Mucopolysaccharidoses
diagnostic approaches

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OCCURRENCE OF URINARY ACID MUCOPOLYSACCHARIDES IN THE HURLER SYNDROME*

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The presence of abnormal amounts of a chemically unidentified substance in tissues of patients with the Hurler syndrome (lipochondrodystrophy gargoylism dysostosis multiplex) has been recognized.[1] Brante[2] recovered a substance with characteristics of an acid mucopolysaccharide from tissues obtained at autopsy (liver, meninges) of patients with this disorder. He suggested that this material, which was metachromatic and contained 3.9 per cent sulfur, 27 per cent hexosamine, and 26 per cent glucuronic acid, was similar to or identical with chondroitinsulfuric acid. Dawson[3] and Uzman[4] have also suggested that the "storage material" in
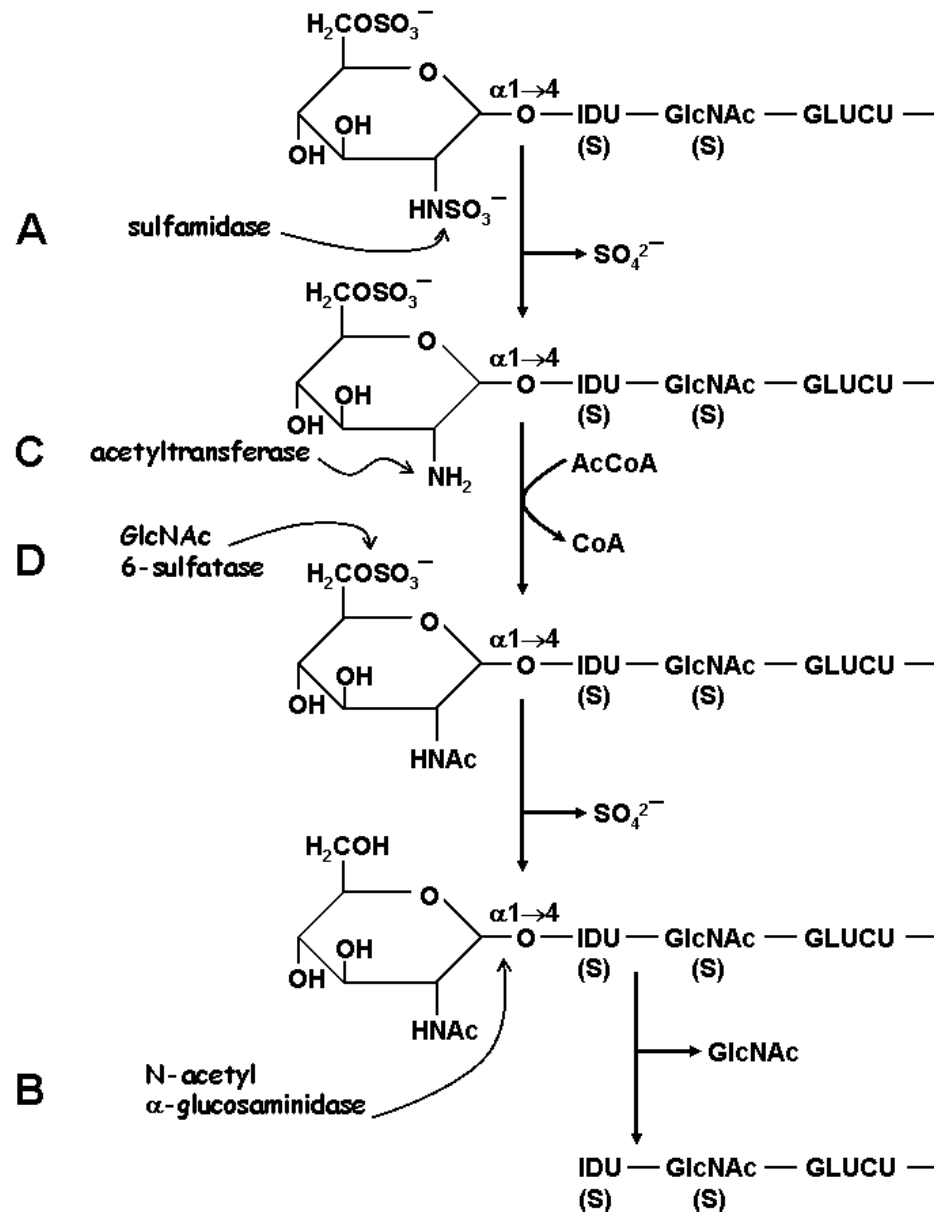
Mucopolysaccharidoses

MPS I		Hurler	α-L-iduronidase
MPS II		Hunter	iduronate sulfatase
MPS III	A	Sanfilippo	heparan N-sulfatase (sulfamidase)
	B		α-NAc-glucosaminidase
	C		AcCoA:α-glucosaminide Ac trf.
	D		GlcNAc 6-sulfatase
	E ?		glucuronate sulfatase
MPS IV	A	Morquio	Galactose 6-sulfatase
	B		β-galactosidase
MPS VI		Maroteaux-Lamy	GalNAc 4-sulfatase (arylsulfatase B)
MPS VII		Sly	β-glucuronidase
MPS IX ?		Natowicz	hyaluronidase
			(endo-hexosaminidase)

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Degradation of glycosaminoglycans (GAG; mucopolysaccharides)
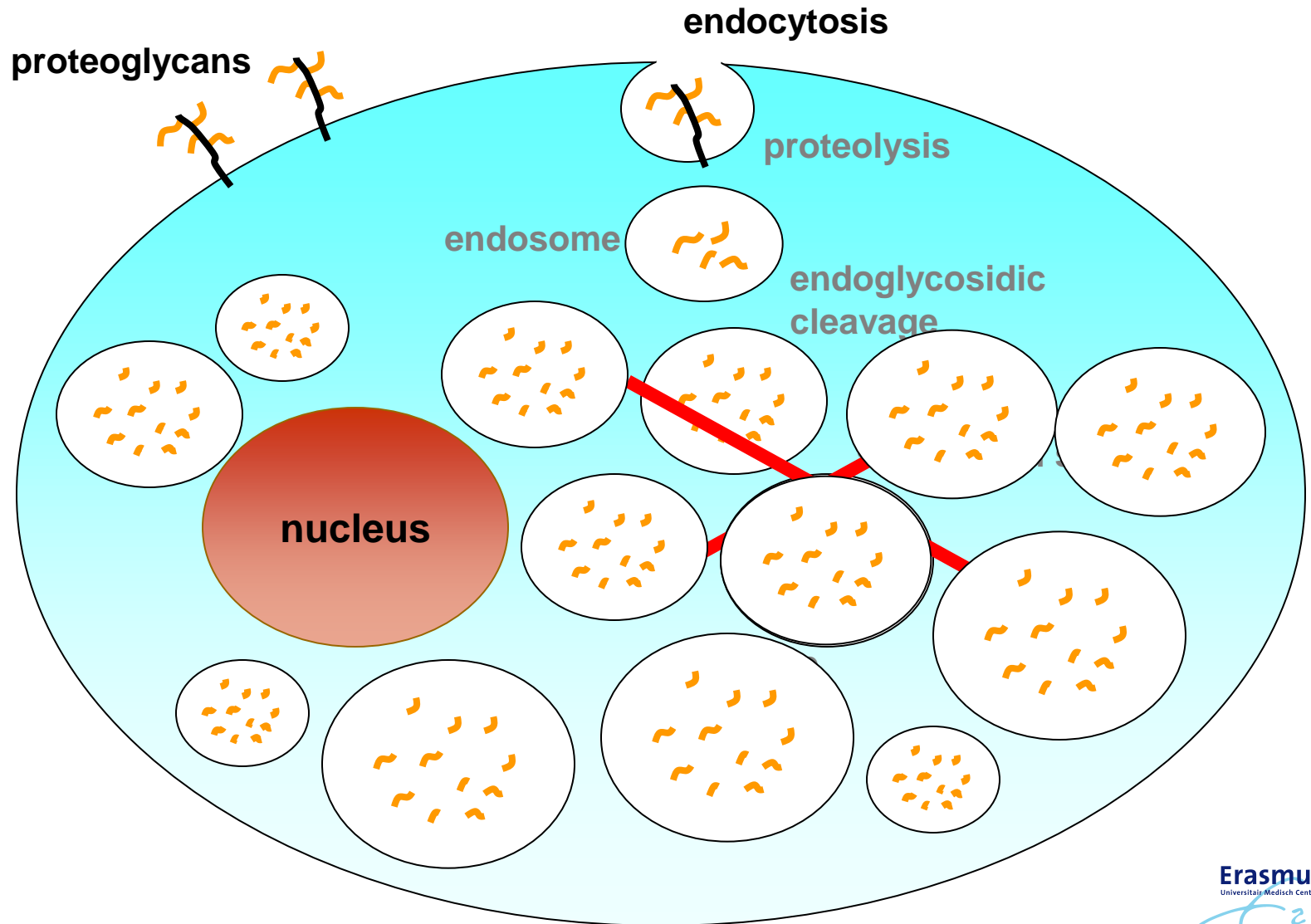
Heparan sulfate degradation

GAG in urine

Type	GAG storage
Normal	-
MPS I	DS + HS
MPS II	DS + HS
MPS III	HS
MPS IV	KS + C6S
MPS VI	DS
MPS VII	CS + DS + HS

CS	**Chondroitinsulfate**
DS	**Dermatansulfate**
HS	**Heparansulfate**
KS	**Keratansulfate**

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Diagnostic tests for mucopolysaccharidoses

Screening in urine

Quantitative analysis of total GAG

Qualitative analysis: separation of GAG subfractions

Enzyme tests in leukocytes/fibroblasts to establish subtype
Activity assays of 1 to 4 enzymes

