Identification of Newborn Infants at Risk for a Lysosomal Storage Disease by Tandem MS/MS

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1.A collaborative project between the Univ.Washington, Washington State Newborn screening laboratory, and PerkinElmer

Disclosure Information

WORLD Symposium™ 2015

(C Ronald Scott)

I have the following financial relationships to disclose:

Consultant for: Genzyme Corp.

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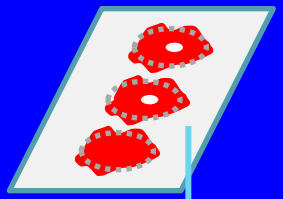
2RO1DK067859

Genzyme Corp / Shire Corp /PerkinElmer

Employee of: Univ Washington

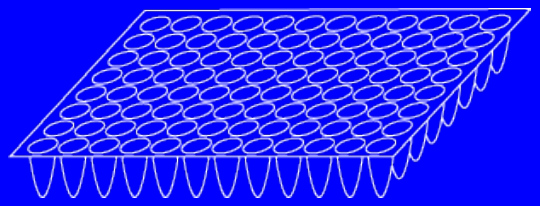
I will not discuss off label use and/or investigational use in my presentation.

PE-UW-6plex Procedure

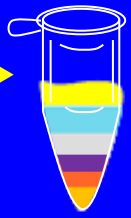


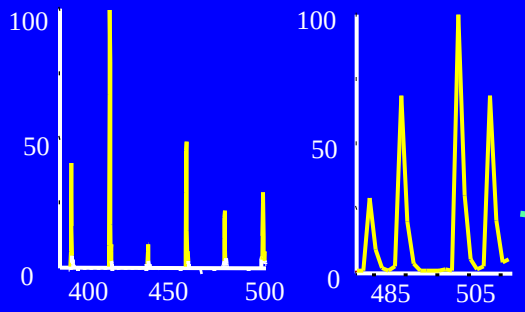
Anonymous blood spots

Fabry, Pompe, MPS-1,
N-P, Gaucher, Krabbe



Assay plate

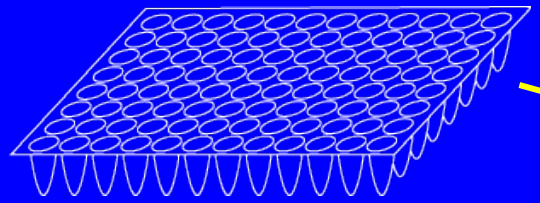




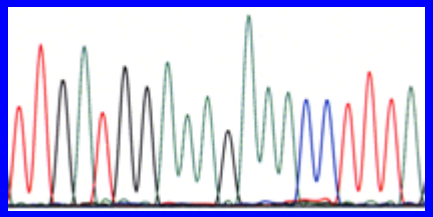
MS/MS

normal

abnormal



Duplicate plate



DNA sequencing (GLA,
GAA, IdUA, GBA, SMPD1,
GALC) for genotype

unaffected

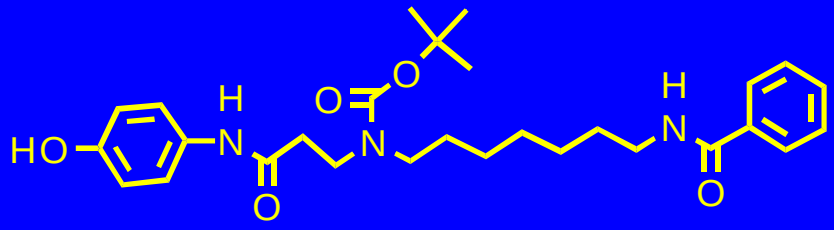
affected

Pompe Assay

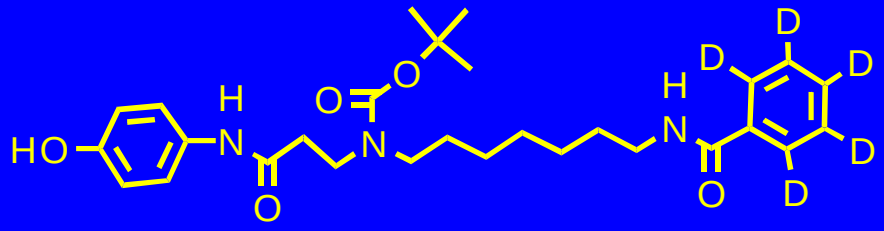
Substrate

Acid alpha-Glucosidase (α-Glu)

(Pompe)

