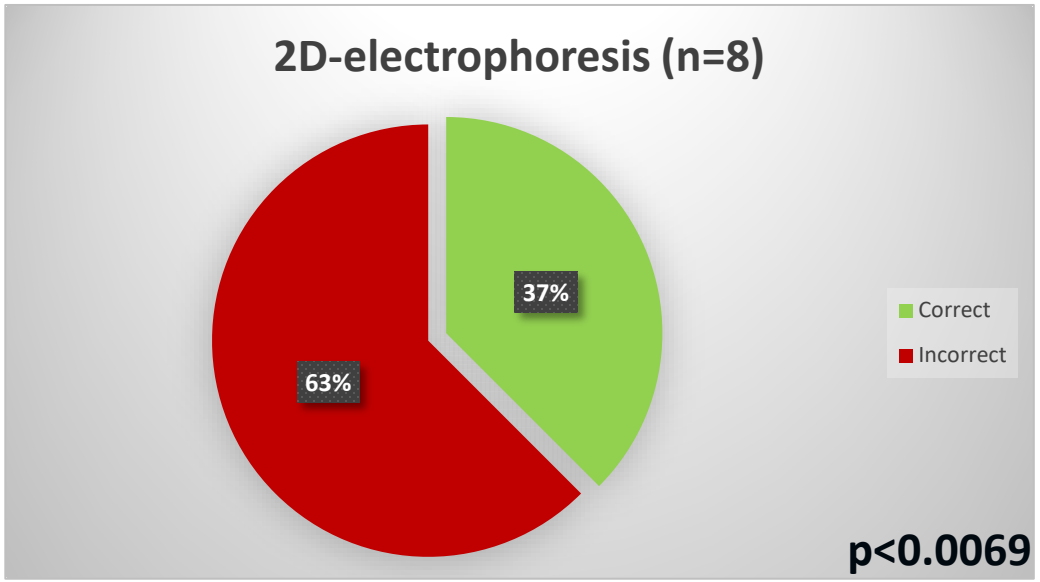
GAG-subtyping: MPS-IVA (F, 22 years old, circulated in 2023)

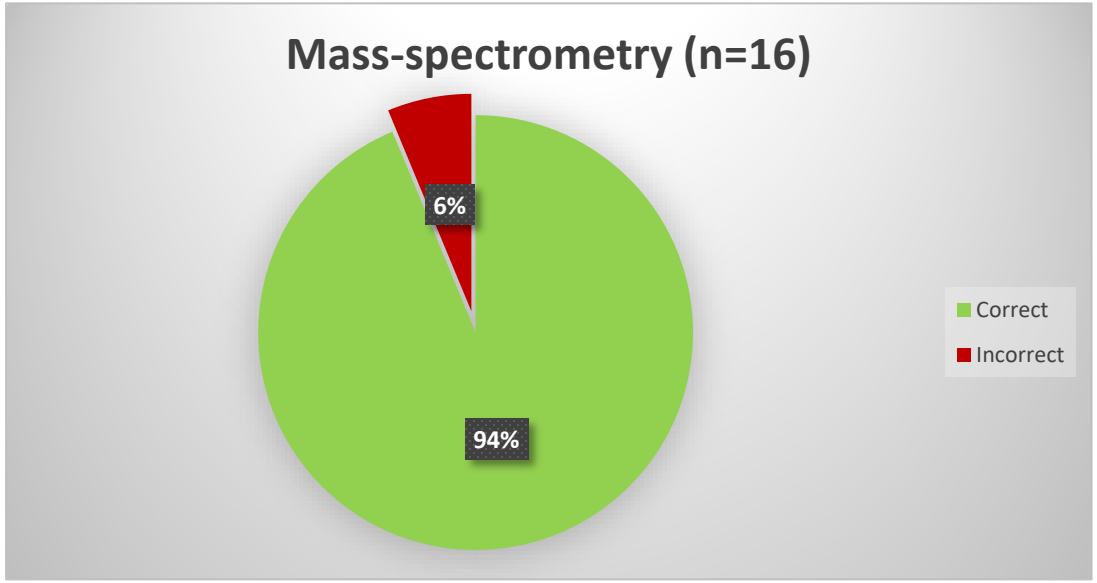
Celebrating 30 years of ERNDiM 1994–2024