Mutation analysis

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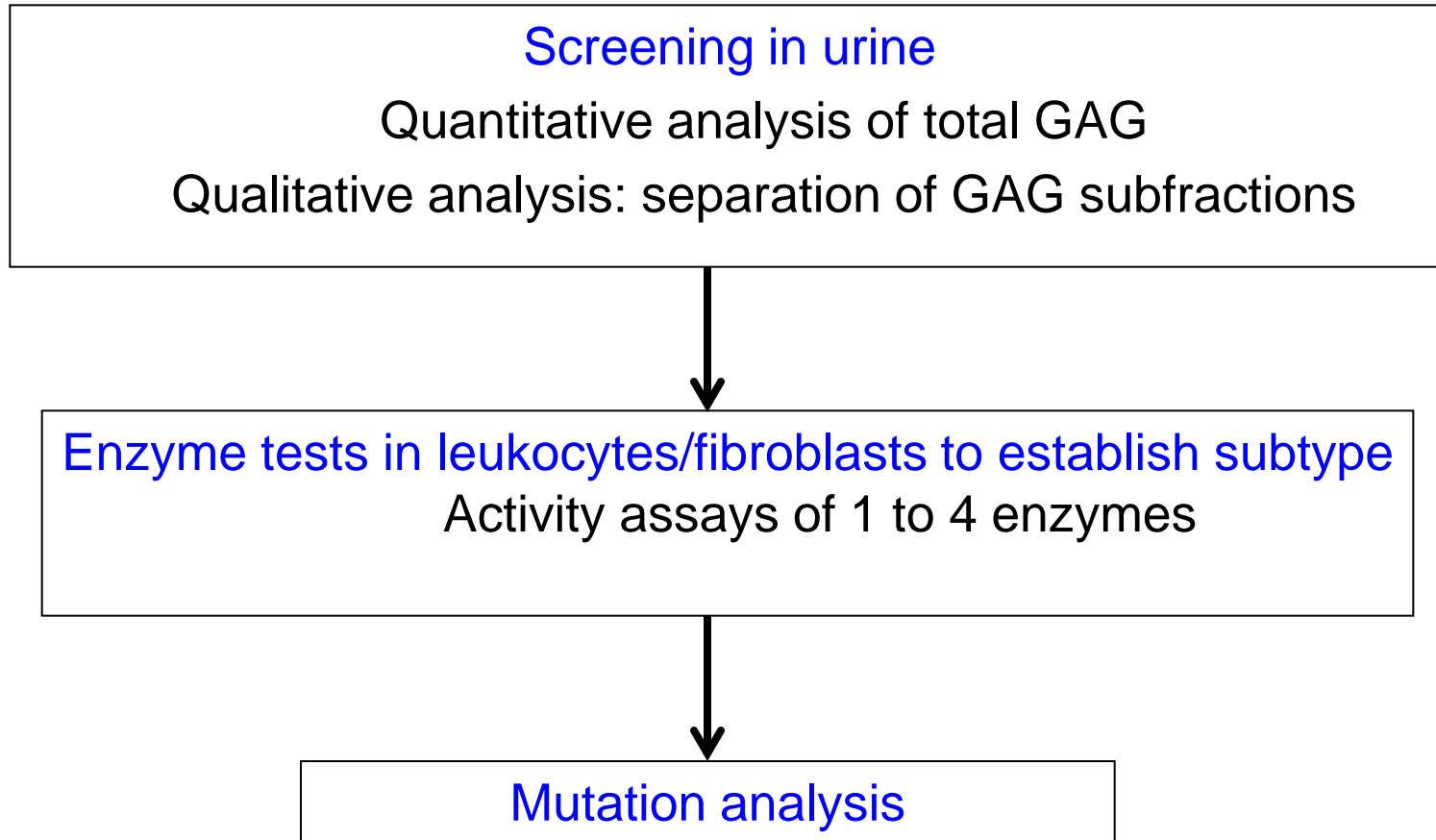
DMB test; normal controls vs. MPS
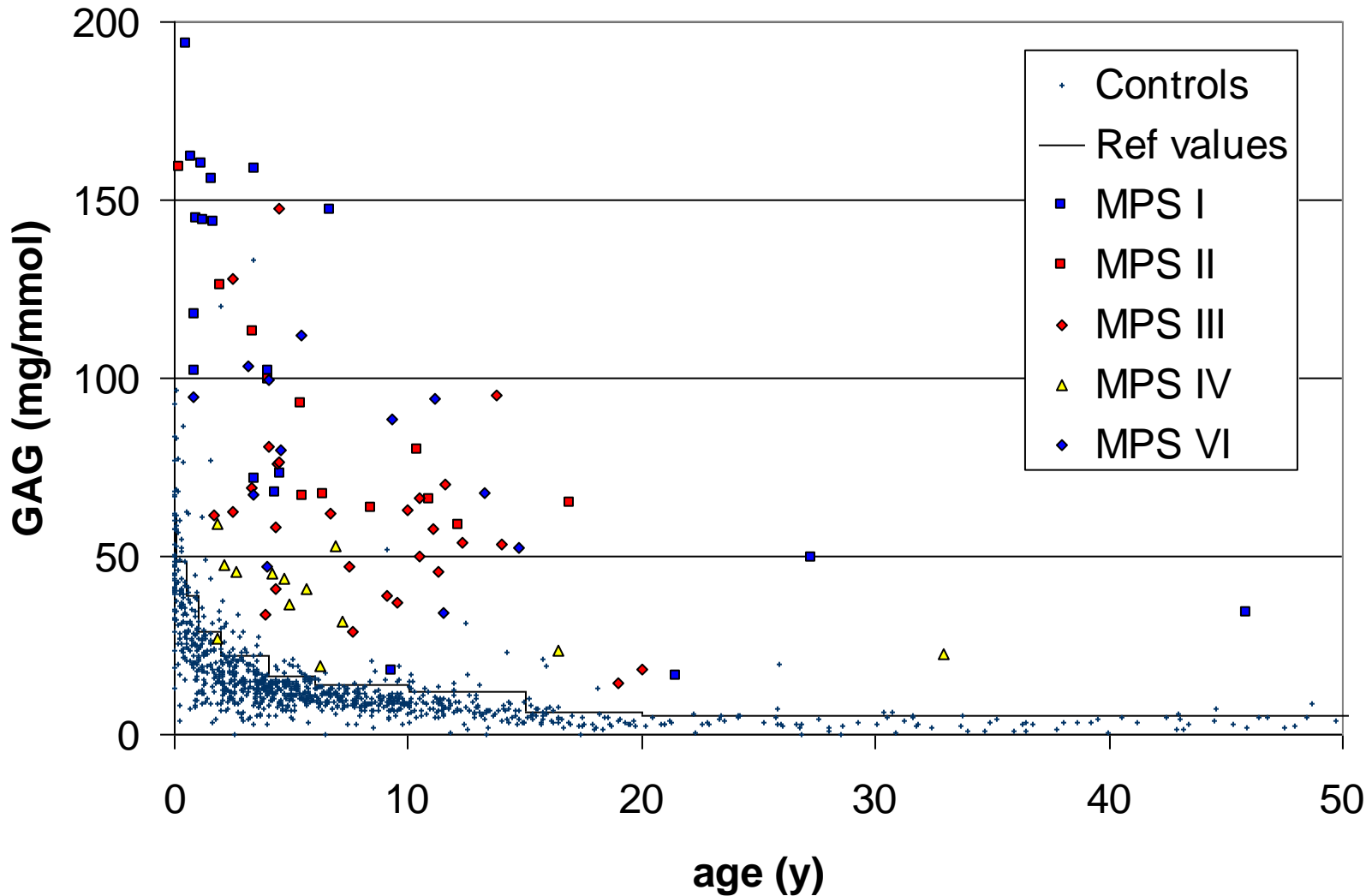
False positive results:
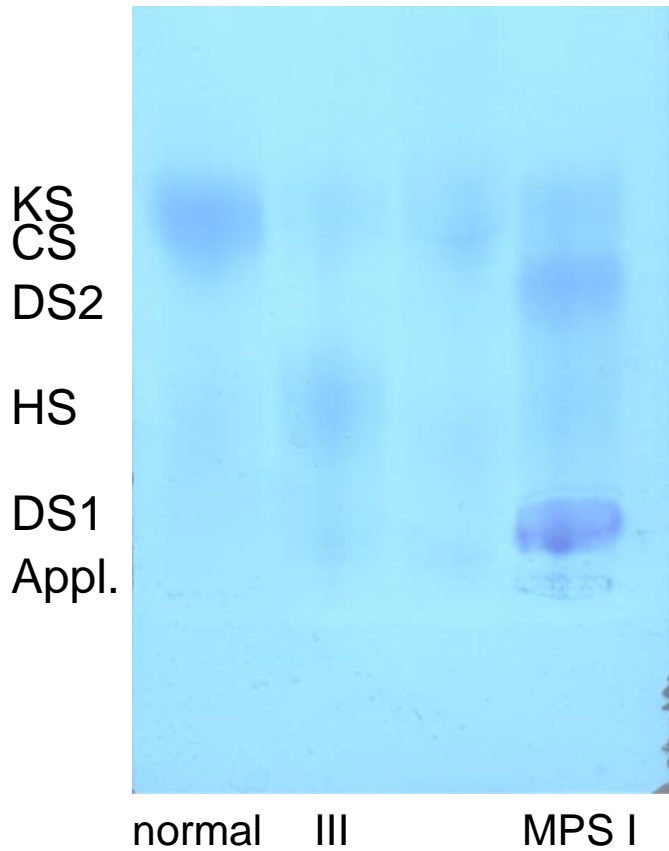rheumatoid arthritis, heparin, glue from collection bags

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What deviation from normal is suspect?

$$X = \frac{\text{Measured value} - \text{mean of age matched reference values}}{\text{Standard deviation in age matched reference values}}$$
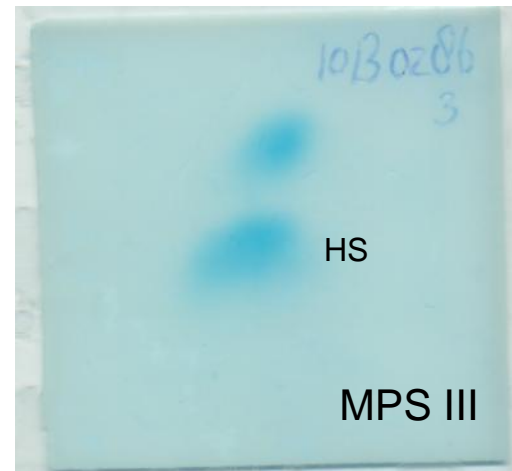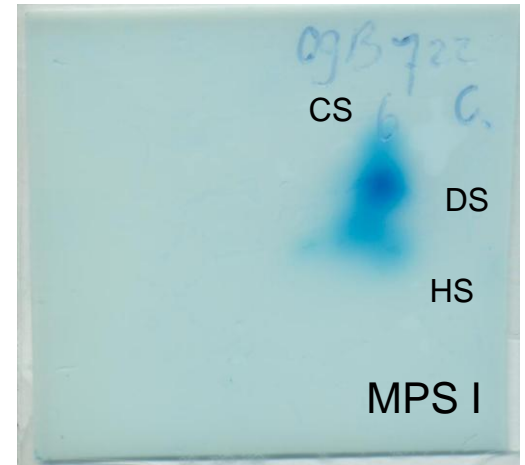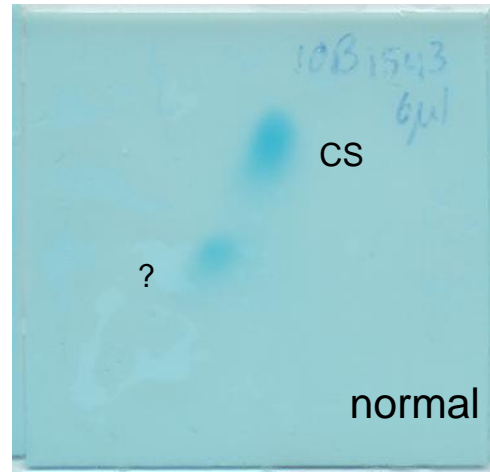
X = no. of SD above average normal

