Screening for three lysomal storage diseases in a NBS laboratory, and the potential to expand to a nine-plex assay

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2. Washington State Newborn Screening Laboratory

Potential candidates for newborn screening of Lysosomal disorders.

Disorder	Rx	Requires early detection
Gaucher	ERT/SM	
Fabry	ERT/SM	+/-
MPS-I	ERT/BMT	+
Pompe	ERT/SM	+
MPS-II	ERT	+
MPS-IVA	ERT	+
MPS-VI	ERT	+
Krabbe	BMT	
Niemann-Pick B	?	
MLD	BMT	+

Product Description

Substrates and internal standards are available without charge through the CDC

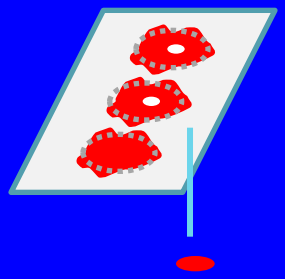
Each vial contains the optimized ratio of substrate : internal standard for 1200 tests for:

- Gaucher disease
- Pompe disease
- Fabry disease
- Niemann-Pick A/B
- Krabbe disease (600 tests)
- MPS-I

Each box contains 6000 tests

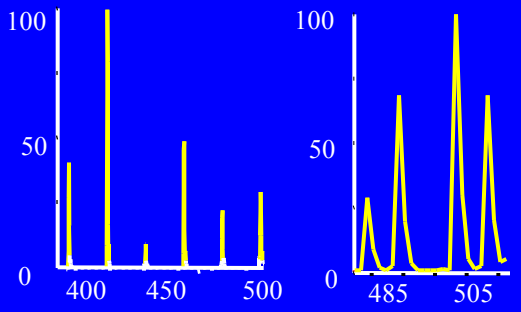
Reagents for each disorder are packaged separately to allow end-user to choose menu-style

Triplex Procedure



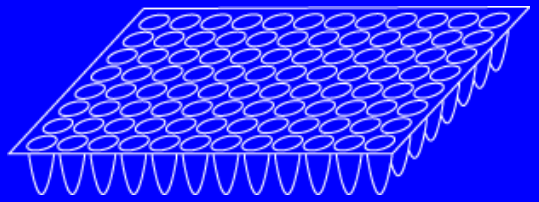
Anonymous blood spots

Fabry, Pompe, MPS-1



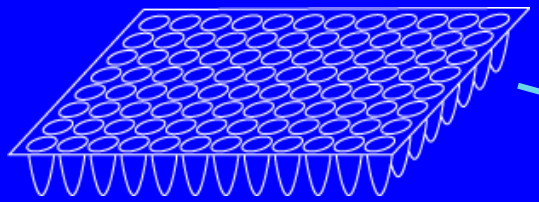
MS/MS

normal

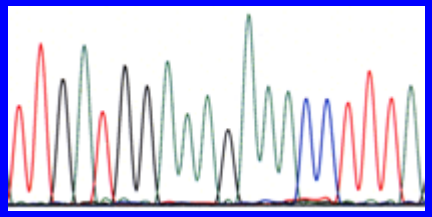


Assay plate

abnormal



Duplicate plate



DNA sequencing (GLA, GAA, IdUA) for genotype

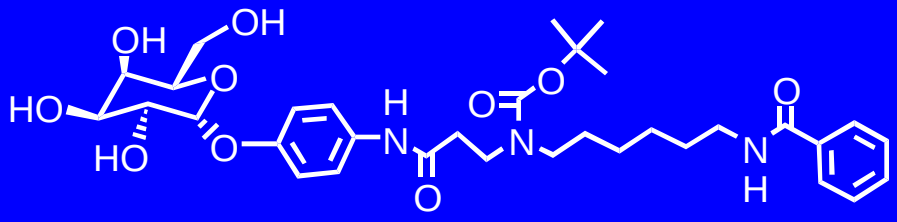
unaffected

affected

Fabry Disease

- X-linked
- Deficiency of acid α-galactosidase
- Shortened life expectancy from:
  - Renal failure at 30-40 years
  - Hypertrophic cardiomyopathy
  - CNS strokes
- Childhood symptoms of:
  - Peripheral pain
  - Fatigue

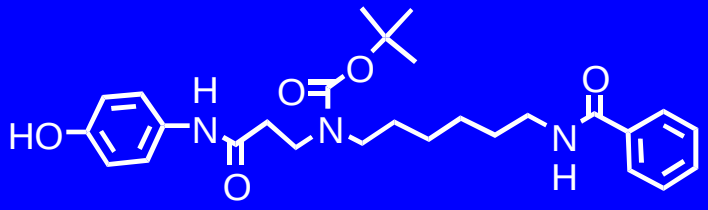
Fabry Assay



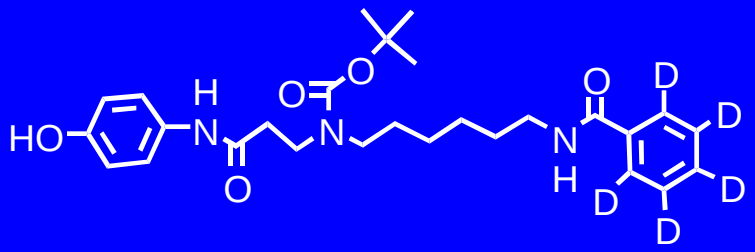
Substrate

Acid alpha-Galactosidase (a-Gal)