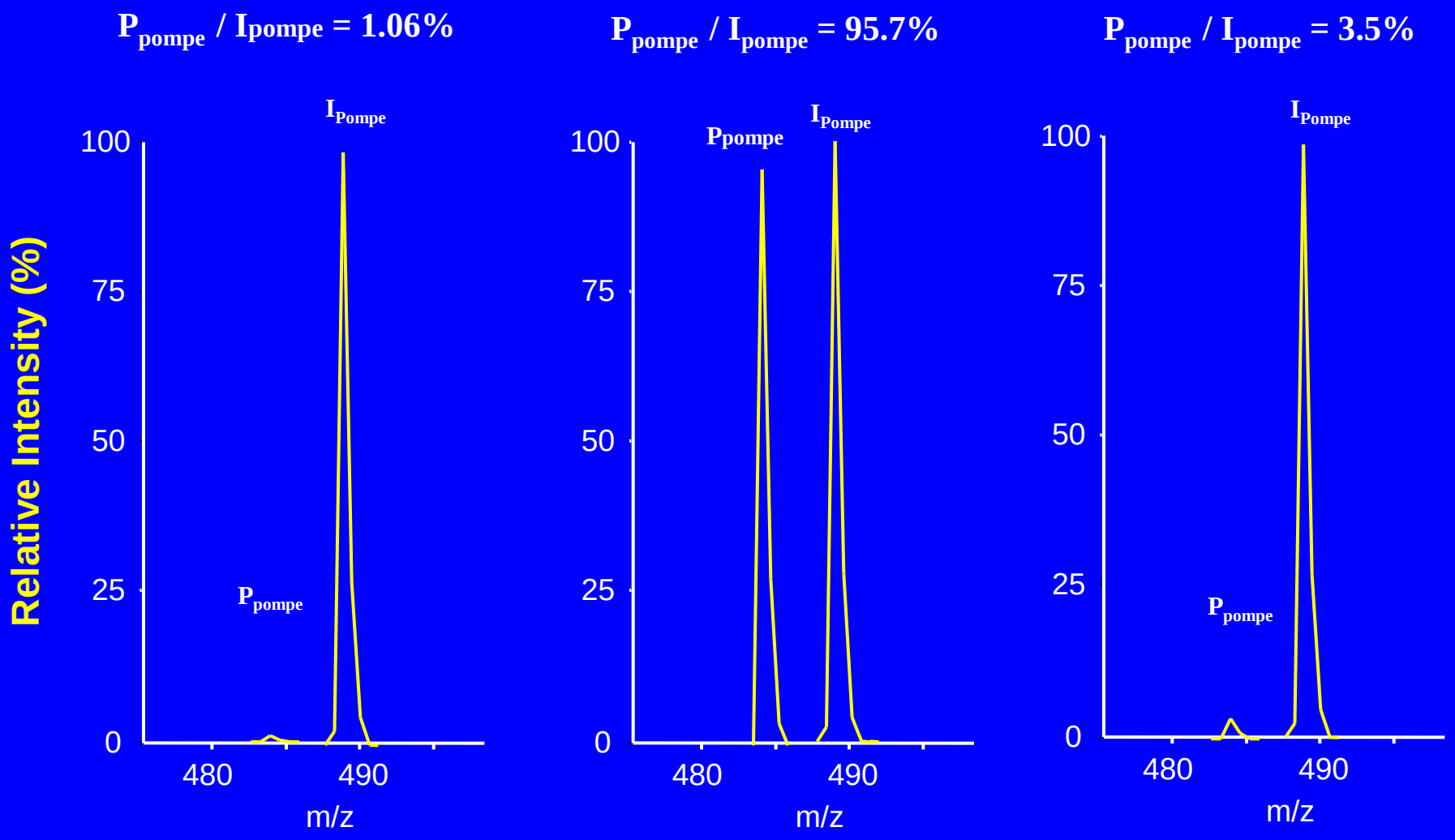


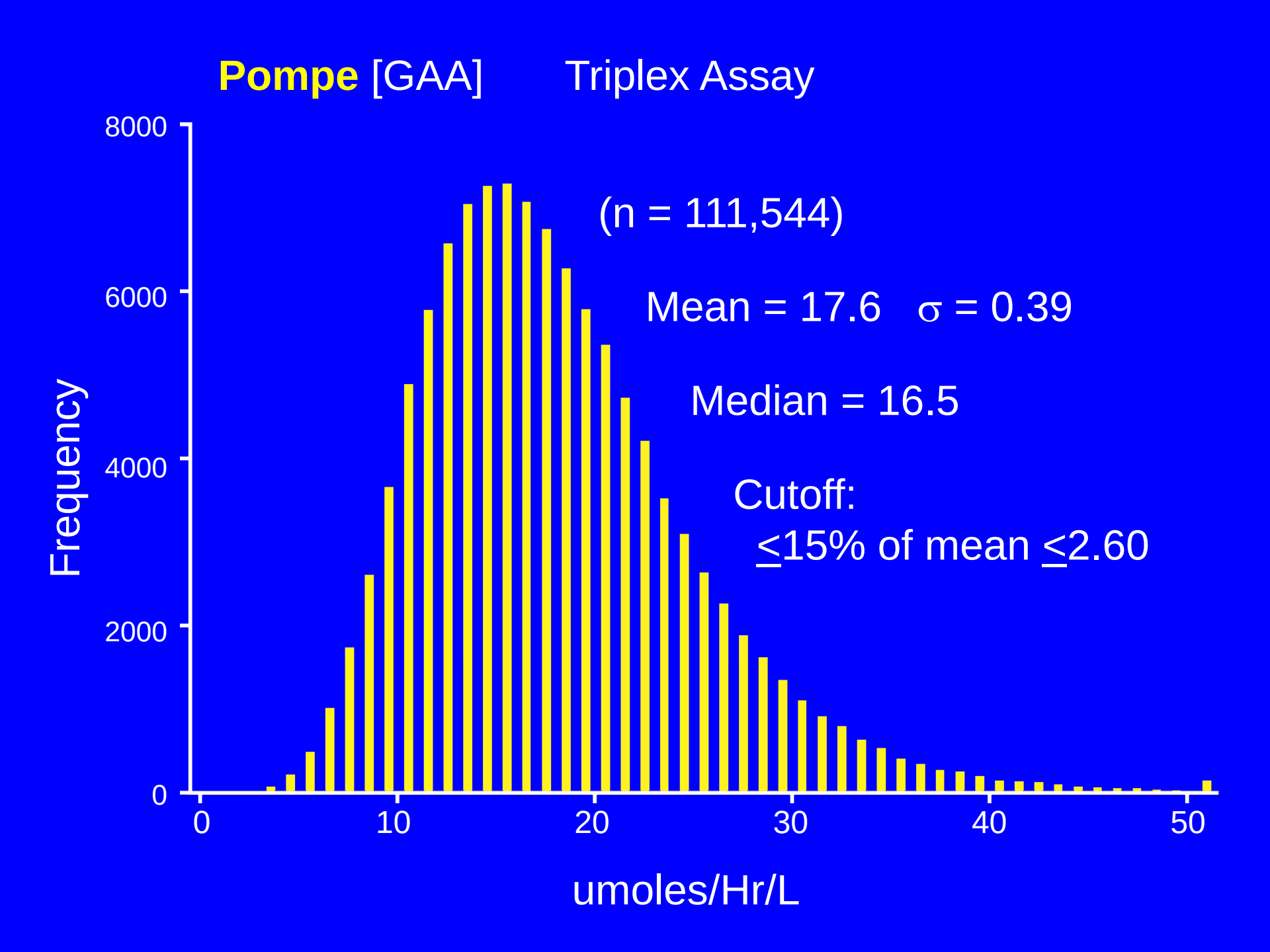
Product



Internal standard

Pompe


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


Pompe [GAA] Triplex Assay
8000
6000
4000
2000
0
Frequency
(n = 111,544)
Mean = 17.6 σ = 0.39
Median = 16.5
Cutoff:
≤15% of mean ≤2.60
0
10
20
30
40
50
umoles/Hr/L

Pompe Disease (n=4) Triplex Assay:

Enzyme Activity (mmol/hr/L blood)	cDNA	Amino acid
2.53	IVS1-13t>g	
	IVS1-13t>g	
1.70	c.365T>A	p.Met122Lys
	c.1925T>A	p.Val642Glu
1.57	IVS1-13t>g	
	IVS1-13t>g	
1.43	IVS1-13t>g	
	c.1-17C>T	

Pompe Disease (n=4) Triplex Assay:

Unaffected with low activity (n=13):

Enzyme Activity

2.45 - 2.20	carrier / wt	n=4
2.38 - 2.20	carrier / pseudo def	n=3
2.44 - 1.49	pseudo def / wt	n=6

Reason for modified MS/MS assay?