MPS	X		n
	median	range	
I	17	3.0 – 50	22
II	19	2.9 – 31	18
III	14	3.6 – 66	39
IV	7	1.0 – 16	19
VI	18	4.5 – 30	14

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1D/2D electrophoresis

1-dimensional

KS
CS
DS2

HS

DS1
Appl.

normal   III   MPS I

2-dimensional

CS

?

normal

CS

DS

HS

MPS I

HS

MPS III
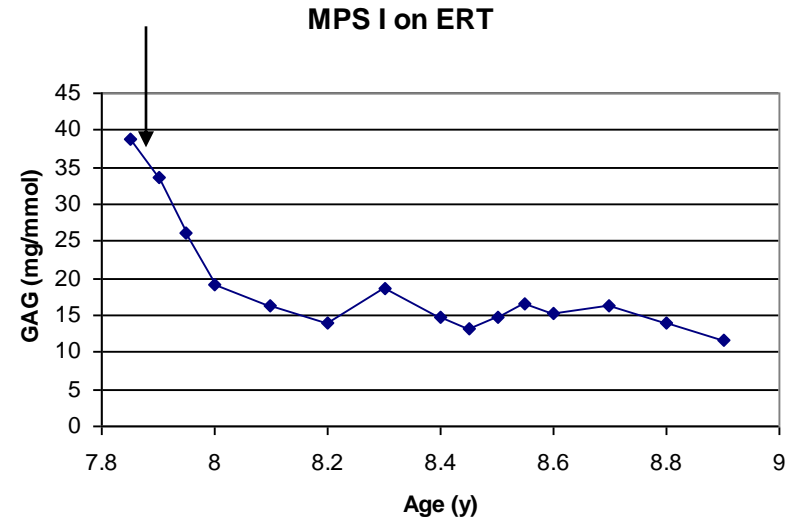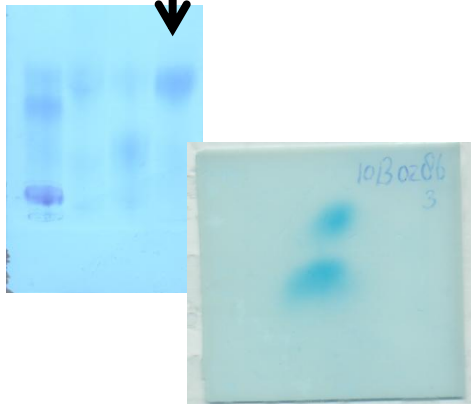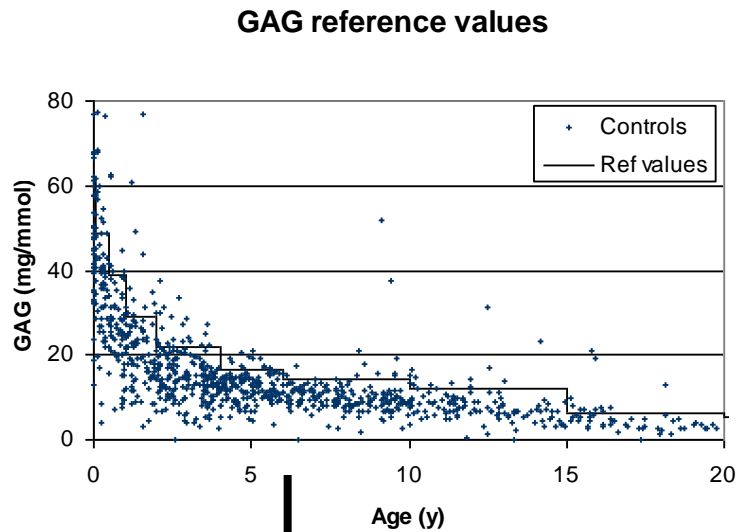
Sensitivity/specificity of current quantitative GAG analysis is not sufficient

Novel methods for GAG diagnostics in urine

- ELISA
  e.g. Tomatsu et al (2005) J Inherit Metab Dis 28:743-57

- LC-MS-MS of 1-phenyl-3-methyl-pyrazolon (PMP)-derivatised oligosaccharides
  e.g. Fuller et al (2004) Ped Res 56:733-738

- GAG degradation by bacterial GAG lyases followed by LC-MS-MS of disaccharides
  e.g. Tomatsu et al (2010) Mol Gen Metab 99:124-131

- GAG degradation by methanolysis followed by LC-MS-MS of disaccharides
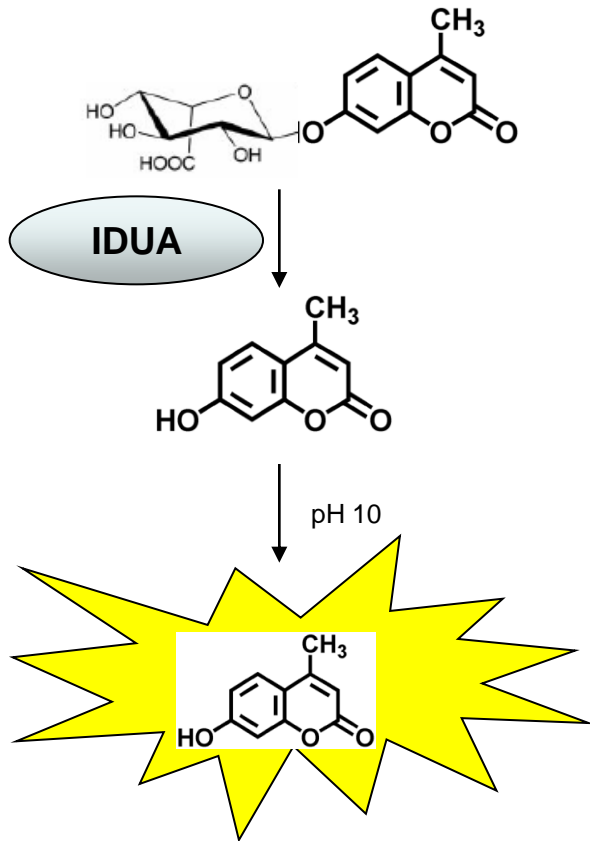 e.g. Auray-Blais et al (2011) Mol Gen Metab 102:49-56

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Enzyme assays for MPS

		4MU	
MPS I	α-L-iduronidase	+	
MPS II	iduronate sulfatase	+ (2 step)	
MPS III A	sulfamidase	+ (2 step)	
B	α-NAc-glucosaminidase	+	
C	AcCoA:α-glucosaminide Ac trf.	+ (2 step)	
D	GlcNAc 6-sulfatase	+ (2 step)	
E ?	glucuronate sulfatase	+ (2 step)	
MPS IV A	GalNAc 6-sulfatase	+ (2 step)	
B	β-galactosidase	+	
MPS VI	arylsulfatase B	(-)	pNCS
MPS VII	β-glucuronidase	+	

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Chemistry of enzyme assays using synthetic substrates
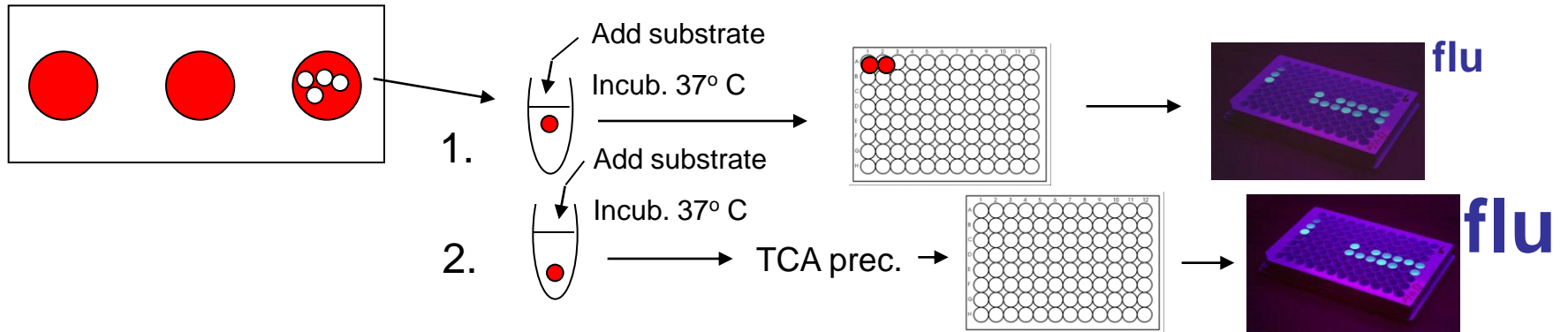
Fluorogenic 4MU substrate   Lipidated MS-MS substrate
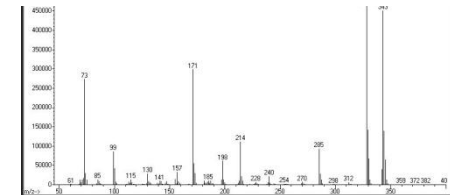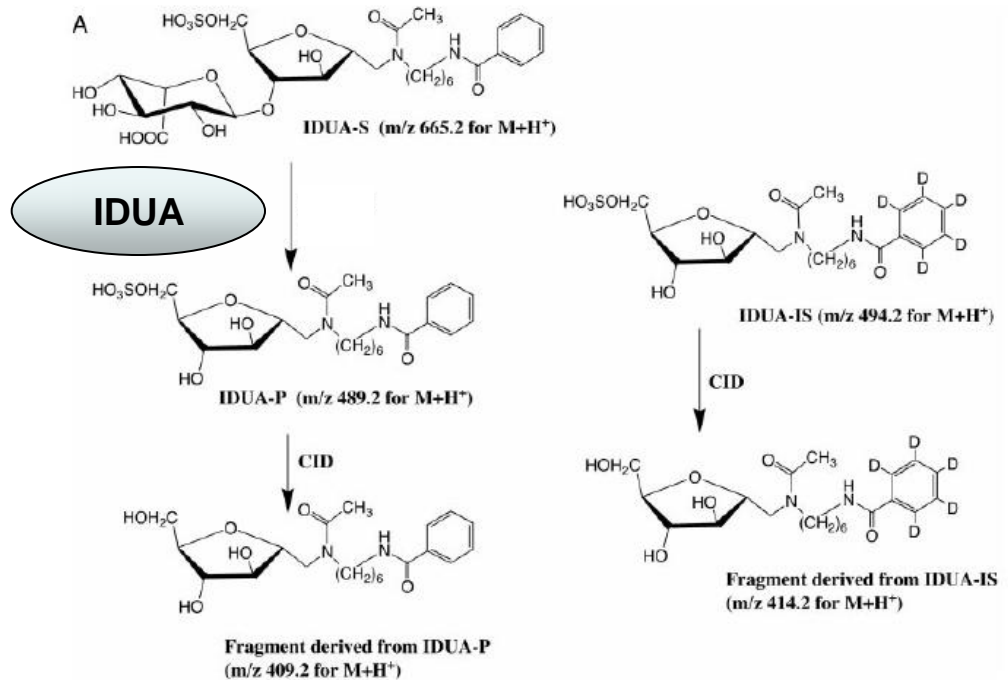
Improved method for 4MU assays in DBS