(Fabry)

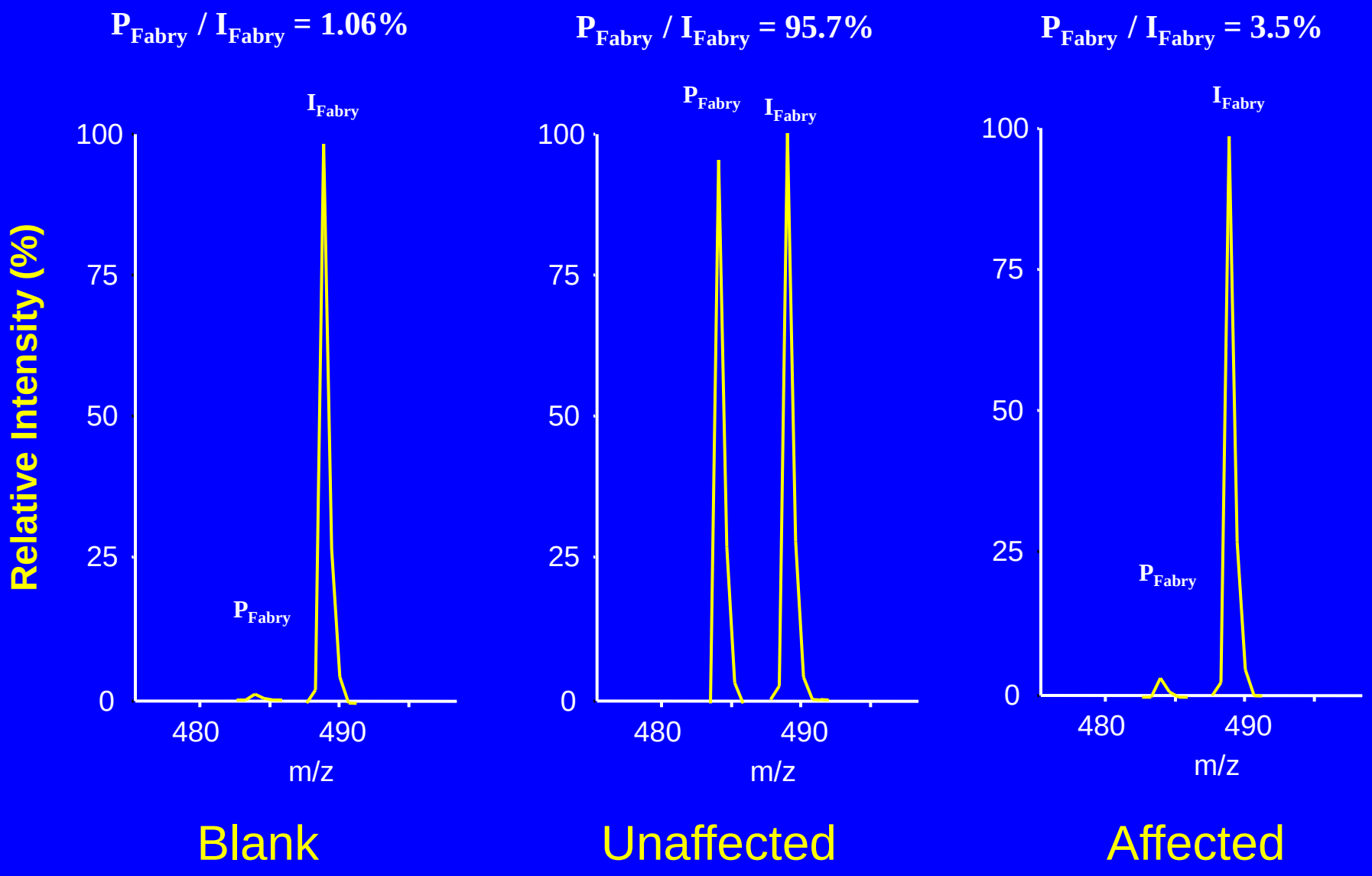


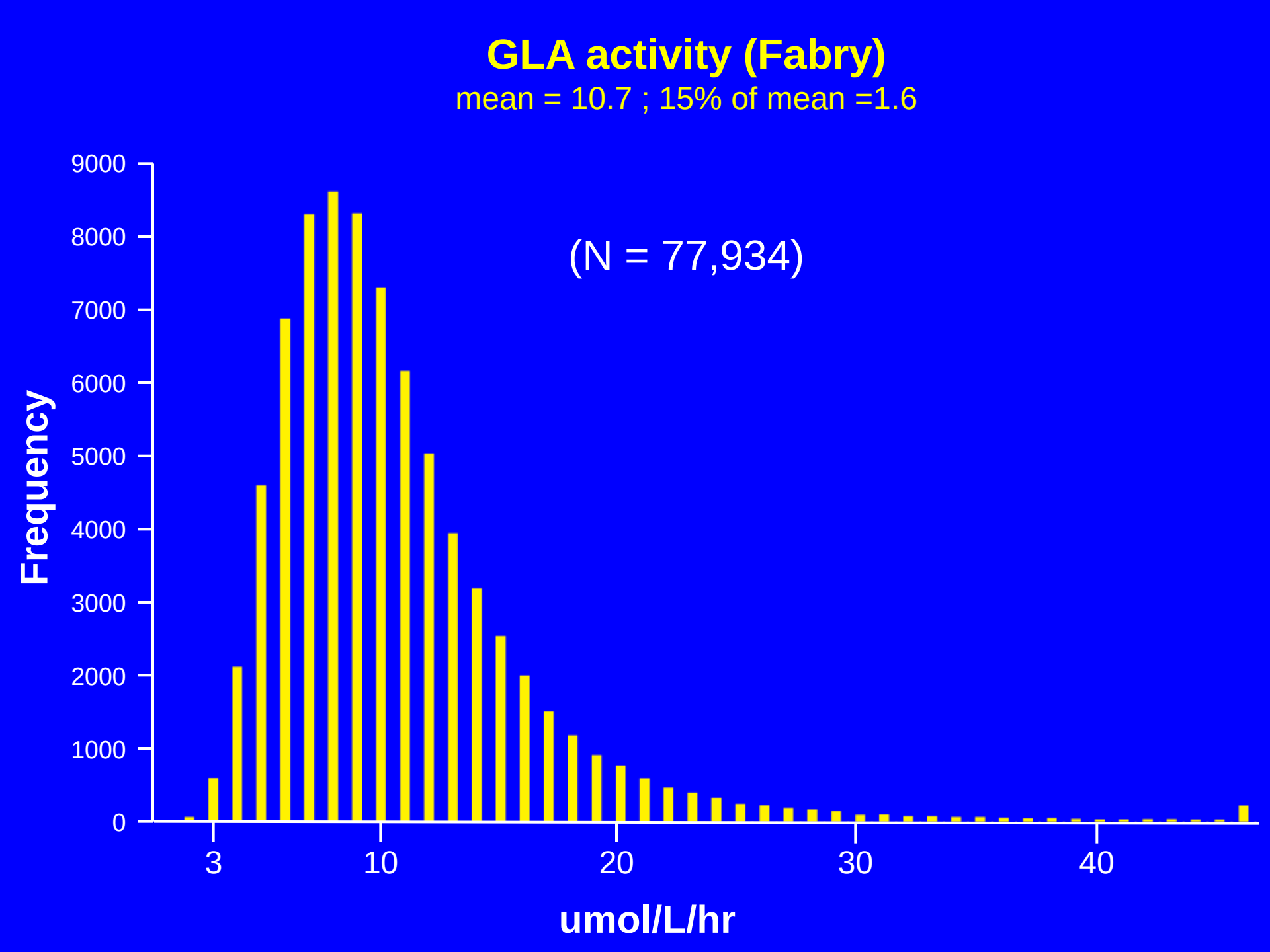
Product



Internal standard

Fabry


P_{Fabry} / I_{Fabry} = 1.06%
P_{Fabry} / I_{Fabry} = 95.7%
P_{Fabry} / I_{Fabry} = 3.5%
Relative Intensity (%)
100
75
50
25
0
480
490
m/z
I_{Fabry}
P_{Fabry}
Blank
Unaffected
Affected


GLA activity (Fabry)
mean = 10.7 ; 15% of mean =1.6
(N = 77,934)
Frequency
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
3
10
20
30
40
umol/L/hr

Fabry Disease

Xq22 13 kb 7 exons 429 AA's

400+ pathogenic mutations

- Prevalence: 1/17,000 - 1/50,000
- Taiwan population: 1/16,000 with hypertrophic cardiomyopathy (IVS4 + 919g>a)
- Italian newborn screening: 1/3,100

Table 1: Fabry Disease

Cut off at 15% of assay mean: 1.5 µmole/hr/L	Enzyme activity (µmole/hr/L)	% of mean $\bar{X}$=10.5	Mutations
	1.72	16.1	p. Ser 334Asn/wt (F)
	1.50	14.0	wt
	1.38	12.9	wt
	1.32	12.3	p.Ala143Thr (M)
	1.12	10.5	wt
	0.89	8.3	p.Asp313Gly (M)
	0.58	5.4	p.Asn215Ser (M)
known affected (n=5)	0.13 – 0.50	1.2 – 4.7	

(M) = male
(F) = female

Positive predicted Value = 0.42
Specificity ~1.0
Sensitivity ~ ?

clinical prevalence: 1/ 40,000 males

NBS prevalence: 1/ 12,000 males

Pompe

Deficiency of lysosomal acid α –glucosidase

Clinical symptoms:

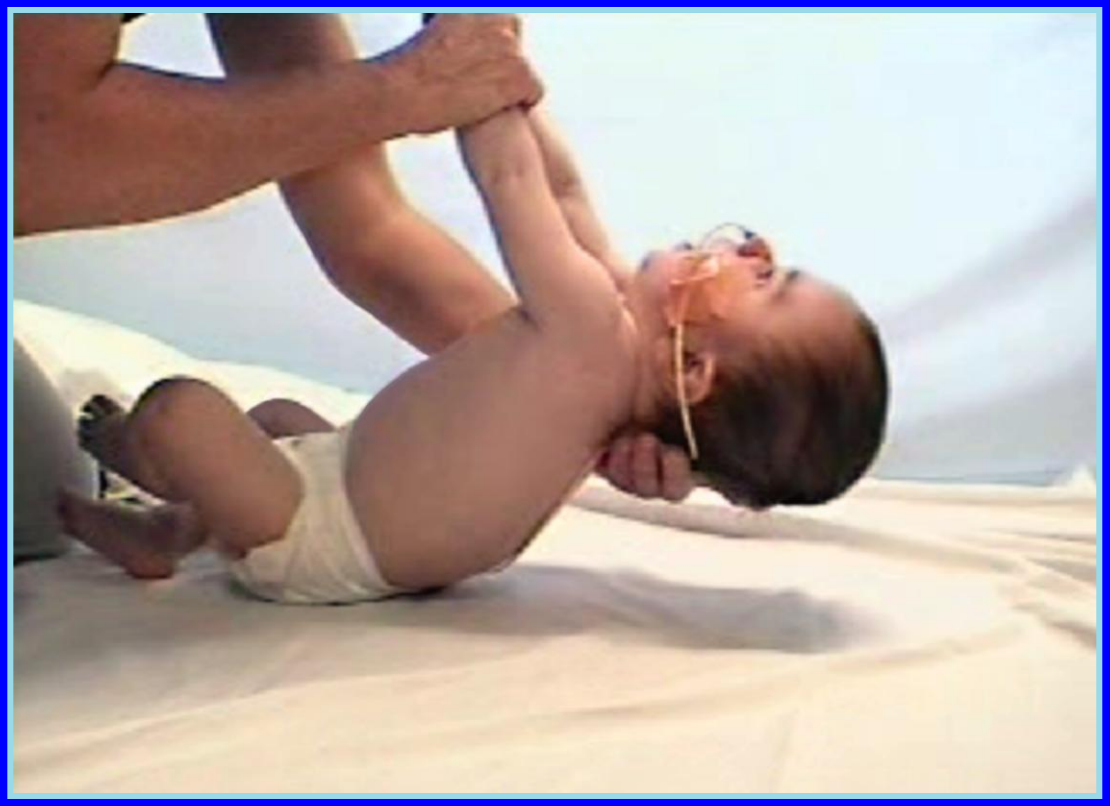
- Progressive muscle weakness with variable onset; 3 mo to adulthood
- Cardiac failure in infancy

In adults:

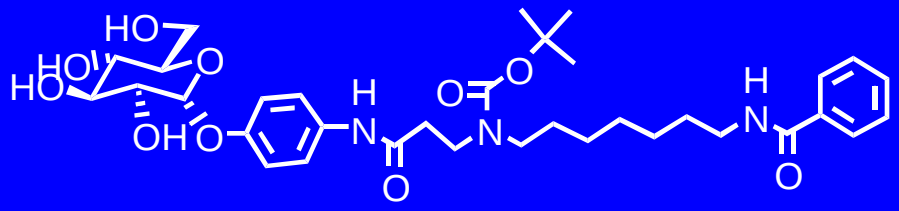
- Progressive muscle weakness
- Respiratory failure

Infantile-Onset Pompe Disease

Head Lag



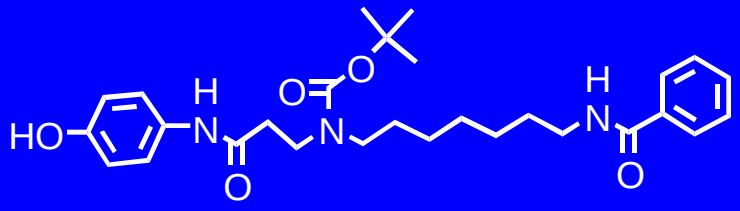
Pompe Assay



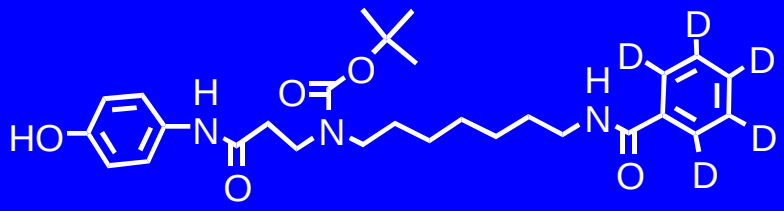
Substrate

Acid alpha-Glucosidase (a-Glu)