1) Improve enzyme accuracy

2) Expand “dynamic range”(mean/blank)

3) Goal: minimize false positive rate

4) Accomplished by: new substrate or IS design, and improved assay buffer

Improvement in Dynamic Range

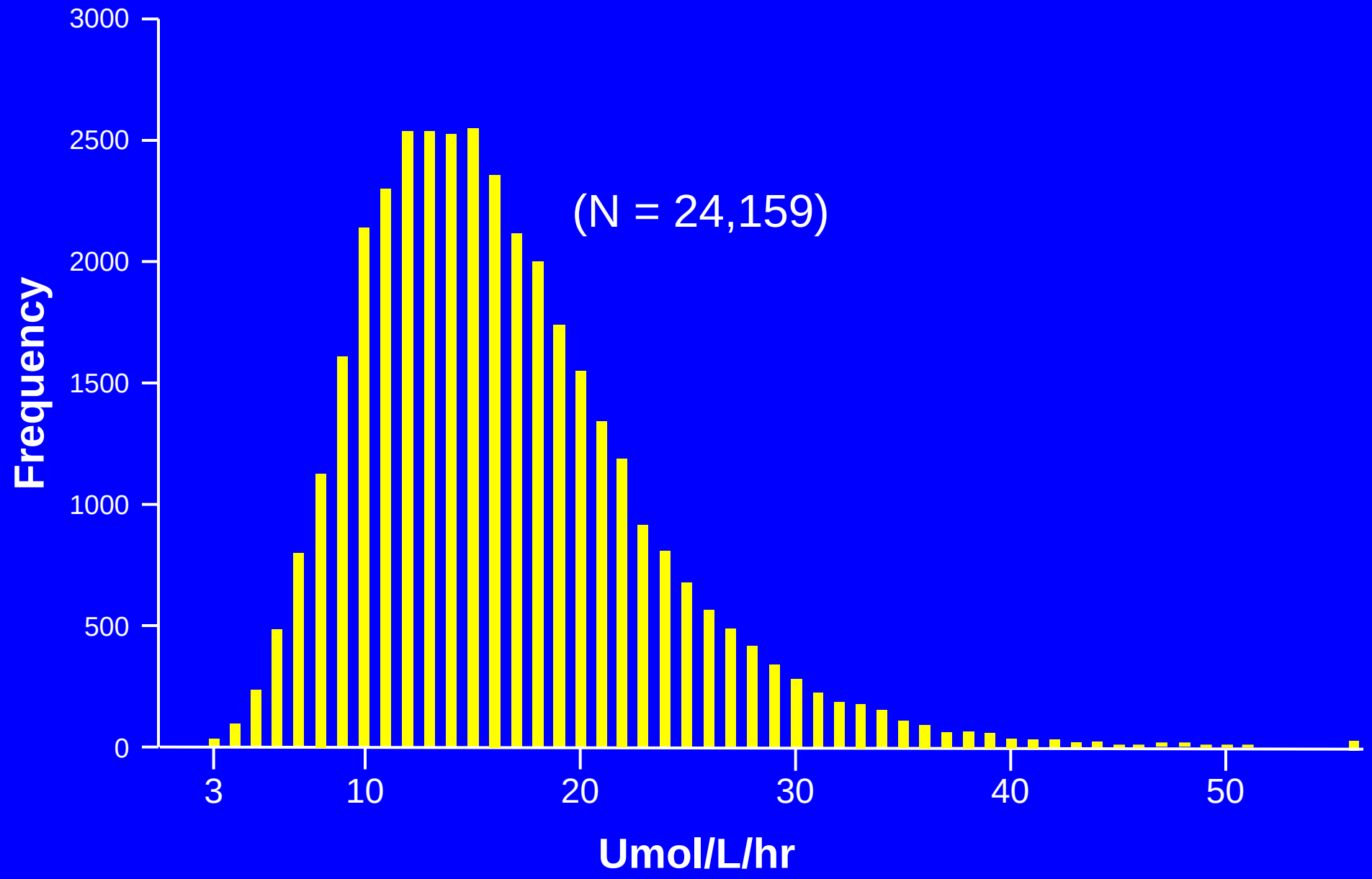
Disorder		Dynamic range (mean/blank)	Improvement
Fabry	UW-6plex CDC-6plex	1049 32	32x
Pompe	UW-6plex CDC-6plex	2358 216	11x
MPS-1	UW-6plex CDC-6plex	585 75	7x

Improvement in Dynamic Range

Disorder		Dynamic range (mean/blank)	Improvement
N-P	UW-6plex CDC-6plex	472 58	8x
Gaucher	UW-6plex CDC-6plex	748 59	12x
Krabbe	UW-6plex CDC-6plex	243 5.6	43x