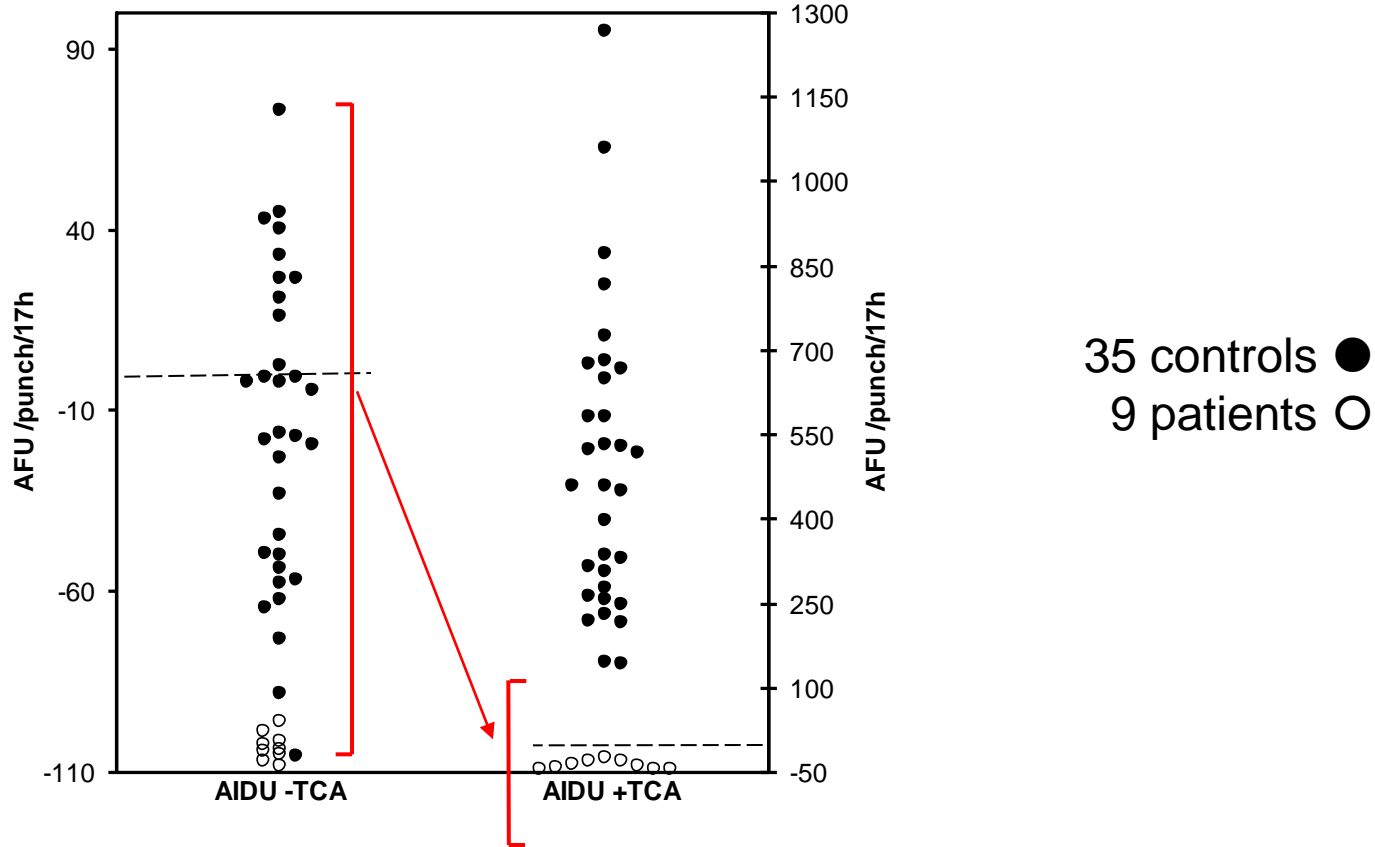
1. Traditional direct fluorescence determination
   Drawback quenching by hemoglobin

2. TCA precipitation hemoglobin after enzyme incubatio
   Quenching decreased, FLU about 8-fold increased
   More sensitive and reliable assay
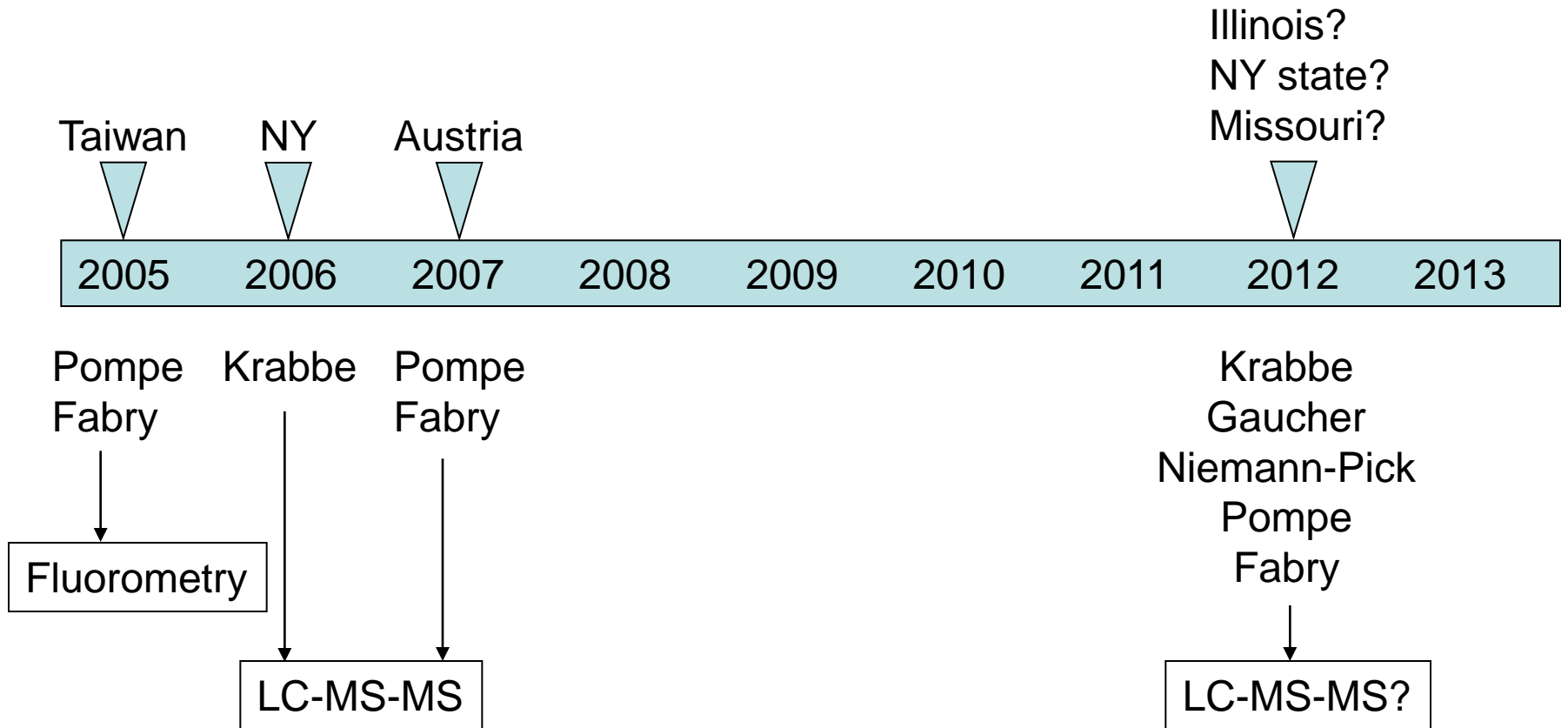
Hurler disease: α-L-iduronidase assay in DBS

35 controls ●
9 patients ○

(Raw fluorescence data AFU/ 3mm punch/ 17h

Current newborn screening for LSDs
No MPS so far

Illinois?
NY state?
Missouri?

Taiwan	NY	Austria						
2005	2006	2007	2008	2009	2010	2011	2012	2013

Pompe
Fabry

Krabbe

Pompe
Fabry

Krabbe
Gaucher
Niemann-Pick
Pompe
Fabry

Fluorometry

LC-MS-MS

LC-MS-MS?

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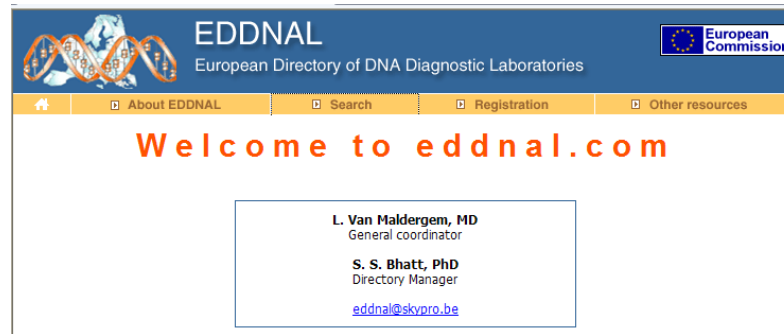
Mutation analysis

- The European Directory of DNA Diagnostic Laboratories (EDDNAL)
  www.eddnal.com