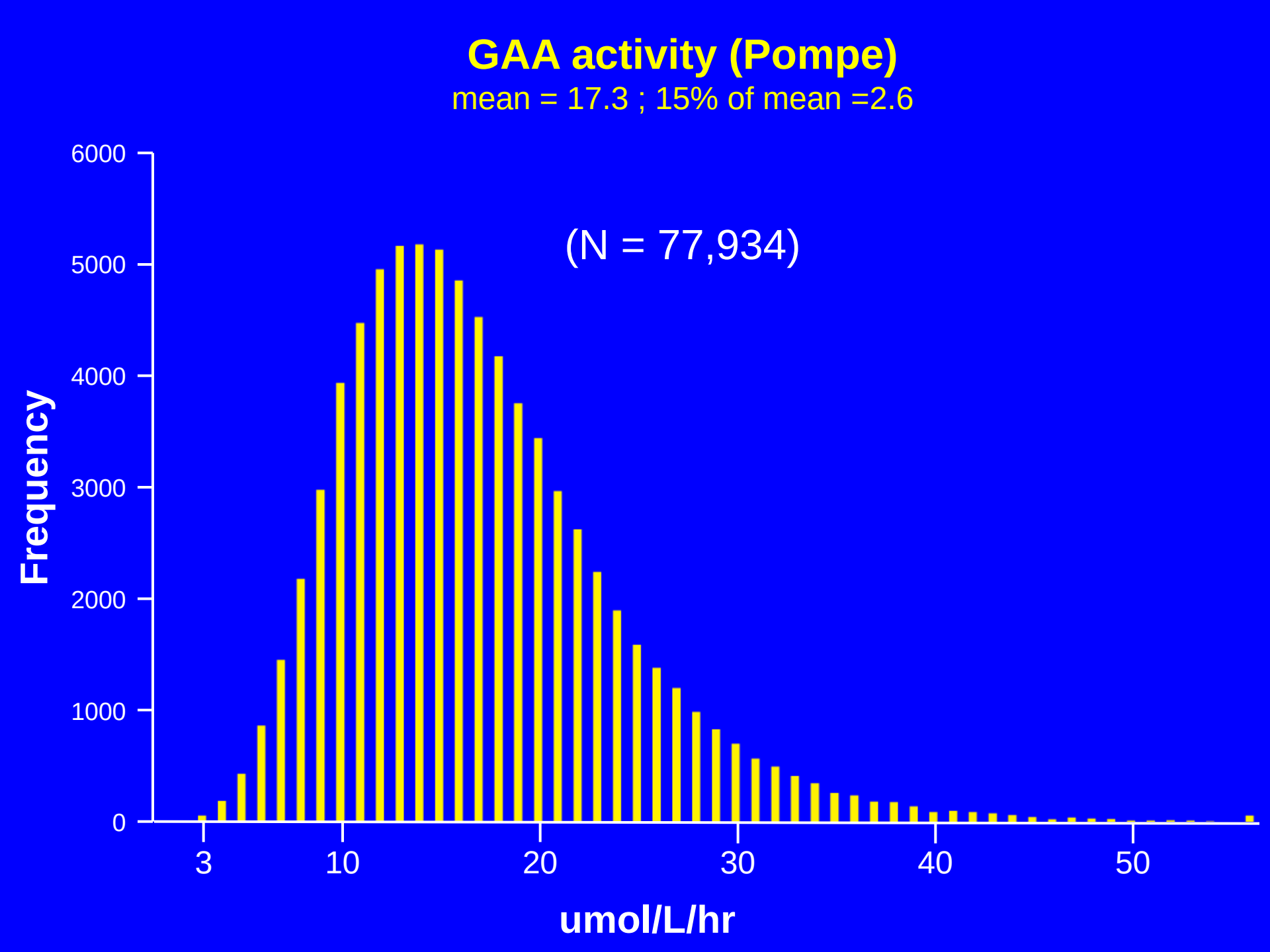
(Pompe)



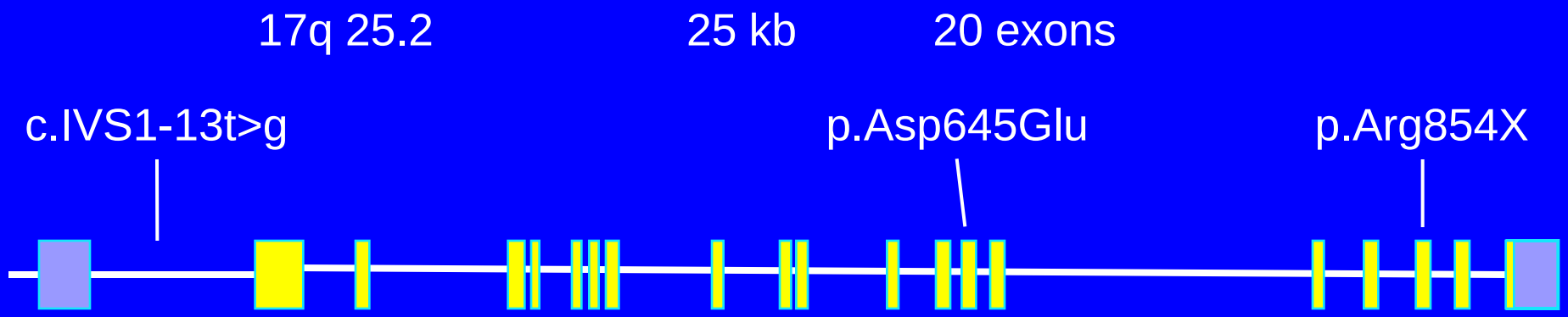
Product



Internal standard


GAA activity (Pompe)
mean = 17.3 ; 15% of mean =2.6
(N = 77,934)
Frequency
6000
5000
4000
3000
2000
1000
0
3
10
20
30
40
50
umol/L/hr

