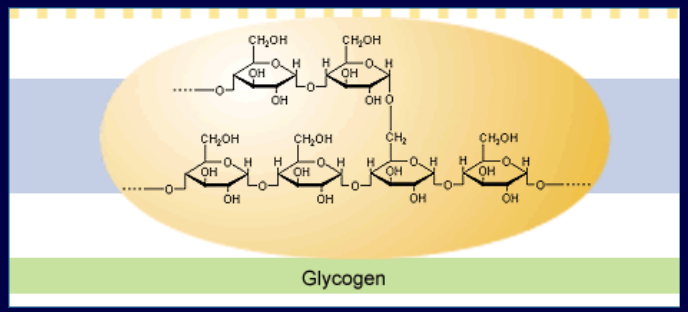
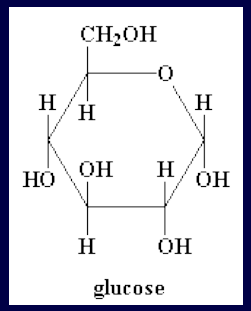
Newborn Screening for Pompe Disease in New York

1. New York Assay(s)

3. Testing algorithm

4. Screening Data





Multiplex MS/MS methods: NY

1. Direct Multiplex Assay of Lysosomal Enzymes in Dried Blood Spots for Newborn Screening

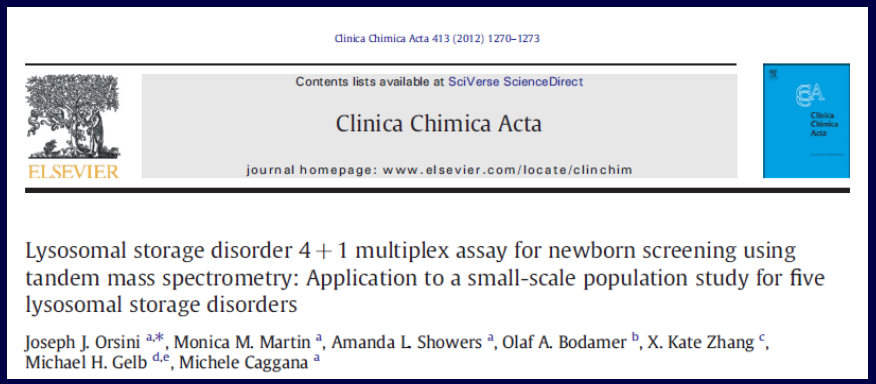
   Yijun Li,[1] C. Ronald Scott,[2] Nestor A. Chamoles,[3] Ahmad Ghavami,[4] B. Mario Pinto,[4] Frantisek Turecek,[1] and Michael H. Gelb[1,5*]

2. Multiplex Enzyme Assay Screening of Dried Blood Spots for Lysosomal Storage Disorders by Using Tandem Mass Spectrometry

   X. Kate Zhang,* Carole S. Elbin, Wei-Lien Chuang, Samantha K. Cooper, Carla A. Marashio, Christa Beauregard, and Joan M. Keutzer

3. A Tandem Mass Spectrometry Triplex Assay for the Detection of Fabry, Pompe, and Mucopolysaccharidosis-I (Hurler)

   Trisha A. Duffey,[1] Garland Bellamy,[2] Susan Elliott,[2] Angela C. Fox,[3] Michael Glass,[2] Frantisek Turecek,[1*] Michael H. Gelb,[1,4*] and C. Ronald Scott[3*]

4. 

Dieter Matern added MPS-I and X-ALD (extra punch)

Pompe (LSD) assays available

1. Fluorescent assay, single enzyme

2. Fluorescent assay, multiplex (digital fluidics/Missouri)

3. Tandem mass spectrometry assays
   a. Optimized enzyme, with/without L/L/SPE (NY)
   b. Multiplexed enzymes, with/without L/L/SPE (Current NY assay is for Krabbe, Pompe, and X-ALD, triplex assay)
   c. Optimized enzyme, followed by on-line cleanup
   d. Multiplexed enzyme, followed by on-line cleanup

MS/MS reagents:

1. *Currently using CDC provided reagents*: use to screen for Krabbe, Pompe, Fabry, Gaucher, MPS-I, Niemann Pick A/B.