- Orphanet
  www.orpha.net

- MPS I, II, III A/B/C/D, IV A/B, VI
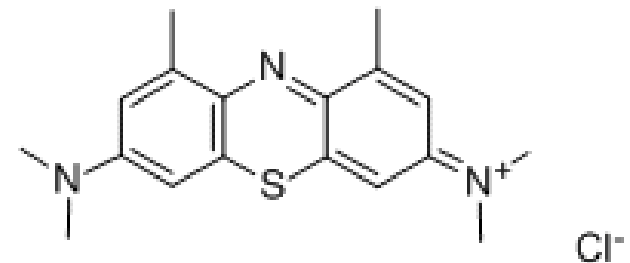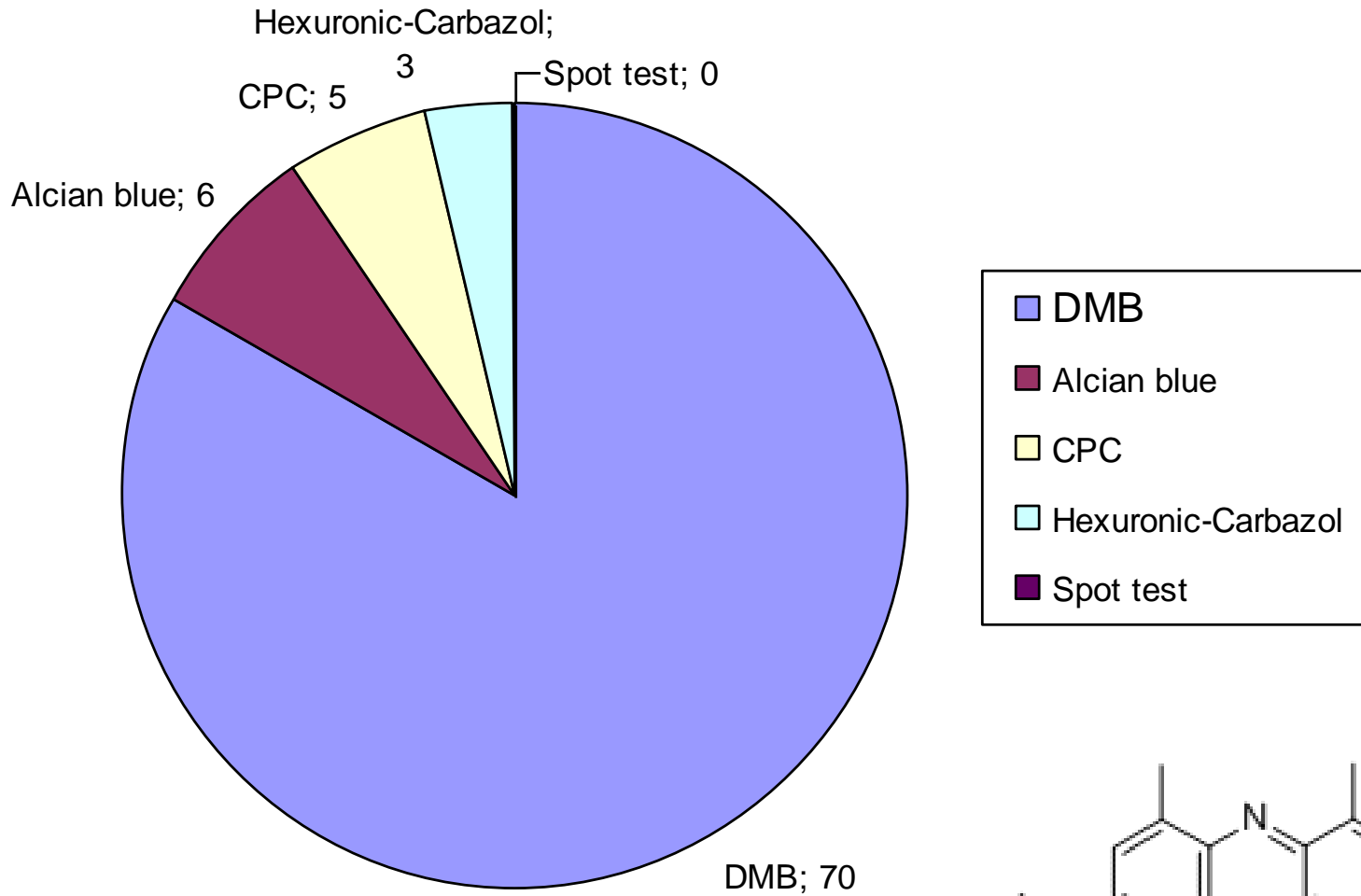
ERNDIM MPS pilot scheme

2010:   8 samples   88 participants

2011:   6 samples   89 participants

- Determine creatinine and GAG concentration
- Qualify GAG level according to age-matched reference values
- (i.e normal or increased)
- Analyse GAG subfractions and qualify
- (i.e. normal or increased CS, HS, DS and KS)
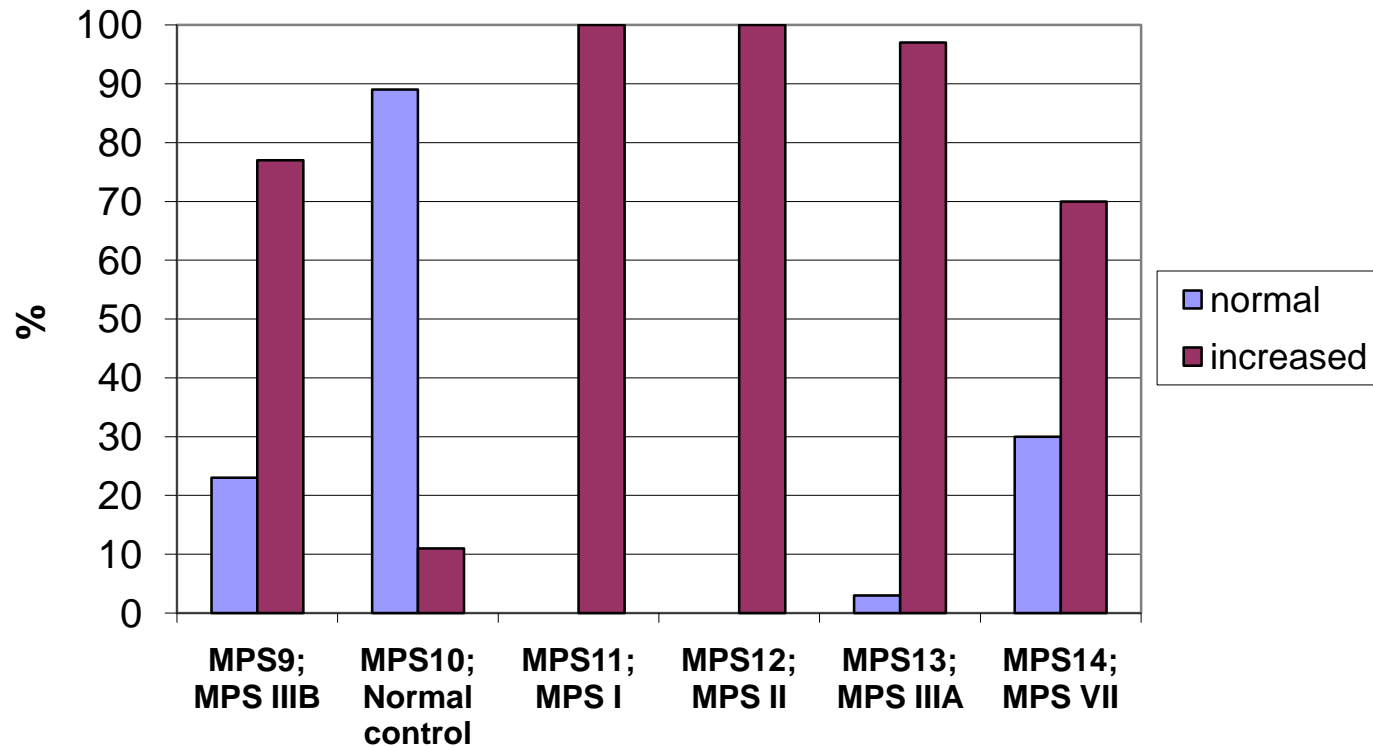- Give most likely diagnosis

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Quantitative analysis of GAG in urine

Hexuronic-Carbazol; 3

CPC; 5

Spot test; 0

Alcian blue; 6

DMB; 70

- DMB
- Alcian blue
- CPC
- Hexuronic-Carbazol
- Spot test

Quantitative GAG results 2011

| Sample ID	MPS9	MPS10	MPS11	MPS12	MPS13	MPS14
Diagnosis Age of patient	MPS III 19 y	Normal 5 y	MPS I 2 y 4 m	MPS II 5 y	MPS III 5 y	MPS VII 19 y
No. of reports	78	78	78	79	79	79
Creatinine (mmol/L) Average SD CV	 21 0.31 14	 6.05 0.56 9	 1.09 0.35 32	 3.14 0.31 10	 1.66 0.22 13	 3.95 0.48 12
GAG (mg/mmol) Average SD CV	 9.4 4.6 49	 10.0 4.1 41	 130 50 38	 54.4 13.2 24	 51.9 15.9 31	 10.9 5.1 47

Interpretation of quantitative GAG results

Performance of quantitative methods
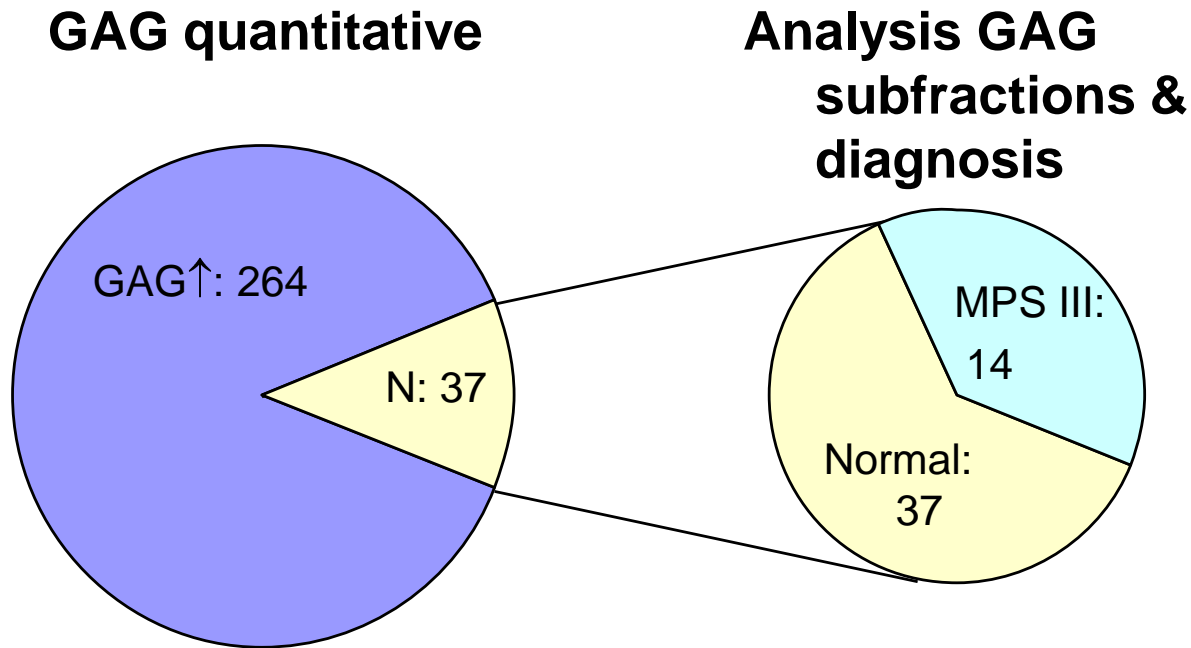
4 MPS samples: 3 x MPS III, 1 x MPS VII