Pompe Disease



Prevalence:

Af. American:	1/14,000	(infant)
US population:	1/40,000	(infant & adult)
European:	1/100,000	(infant)
	1/60,000	(adult)

Table 3: Pompe Disease

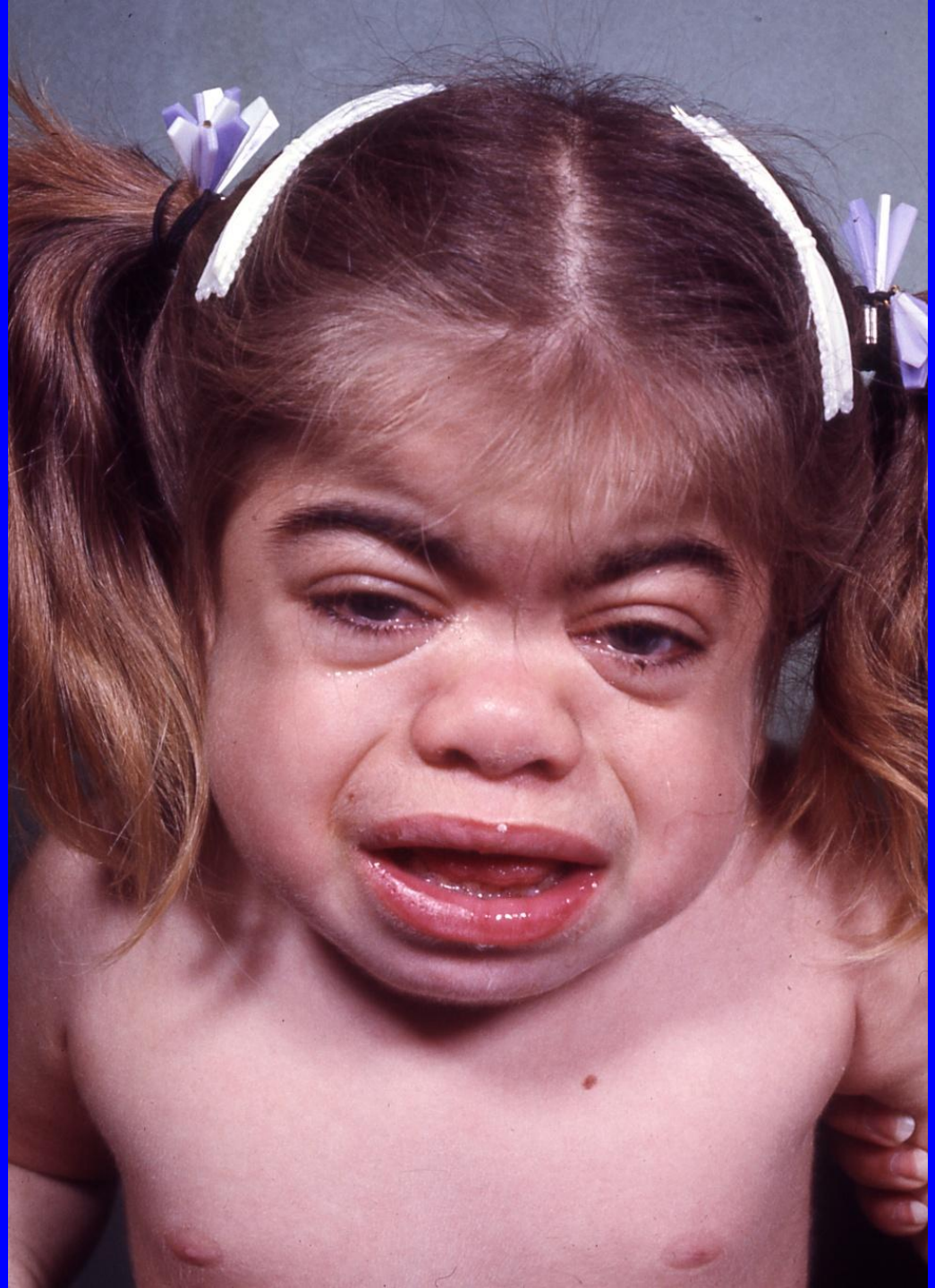
Cut off at 15% of assay mean: <2.6 µmole/hr/L	Enzyme activity (µmole/hr/L)	% of mean $\bar{X}$=17.3	Mutations
	2.53	14.4	*IVS1-13t>g/IVS1-13t>g
	2.46	14.0	wt
	2.44	13.9	p.Gly576Ser;p.Glu689Lys/wt
	2.38	13.5	IVS1-13t>g/p.Glu689Lys
	2.21	12.6	IVS1-13t>g/p.Glu689Lys
	2.2	12.5	p.Gly576Ser;p.Glu689Lys/p.Glu945Lys
	1.77	10.1	p.Glu689Lys/?exon9
	1.70	9.7	p.Met122Lys / p.Val642Asp + psdef
	1.70	9.7	unable to sequence
	1.57	8.9	*IVS1-13t>g/IVS1-13t>g
	1.42	8.1	wt (?)
known affected (n=5)	0.13 – 0.50	1.2 – 4.7	