2. *Perkin Elmer* : Substrate/Internal Standard pairs available
   - NY: recently evaluated/validated materials for Gaucher, NP-A/B, Fabry, and MPS-1 (Pilot study)
   - NY: evaluating Krabbe, Pompe, MPS-1

5-enzymes

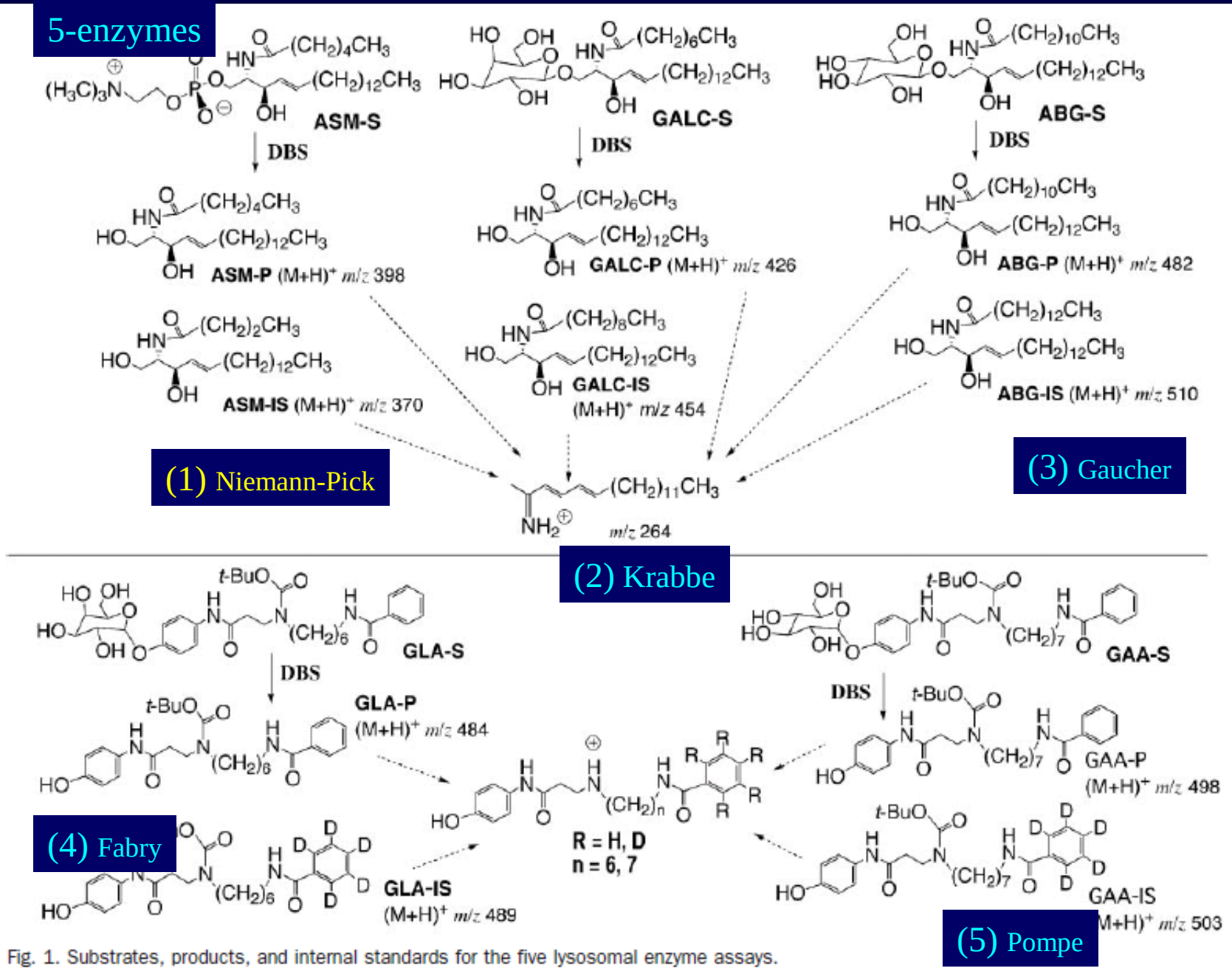
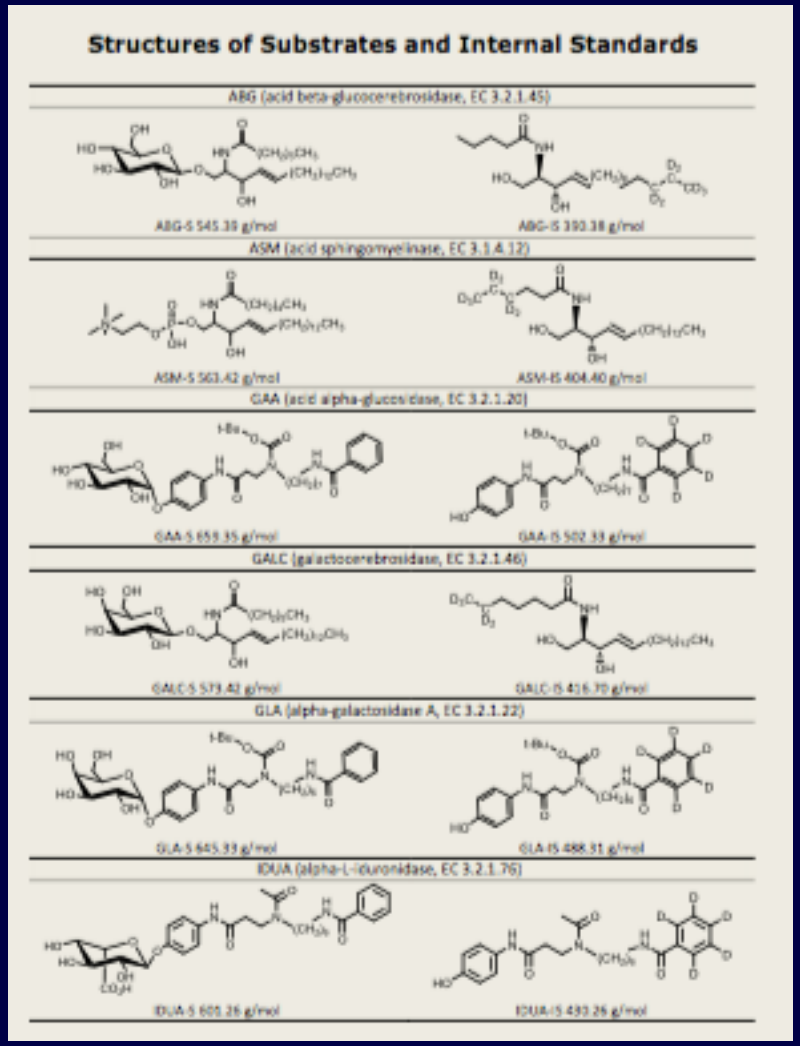