GAA activity (Pompe) PE-UW-6Plex Assay

mean = 11.79, cut off 10% of mean =1.18

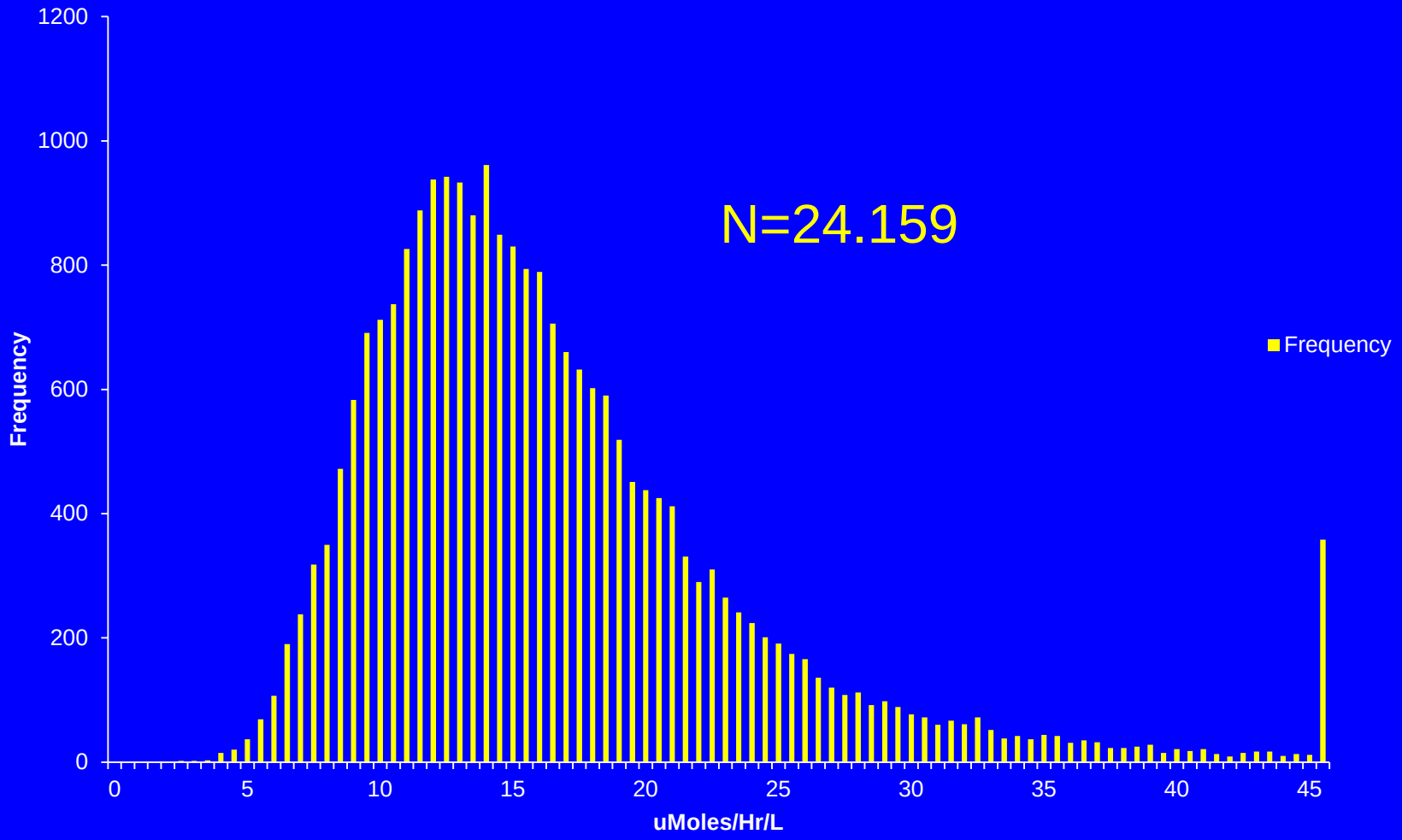


Pompe Disease (n=3) 6Plex Assay:

Enzyme Activity (mmol/hr/L blood)	**Amino acid**
1.05	p.T602I/pseudo
1.56	p.S736A/wt
1.61	c.-32-13 t>g/ pseudo