PPV=0.27

Prevalence=1/27,000

MPS-1 disease

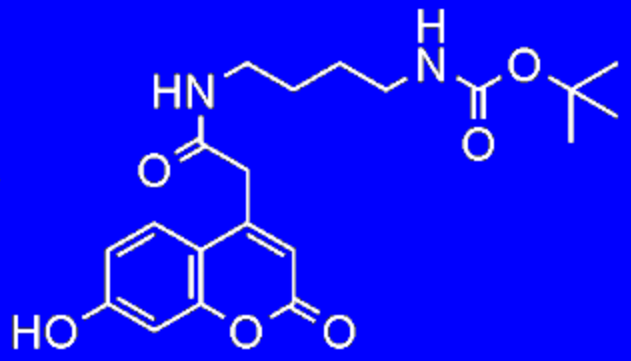
- Hurler phenotype:
  - early diagnosis with:
    - Coarse features
    - Dyostosis multiplex
    - Progressive intellectual loss
    - Cloudy cornea
- ‘attenuated’ phenotype:
    - Mild somatic changes
    - Slow neurological progression
    - Stiff joints
    - Thickened dura
    - Cloudy cornea



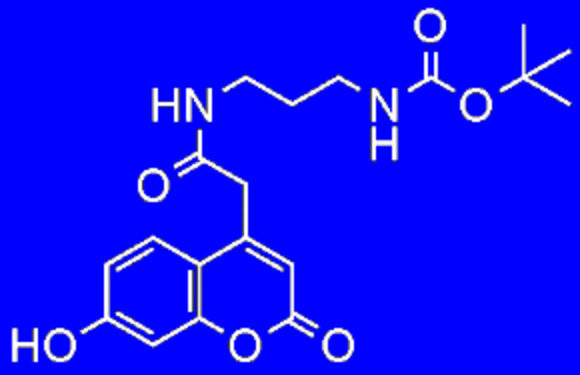
MPS-I Assay

IdA-S (m/z 567.2 for $M+H^+$)