Statistical analysis: Fisher exact test





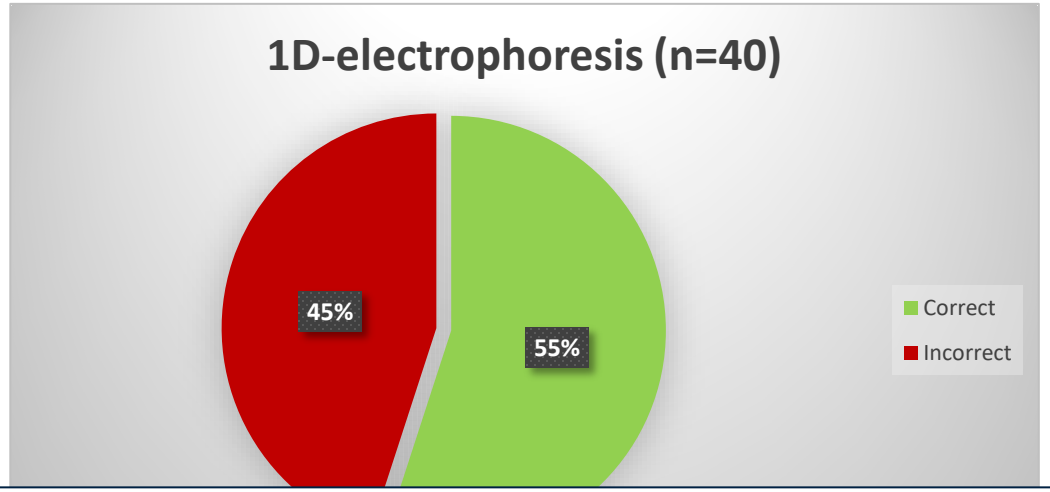


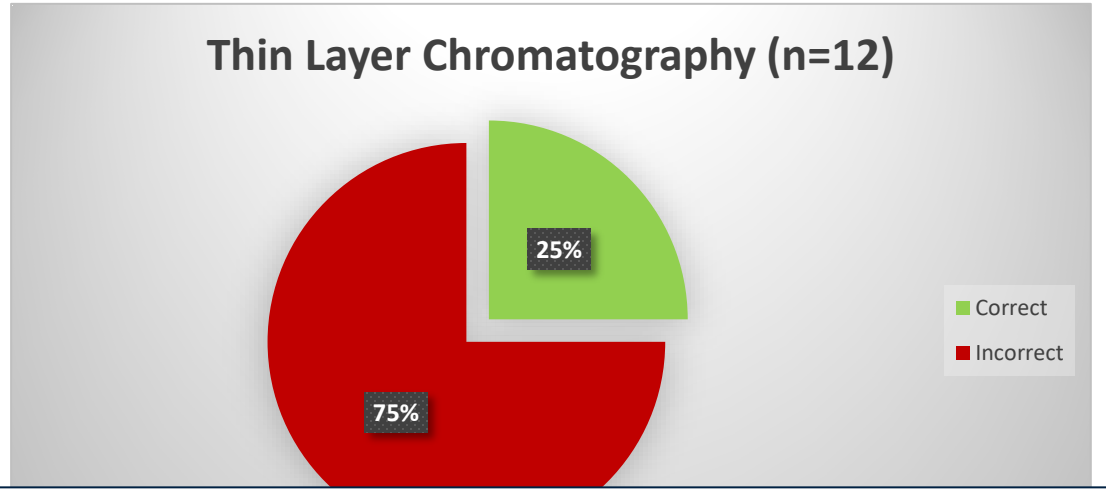


GAG-subtyping: MPS-IVA (F, 22 years old, circulated in 2023)