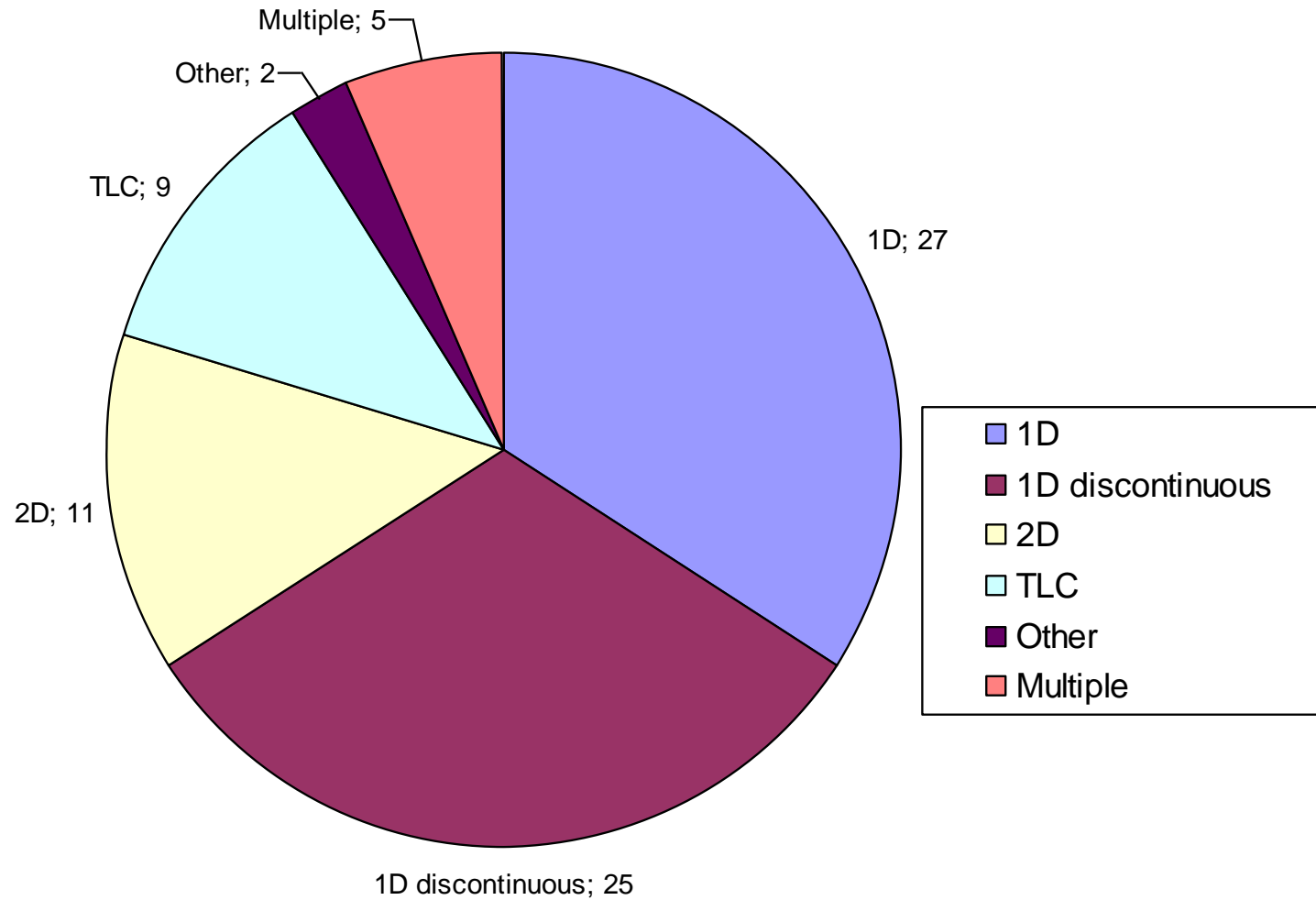GAG concentrations moderately increased

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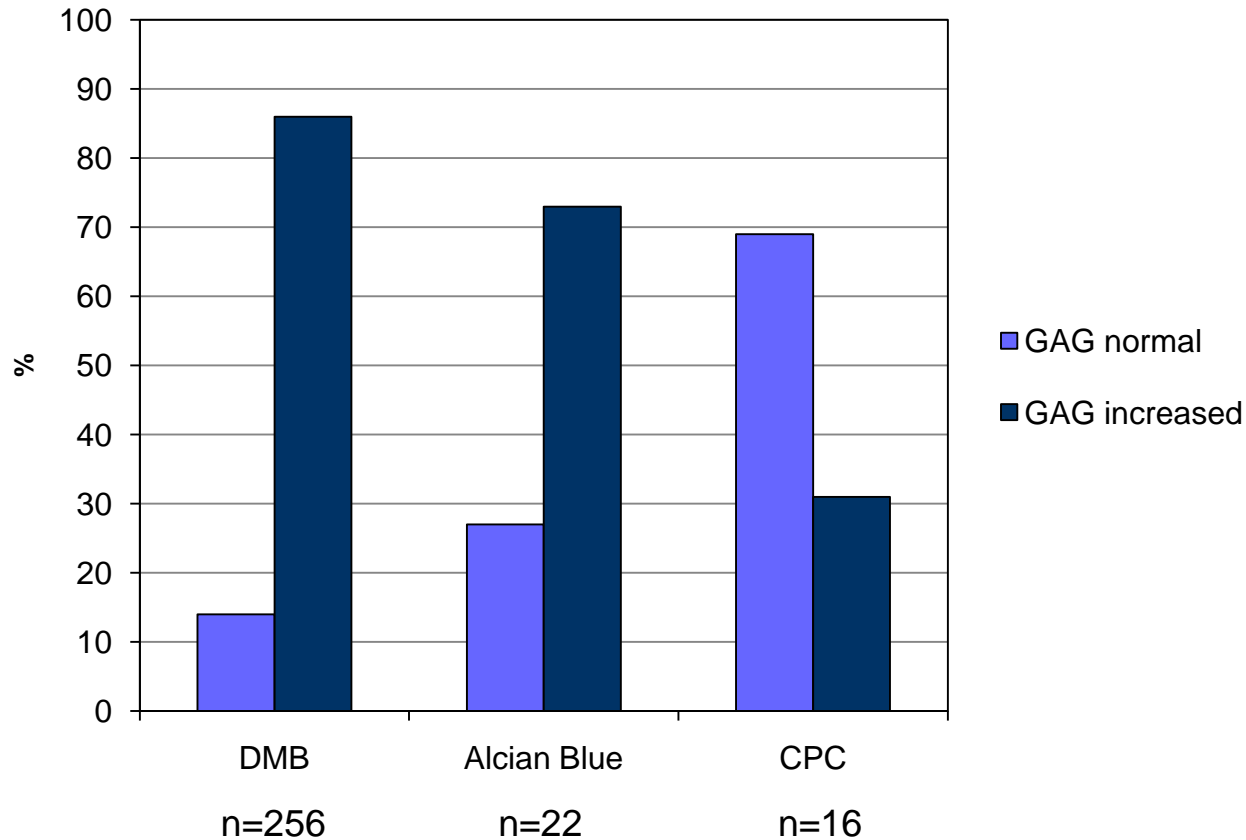
Is quantitative analysis of total GAG sufficient?

MPS III: 4 samples in 2010-2011

Total sample reports: 301

GAG quantitative

GAG↑: 264

N: 37

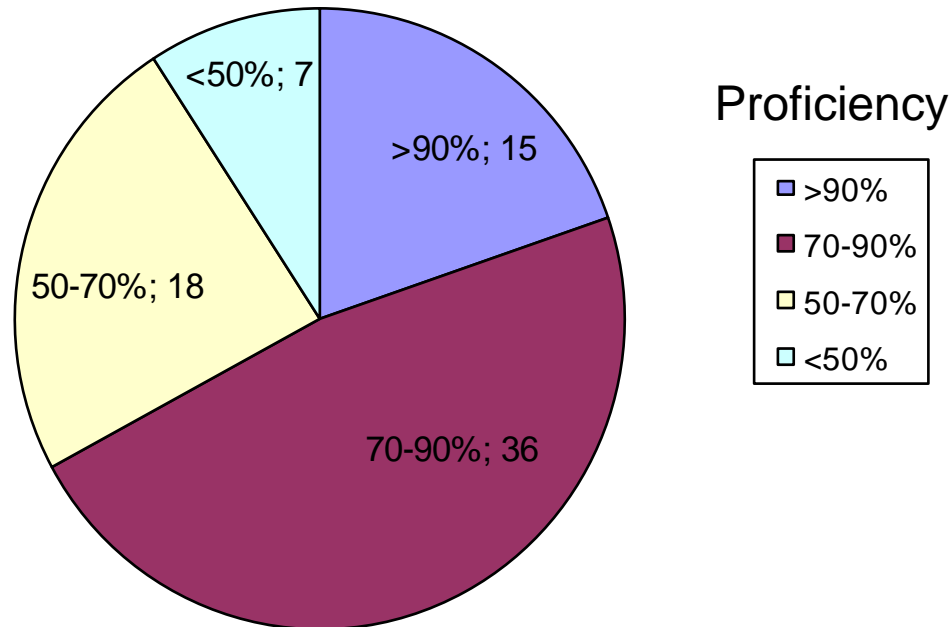**Analysis GAG subfractions & diagnosis**