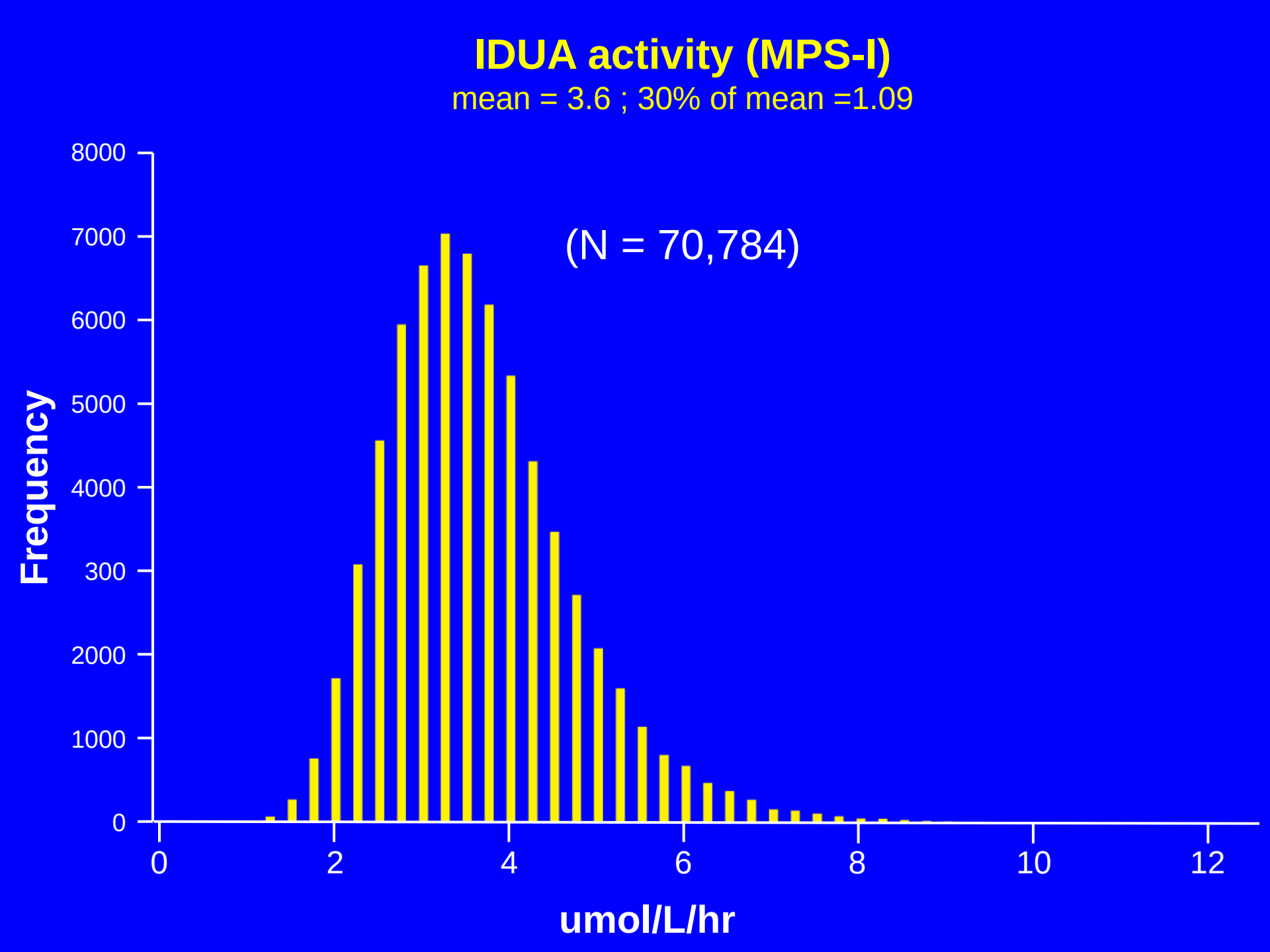
IdA in DBS



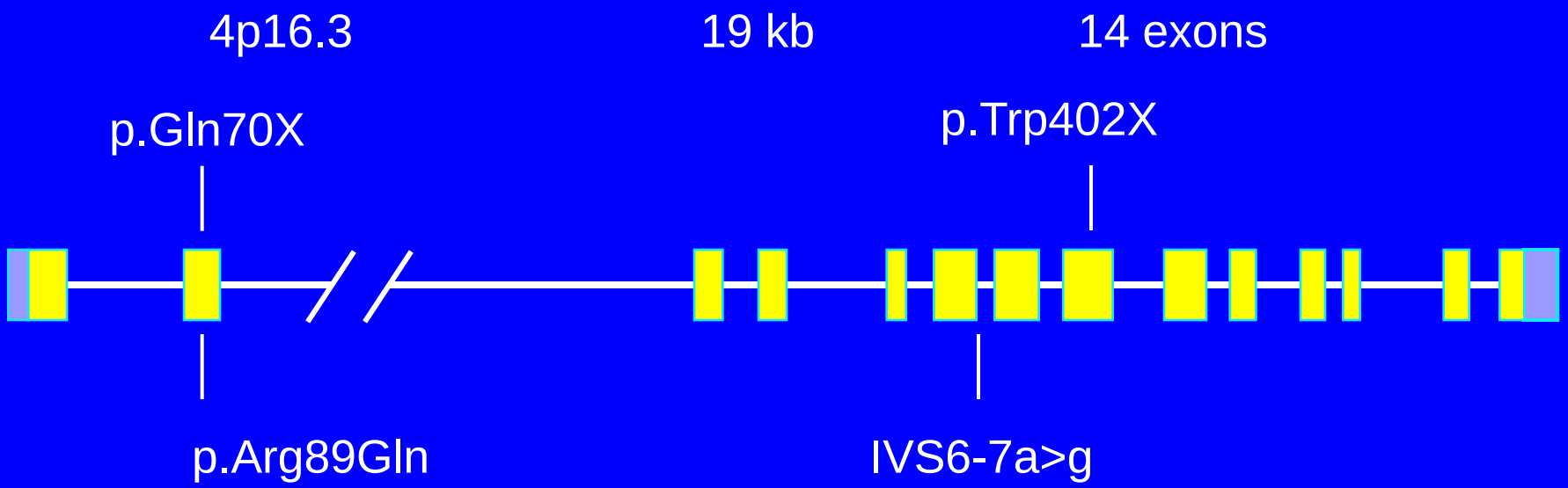
IdA-P (m/z 391.2 for $M+H^+$)



IdA-IS (m/z 377.2 for $M+H^+$)


IDUA activity (MPS-I)
mean = 3.6 ; 30% of mean =1.09
(N = 70,784)
Frequency
8000
7000
6000
5000
4000
300
2000
1000
0
0
2
4
6
8
10
12
umol/L/hr

MPS-1 Disease



Clinical prevalence: ~1/100,000

Table 2: MPS-I

Cut off at 30% of assay mean: < 1.07	Enzyme activity (µmole/hr/L)	% of mean $\bar{X}$=3.6	Mutations
	1.08	29.2	wt
	1.06	28.6	p.Asp119Tyr / p.Glu84Ser
	1.05	28.4	p.Gln70X / p.Gln70X
	1.02	27.6	p.Trp402X / wt
	0.94	25.4	wt (poor punch)
known affected (n=5) known carriers (n=4)	0.58 – 0.83 1.44 – 3.02	18.4 – 22.5 39 – 81	

Positive predictive value = 0.4

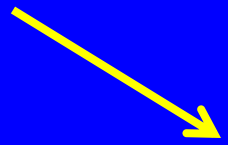
Clinical prevalence: ~1/ 100,000
NBS prevalence : ~1/ 35,000

Summary

- MS/MS assay can easily be adapted to a NBS screening laboratory
- Multiplexing multiple enzymes simplifies the procedure and is a built-in control for sample integrity
- In the first 70,000^{+} samples:
  8 positive newborns identifies with LSD
- Overall prevalence: ~1/9,600

Potential Diseases for LSD Screening By Multiplex Analysis

Fabry	Krabbe	MPS-II
Pompe	Gaucher	MPS-IVA
MPS-I	Niemann-Pick	MPS-VI



organic layer

injection into MS/MS

In queue

MPS-IVB, MLD

CNL1, CNL2

Acknowledgements

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Genzyme Corporation

BioMarin Corporation

Centers for Disease Control