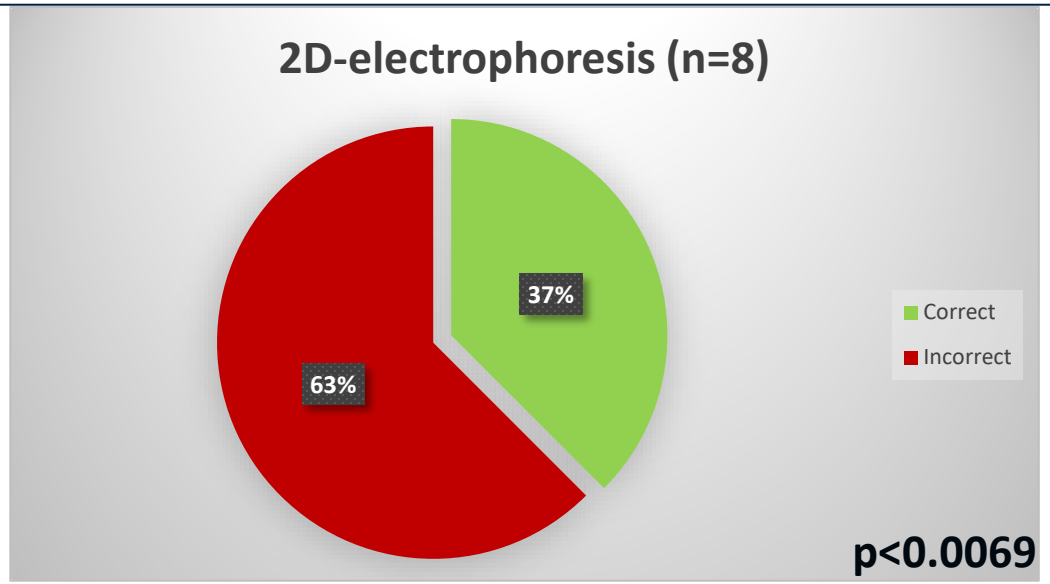
Celebrating 30 years of ERNDiM 1994–2024