MPS III: 14

Normal: 37

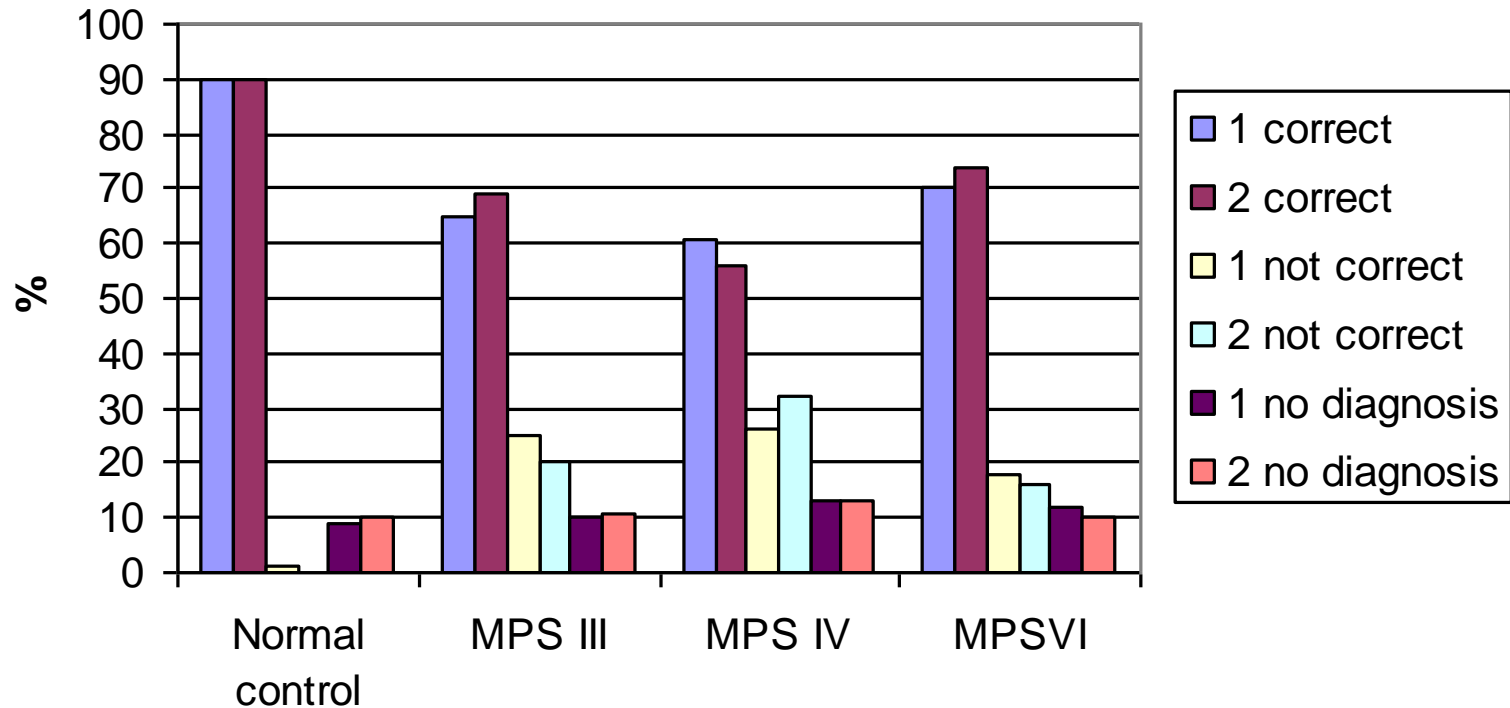# Qualitative analysis of GAG in urine
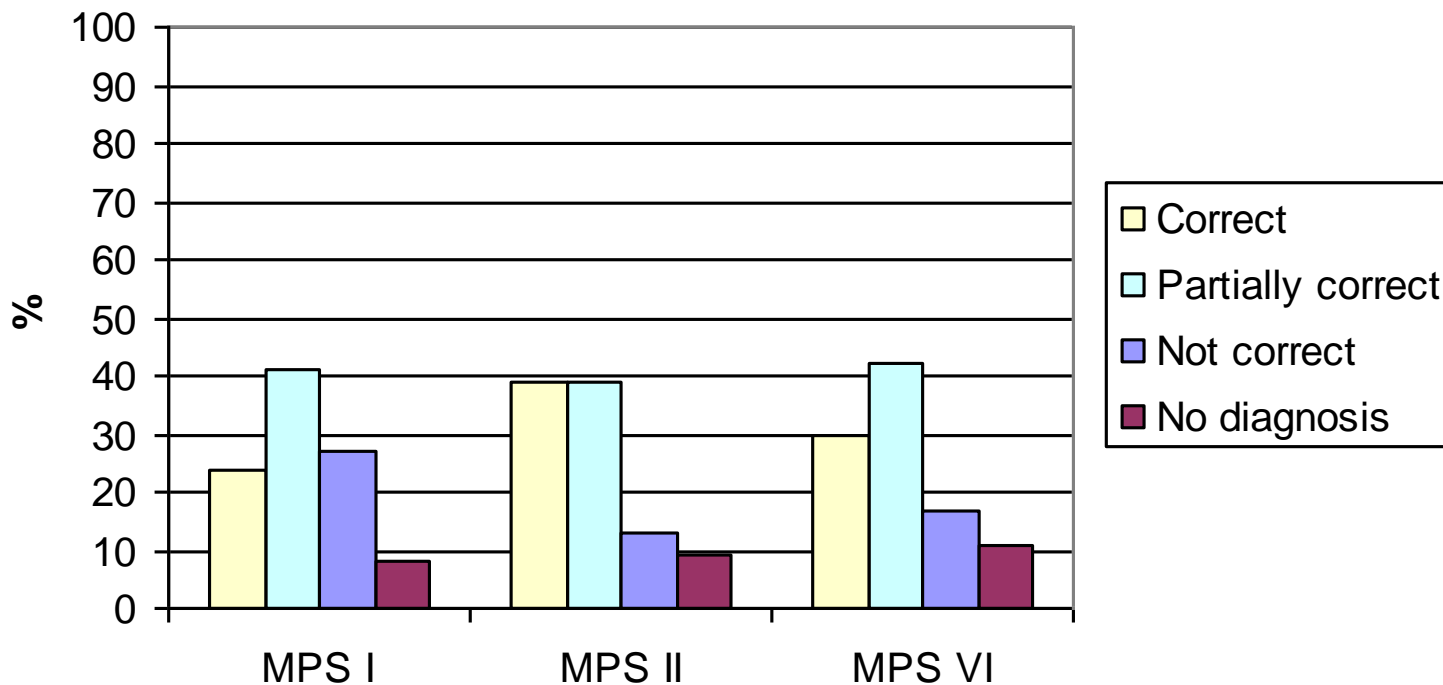
Diagnostic proficiency summarised

Participants who submitted at least 10 out of 14 sample reports: **76**

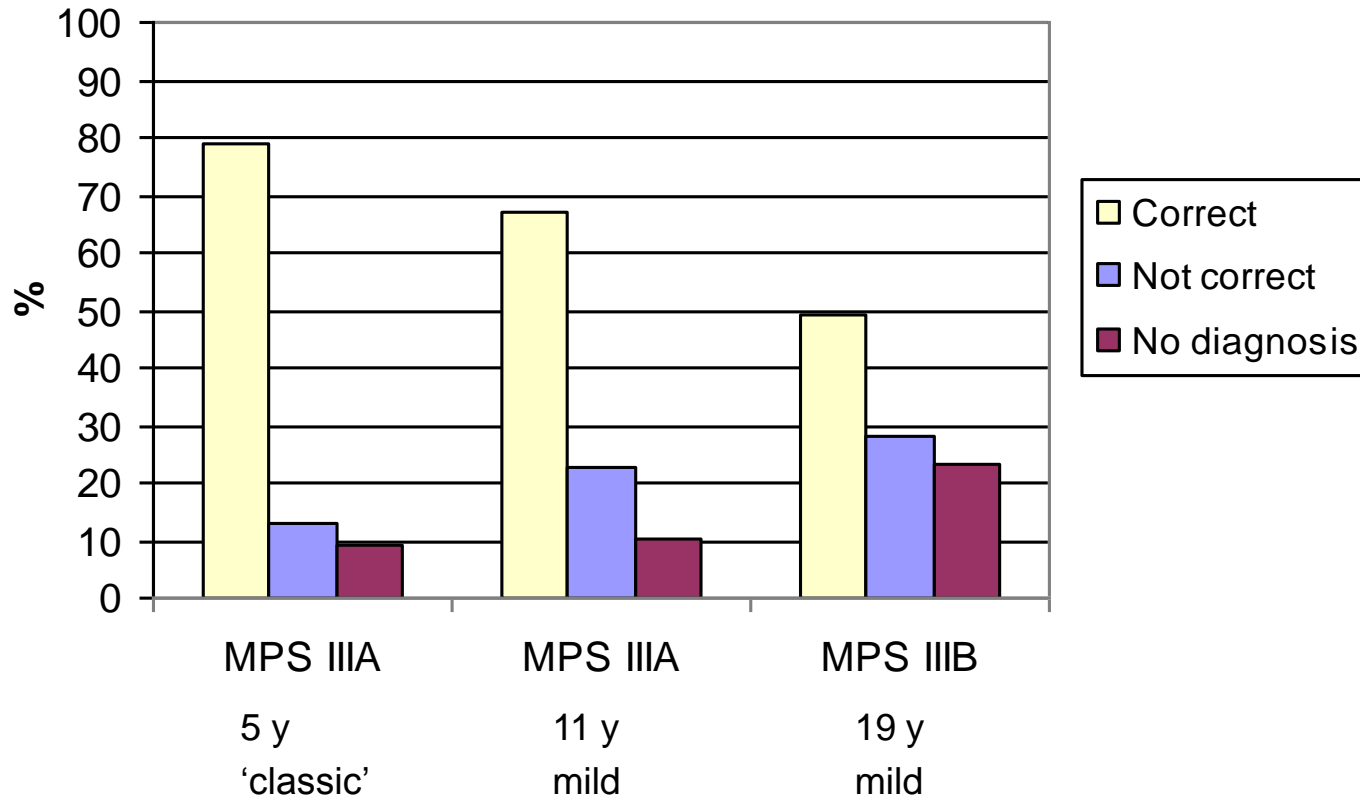# Reproducibility of diagnostic proficiency

Can MPS I, II and VI be distinguished by GAG analysis?

	MPS I	MPS II	MPS VI
Correct	I / II	I / II	VI
Partially correct	I / II / VI	I / II / VI	I / II / VI
DS increased (%)	99	96	91
HS increased (%)	43	77	25

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Diagnostic proficiency of MPS III

	MPS IIIA	MPS IIIA	MPS IIIB
	5 y 'classic'	11 y mild	19 y mild
'Normal' (%)	5	12	23
HS increased (%)	94	80	66