Fig. 1. Substrates, products, and internal standards for the five lysosomal enzyme assays.

(6, 7, 8, 9) MPS-I, MPS-II, MPS IVA, MPS VI

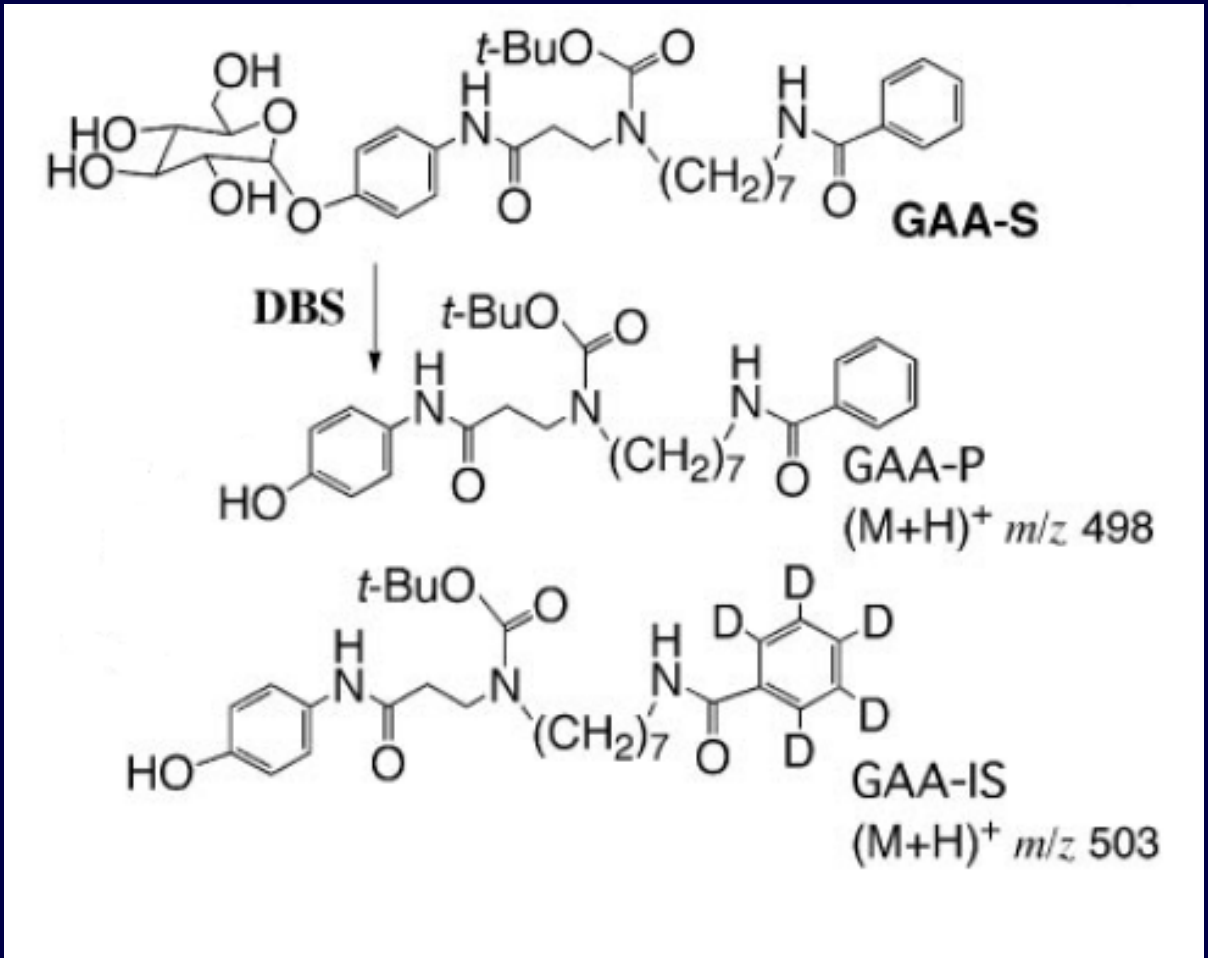
Li/Gelb/Scott et al, 2004

PE Substrates



Potier et. al , APHL Symposium Oct. 2014

Focus on Pompe Assay



New York State LSD Assay

Punch 3-mm specimen, add assay solution reagent and incubate

19 hours

Quench reaction (50/50 MeOH/EtAc), perform Liquid / liquid extraction (EtAc/H20), remove organic phase (50 uL)

Dry plates (10 min)

Reconstitute extract in 19:1 EtOAc/MeOH, perform SPE

Dry plates (40 min)