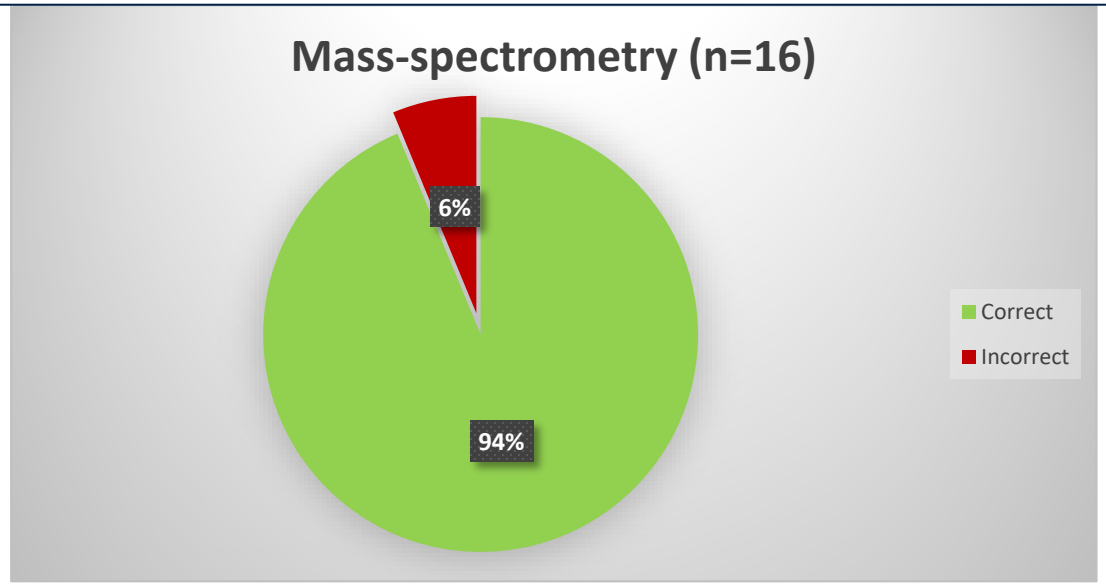
Statistical analysis: Fisher exact test





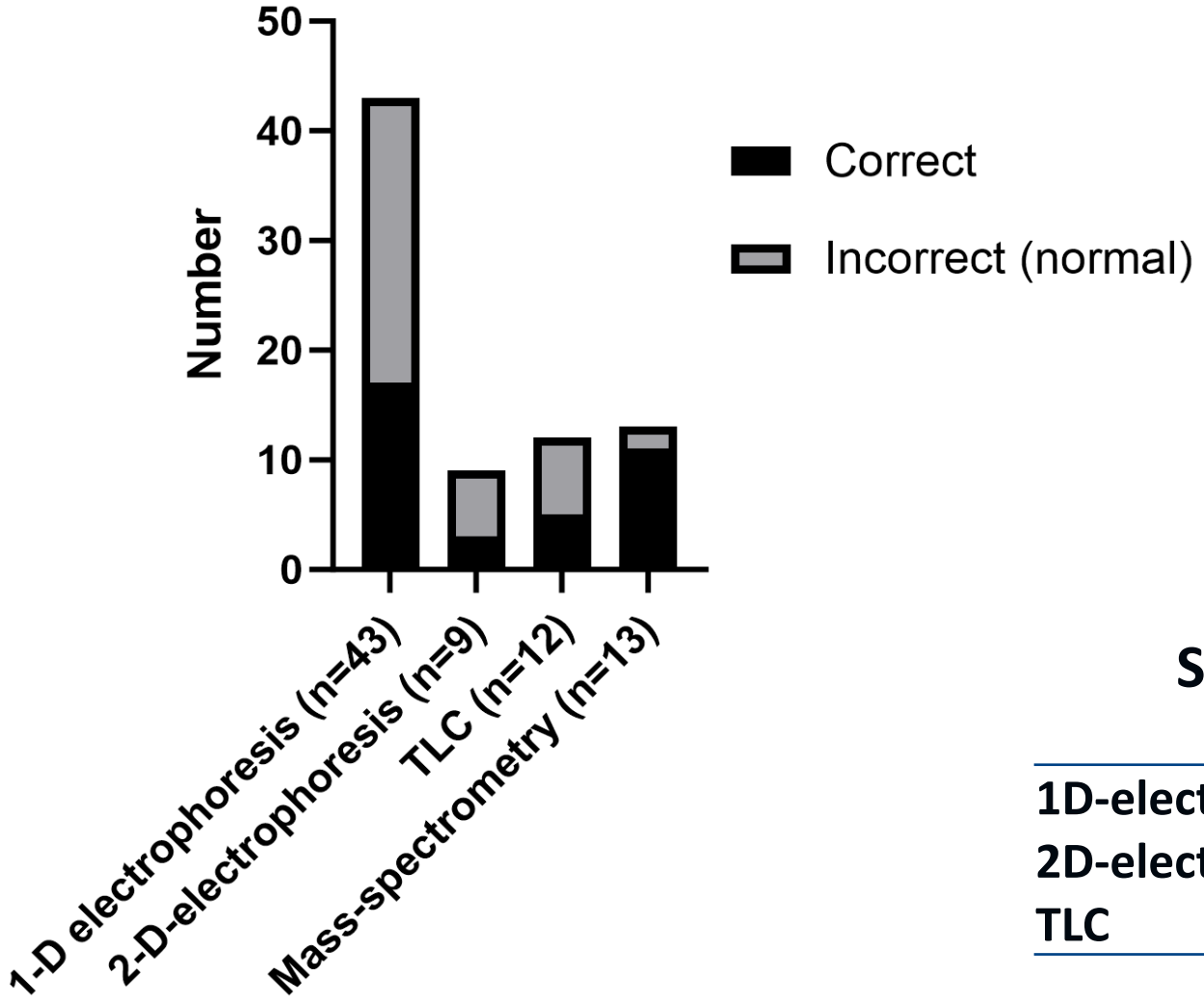
Mass spectrometry is superior to other techniques for diagnosing MPS-IVA





GAG-subtyping: MPS-IVB (F, 51 years old, circulated in 2022)