Legend: Correct, Not correct, No diagnosis

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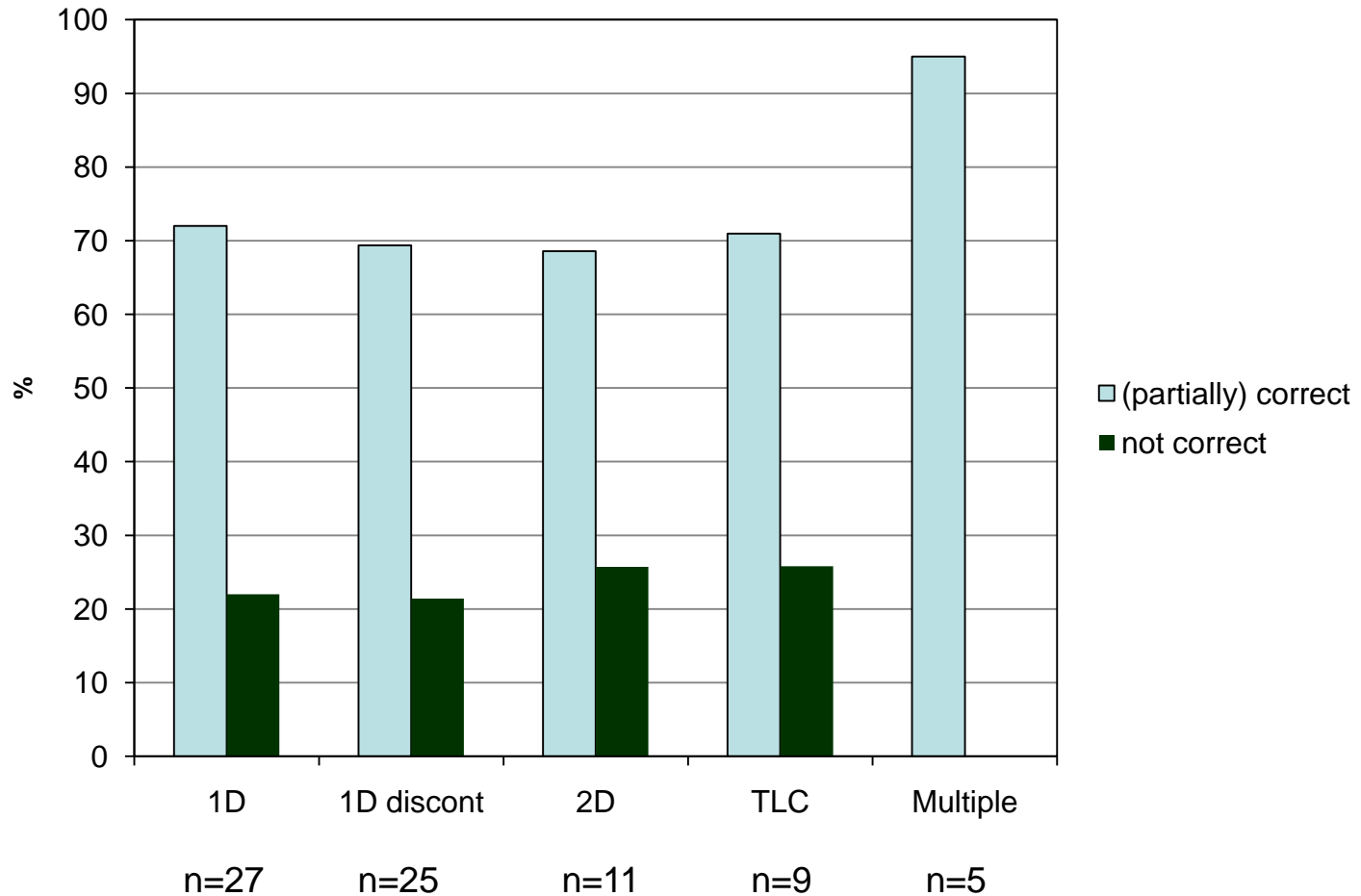Universitair Medisch Centrum Rotterdam

Overall performance of qualitative methods
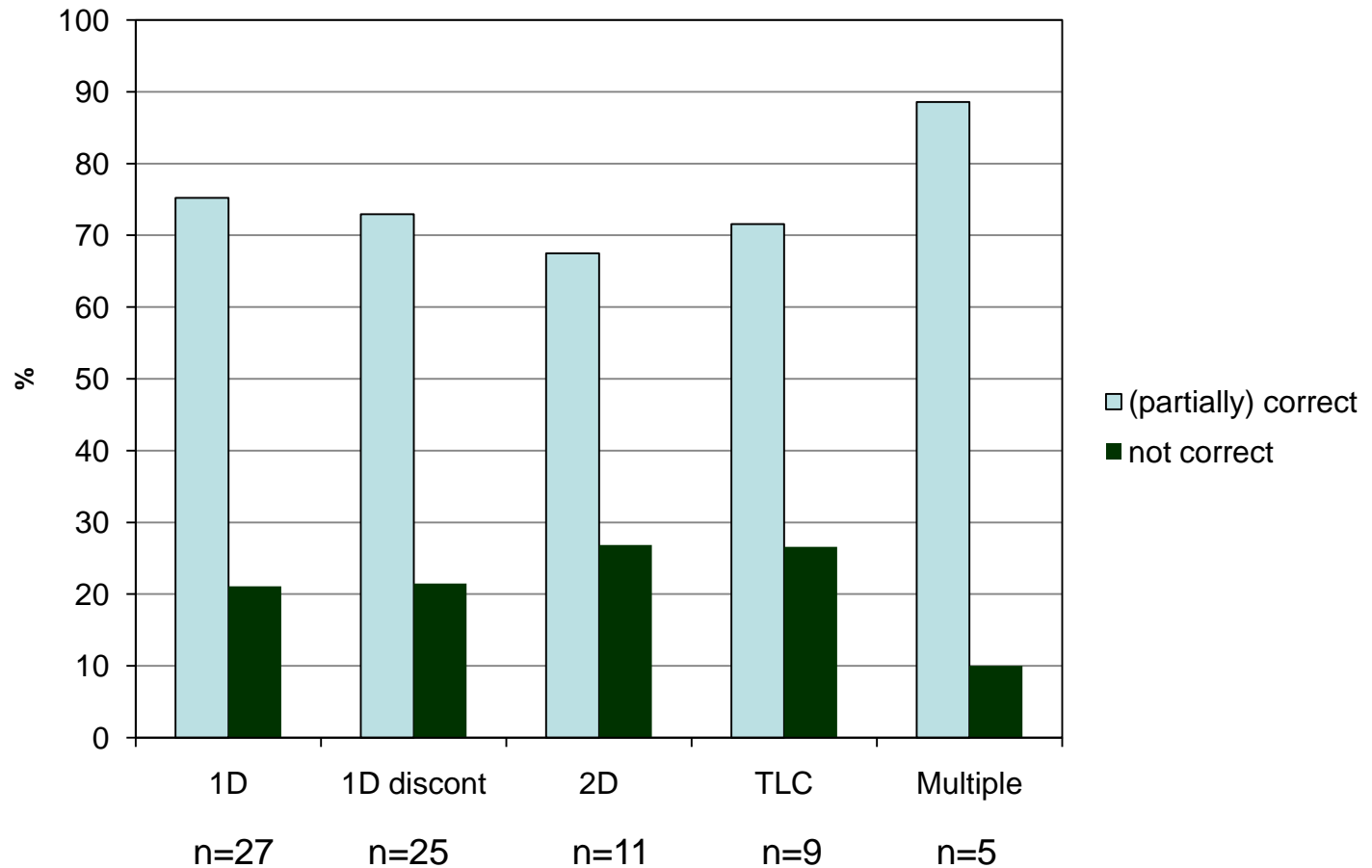
Performance of qualitative methods for MPS IV
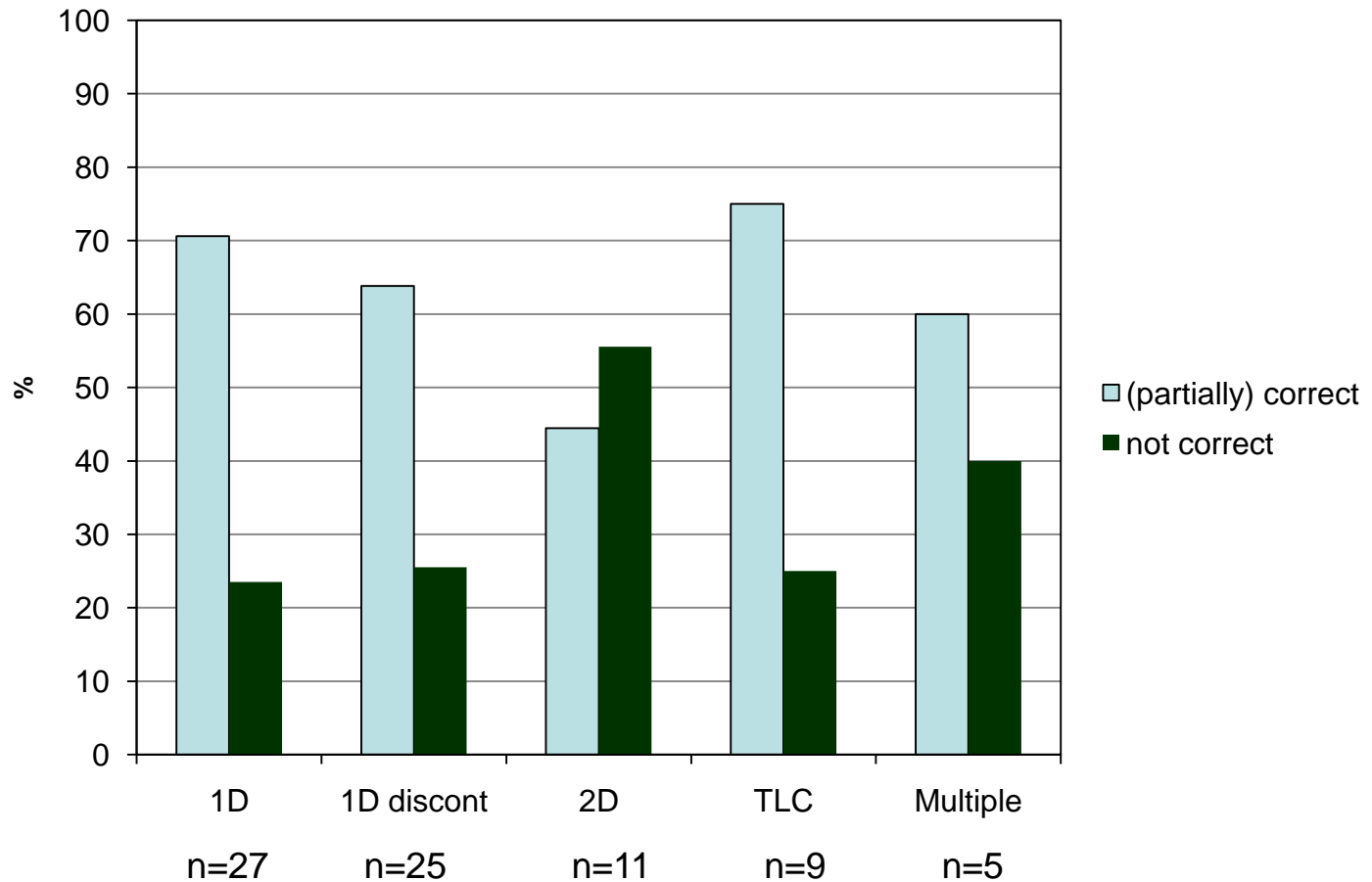
Performance of qualitative methods for MPS III

Summary/conclusions

- MPS pilot scheme started in 2010; ~90 participants

- Quantitative GAG analysis: 83 % DMB

- DMB, Alcian Blue perform better than CPC for mildly elevated GAG

- Analysis GAG sub fractions: 66% 1-dimensional electrophoresis

- Difficult to distinguish MPS I, II and VI on the basis of GAG analysis

- Use of multiple methods for analysis of GAG sub fractions improves diagnostic proficiency

- Sensitivity/specificity of current GAG analysis not sufficient: novel methods required

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Thank you!
Erasmus MC: Jan Huijmans, Rolanda van den Berg, Eric van der Meijden
SKML: Cas Weykamp, Irene de Graaf
All participants of the ERNDIM MPS pilot study