GLA activity (Fabry) PE-UW-6Plex Assay

mean = 16.78, cut off 15% of mean =2.52

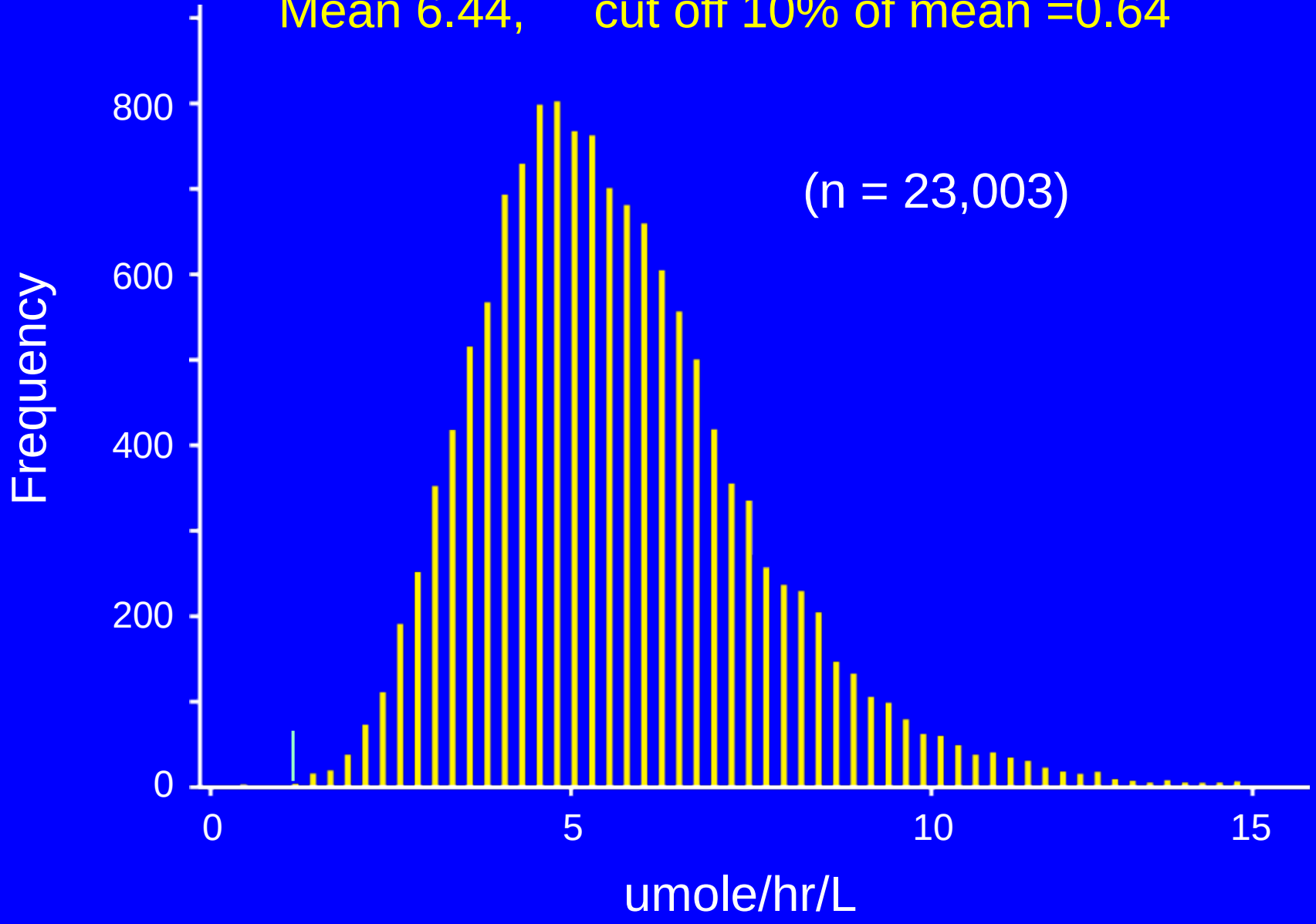


Fabry Disease (n=1) :

Enzyme Activity (mmol/hr/L blood)	**Amino acid**
2.4	p.R356Q

IDUA PE-UW-6Plex Assay

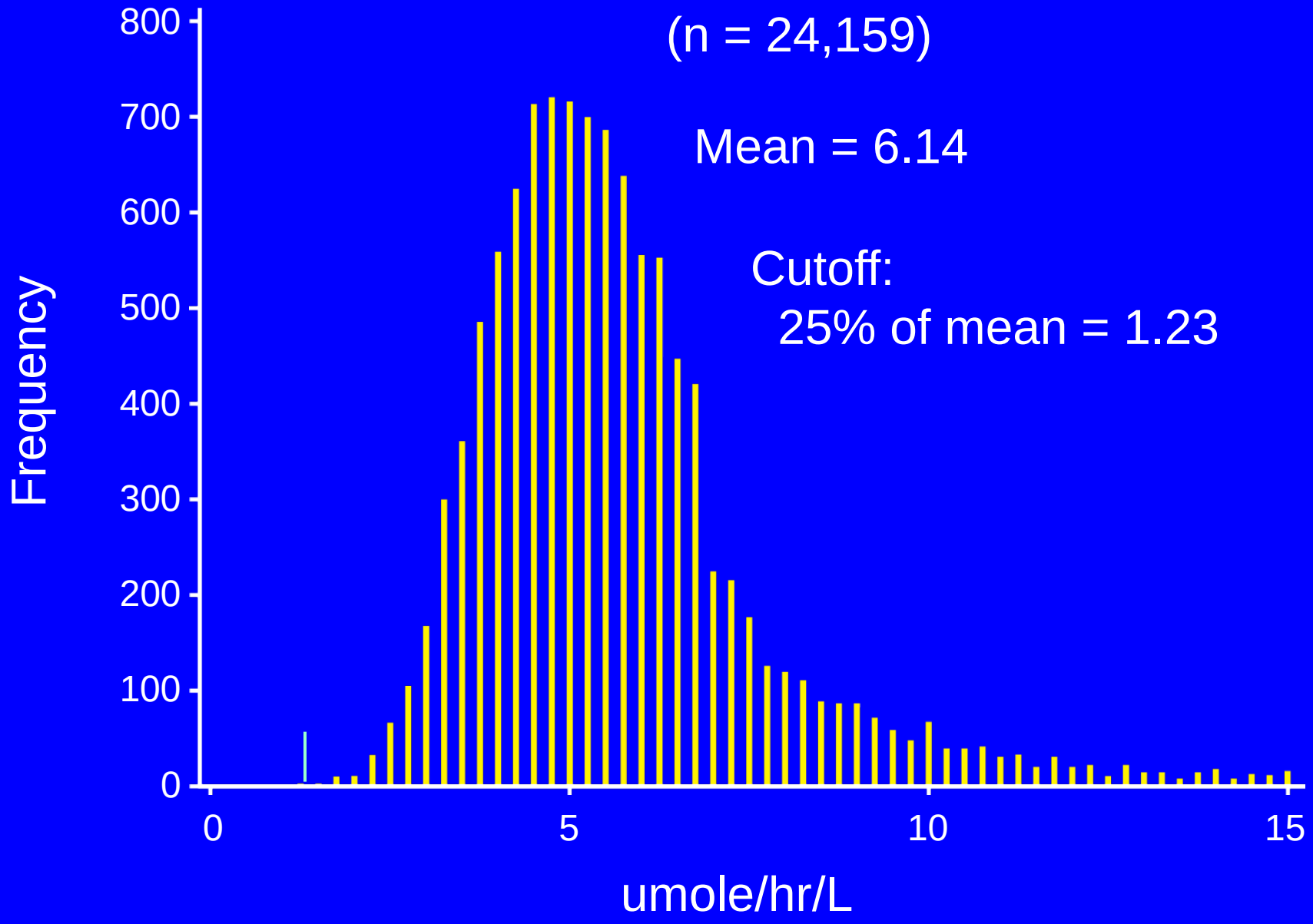
Mean 6.44, cut off 10% of mean =0.64



MPS-I (n=5) 6plex Assay:

Enzyme Activity (mmol/hr/L blood)	**genotype**
0.29	Carrier/ wt
0.45	Pseudo/Pseudo
0.48	Pseudo/Pseudo
0.74	Pseudo/wt
0.86	Pseudo/wt