Celebrating 30 years of ERNDiM 1994–2024

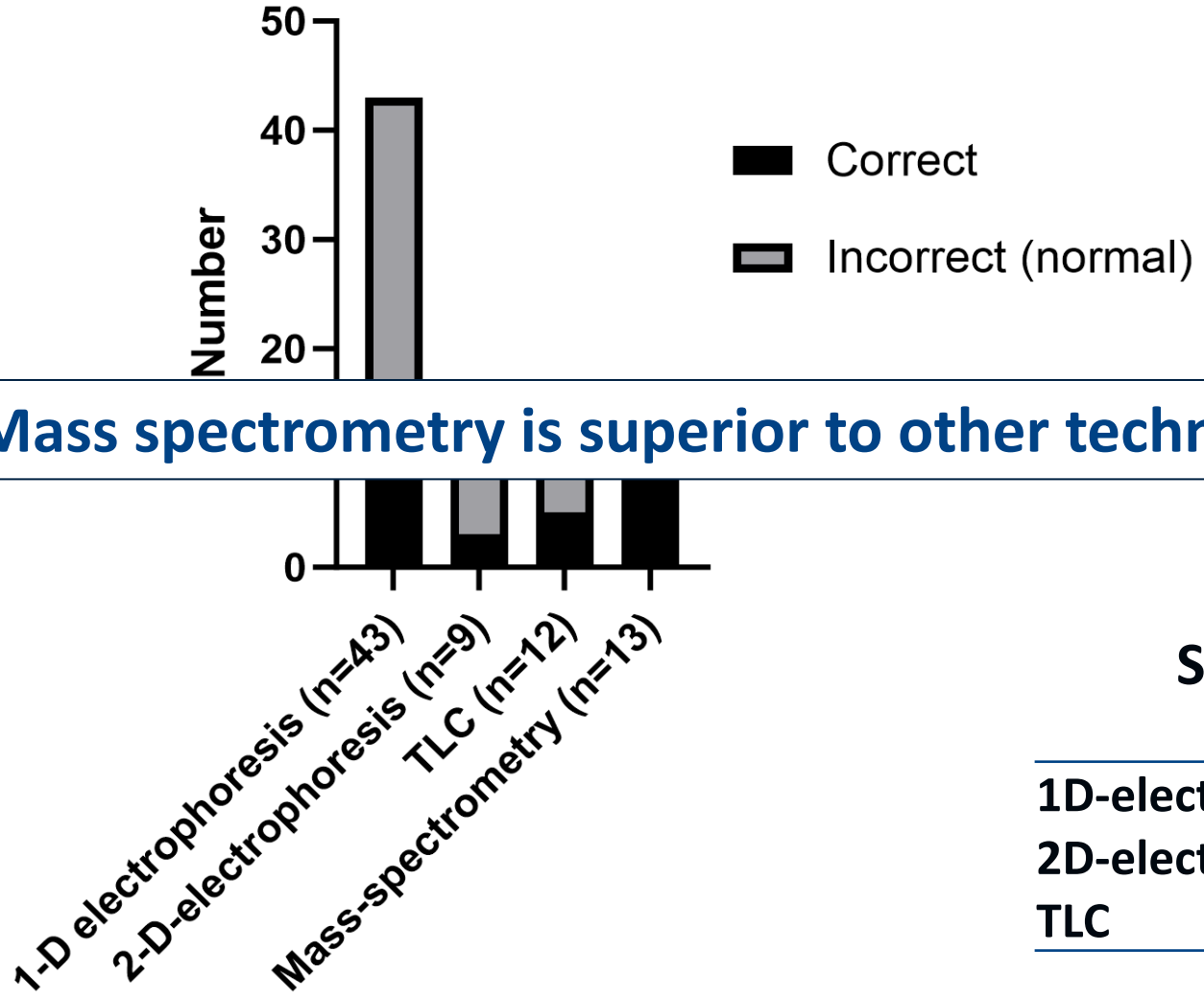


Statistical analysis: Fisher exact test

1D-electrophoresis	Mass-spectrometry	p=0.0091
2D-electrophoresis	Mass-spectrometry	p=0.03
TLC	Mass-spectrometry	p=0.04

GAG-subtyping: MPS-IVB (F, 51 years old, circulated in 2022)

Celebrating 30 years of ERNDiM 1994–2024



Mass spectrometry is superior to other techniques for diagnosing MPS-IVA + B

Statistical analysis: Fisher exact test

1D-electrophoresis	Mass-spectrometry	p=0.0091
2D-electrophoresis	Mass-spectrometry	p=0.03
TLC	Mass-spectrometry	p=0.04

ERNDIM UMPS scheme

Celebrating
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1994–2024

1. Detection of metabolites
2. GAG-subtyping in MPS-IV
3. **Analytical Performance**

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Analytical Performance: (Quantitative GAGs and GAG-subtyping)