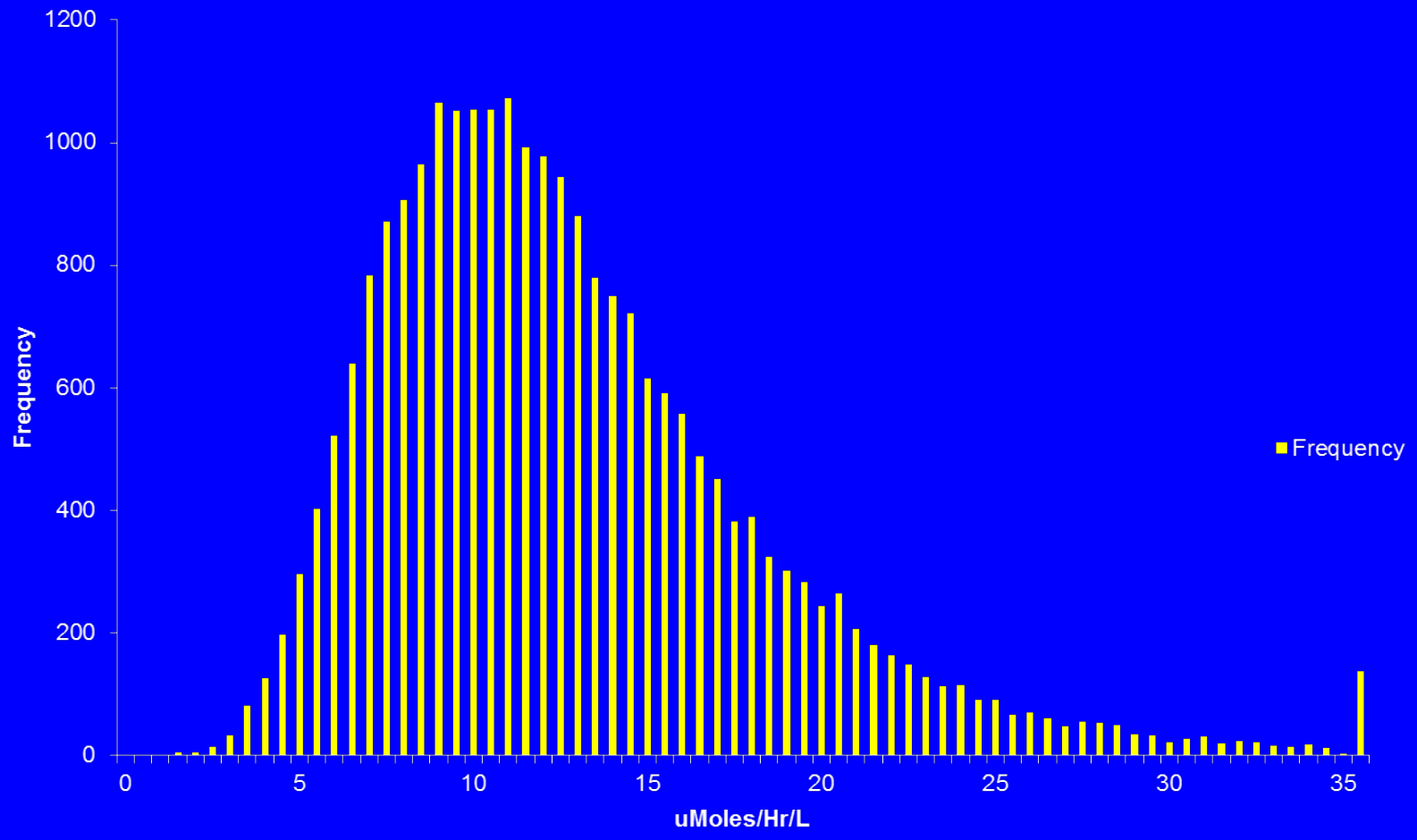
SMPD1 PE-UW-6Plex Assay



SMPD1 Disease (n=5) :

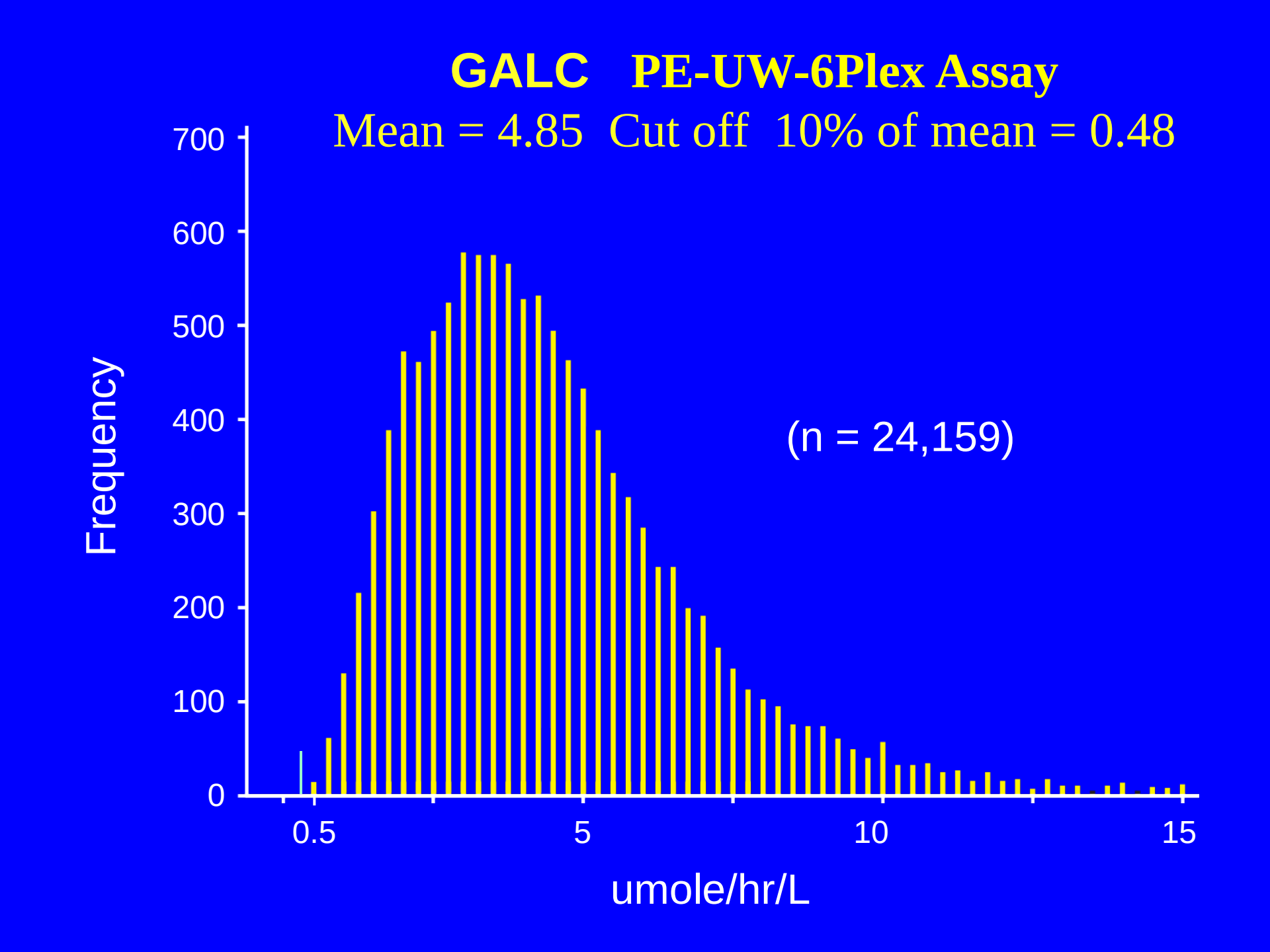
Enzyme Activity (mmol/hr/L blood)	**Genotype**
1.18	c.107_118del (+/+)
1.43	c.143_149del (+/+)
1.47	c.137_143del (+/-)
1.66	wt/wt
1.71	p.Pro331Ser/wt