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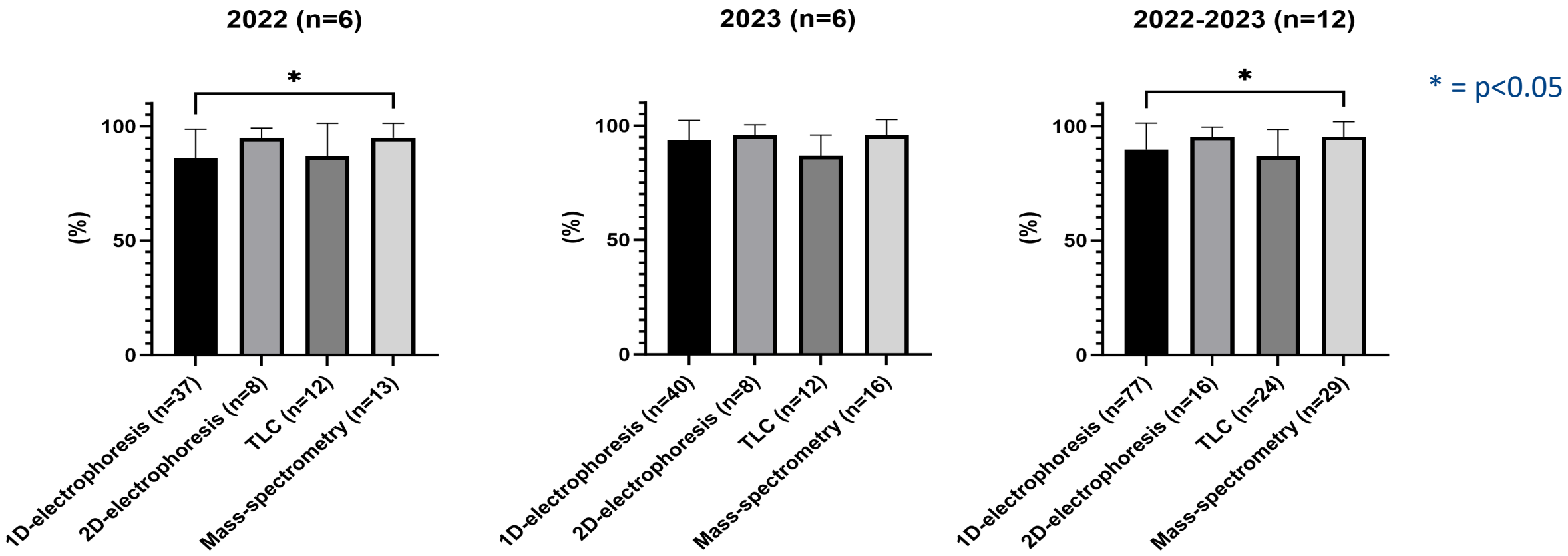
1994–2024



* = p<0.05

Scoring criteria:
GAGs quantitative : 1 mark, GAG-subtyping : 1 mark

2022: A: Control, B: MPS-I, C: MPS-II, D: MPS-IVB, E: Control, F: MPS-III
2023: A: MPS-IVA, B: MPS-I, C: MPS-III, D: MPS-I, E: Control, F: MPS-II
Not included: MPS-VI, MPS-VII

Take home messages

Celebrating
30 years of ERNDiM
1994–2024

Considerations for evaluation of GAG-methodology

- Mass-spectrometry performs both quantitative GAGs and GAG subtyping in a single experiment
- Labspecialists should be aware of the limited availability of consumables (DMB, cellulose-acetate membranes) in certain countries
- Mass-spectrometry can have an added value for diagnosis of IEM with mild disturbances in GAG-metabolism
- Mass-spectrometry detect more metabolites than conventional methods, metabolites that are relevant for diagnosis
- Mass-spectrometry is superior to other techniques for diagnosing MPS-IVA and MPS-IVB (GAG-subtyping)
- Analytical Performance is better for mass-spectrometry compared with 1D-electrophoresis (2022-2023)

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Thank you for your attention

Celebrating 30 years of ERNDiM 1994–2024

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