GBA PE-UW-6Plex Assay
(n=24,159) Mean= 12.71 10%of mean = 1.27



GBA Disease (n=3) :

Enzyme Activity (mmol/hr/L blood)	**Genotype**
0.97	affected
1.43	wt/wt
1.59	wt/wt


GALC PE-UW-6Plex Assay
Mean = 4.85 Cut off 10% of mean = 0.48
(n = 24,159)
Frequency
700
600
500
400
300
200
100
0
0.5
5
10
15
umole/hr/L

GALC Disease (n=4) :

Enzyme Activity (mmol/hr/L blood)	**Genotype**
0.37	pseudo/pseudo
0.38	wt/wt
0.46	wt/wt
0.48	wt/wt

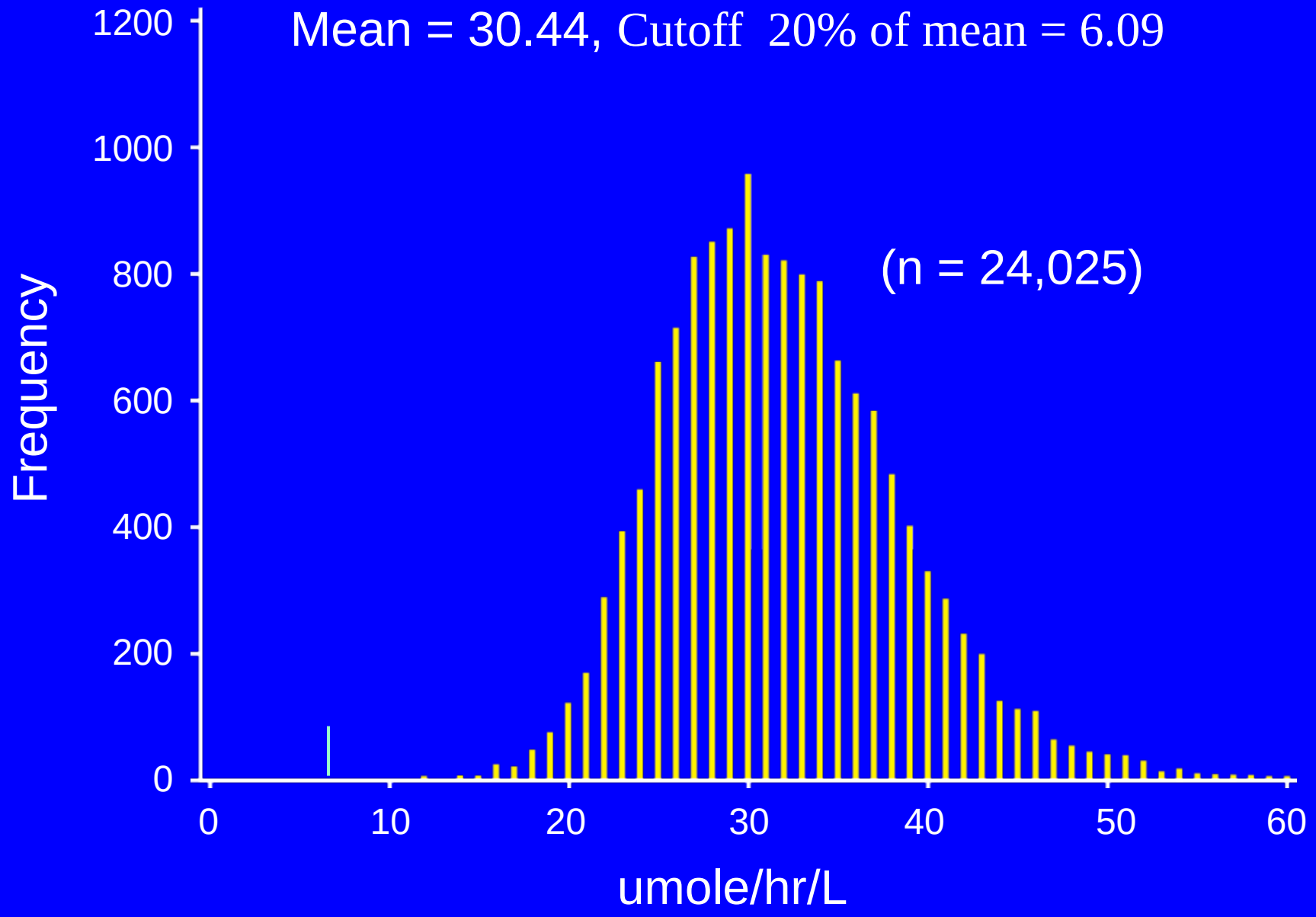
Identified Low Enzyme Activity by PE-UW-6plex (24,159 samples)

	Low Activity	Affected	Predicted Phenotype
Fabry	1	1	1 affected
Pompe	3	0	1 carrier/pseudo 1 pseudo 1 benign variant unknown significance
MPS-1	5	0	1 carrier, 4 pseudo
N-P	5	2	2 affected, 2 carrier, 1 wt
Gaucher	3	1	1 affected, 2 wt
Krabbe	4	0	1 pseudo, 3 wt
Total	**21**	**4**	

Potential candidates for newborn screening of Lysosomal disorders.

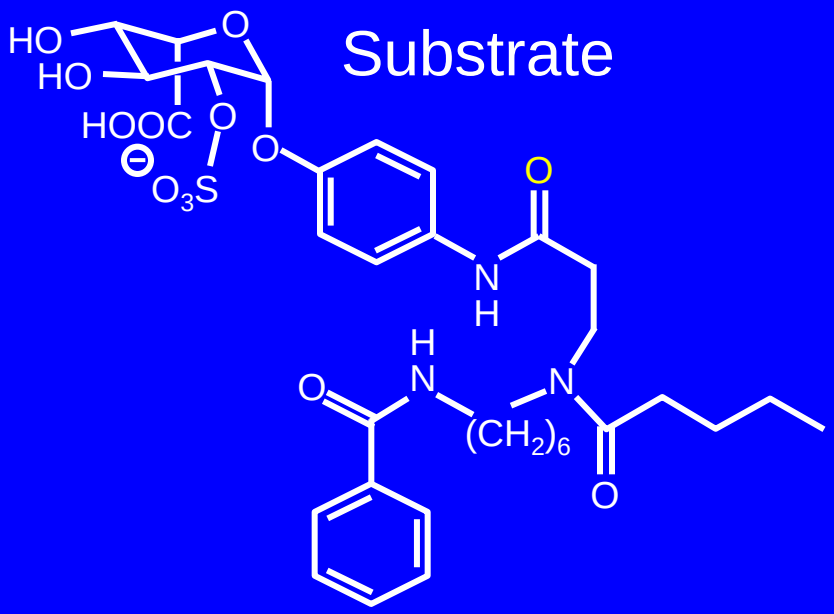
Disorder	Rx	Requires early detection
Gaucher	ERT/SM	+/-
Fabry	ERT/SM	+/-
MPS-I	ERT/BMT	+
Pompe	ERT/SM	+
Krabbe	BMT	+
Niemann-Pick B	?	?
• MPS-II	ERT	+
• MPS-IVA	ERT	+
• MPS-VI	ERT	+

MPS II

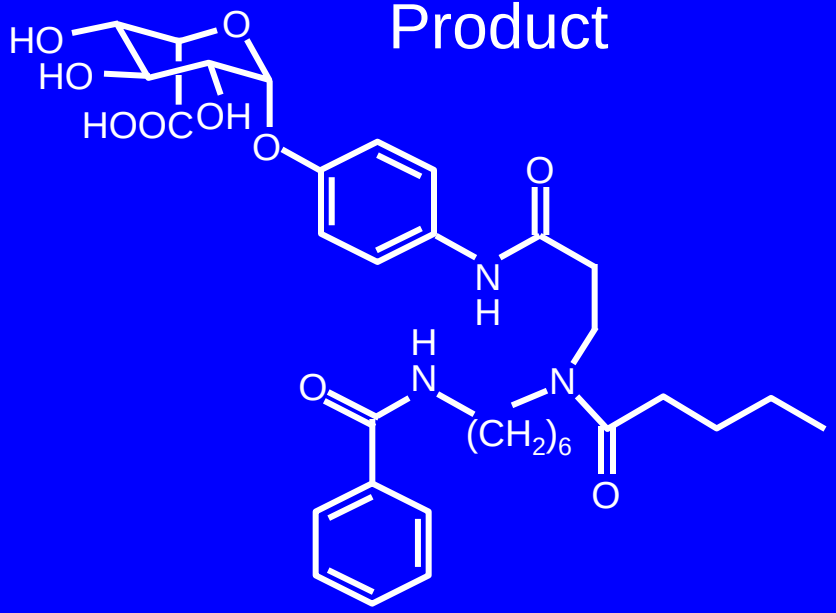

Mean = 30.44, Cutoff 20% of mean = 6.09
(n = 24,025)
Frequency
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
umole/hr/L

MPS II Assay

Substrate



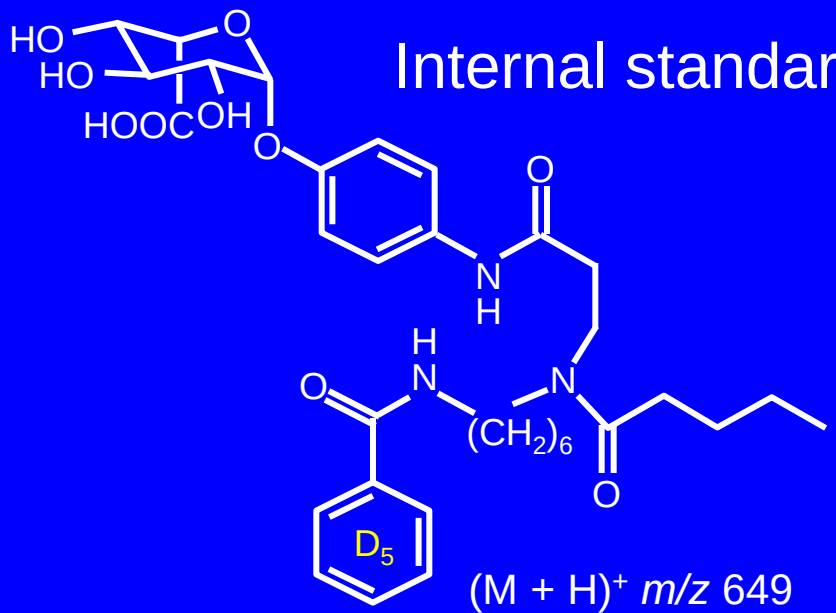
Product



$C_{33}H_{45}N_3O_{10}$

$(M + H)^+$ *m/z* 644

Internal standard



$(M + H)^+$ *m/z* 649

Summary

1. The new PE-UW-6-plex assay to detect infants at risk for Fabry, Pompe, MPS-I, Gaucher, Niemann-Pick and Krabbe is robust and is being successfully performed in a newborn screening laboratory using MS/MS.
2. All six enzymes are assayed simultaneously from a single 3.2 mm blood spot with a minimum of false positives.
3. From 143,798 assays (24,159 NBS) only 21 had low activity. Four were confirmed to be affected (Fabry, Gaucher & N-P).
   The false positive rate is only 1/8500 assays.

Co-investigators

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