Re-dissolve in MS suitable solvent (99/1 MeOH/H20)/Combine with X-ALD extract

Analyze samples, 1.5 minutes per sample

Calculate activity/sample, daily mean activity, *% of daily mean act/sample*

New York State Assay: *(ALD)*

Punch 3-mm specimen, add 200 µL methanol with d4-C26:0 LPC

↓ **1 hour extraction**

Remove 25 µL of extract and combine with LSD extract (optional)*

↓

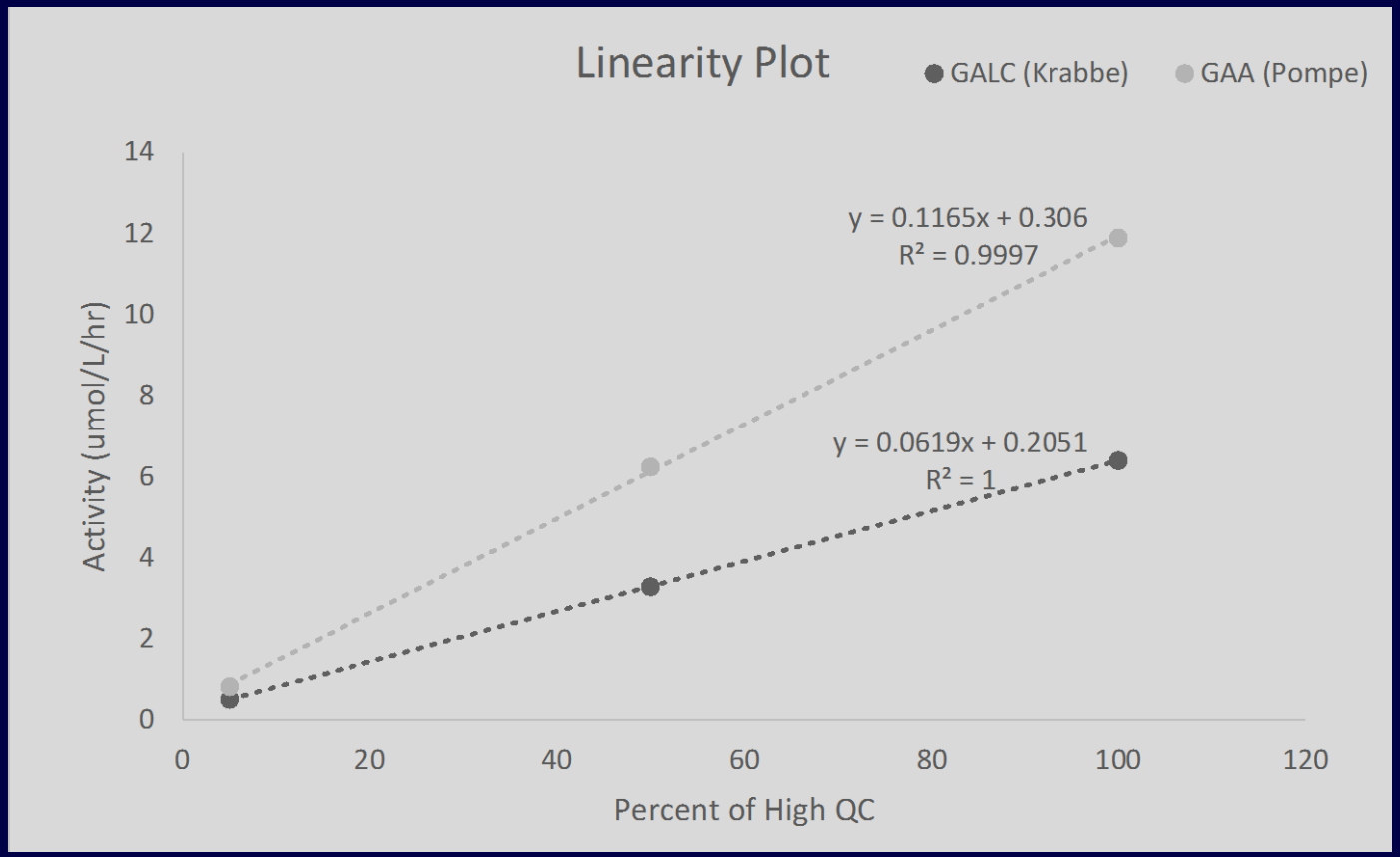
Analyze samples, 1.5(1.0) minutes per sample/Marker is C26:LPC (C20,22,24,26)

↓

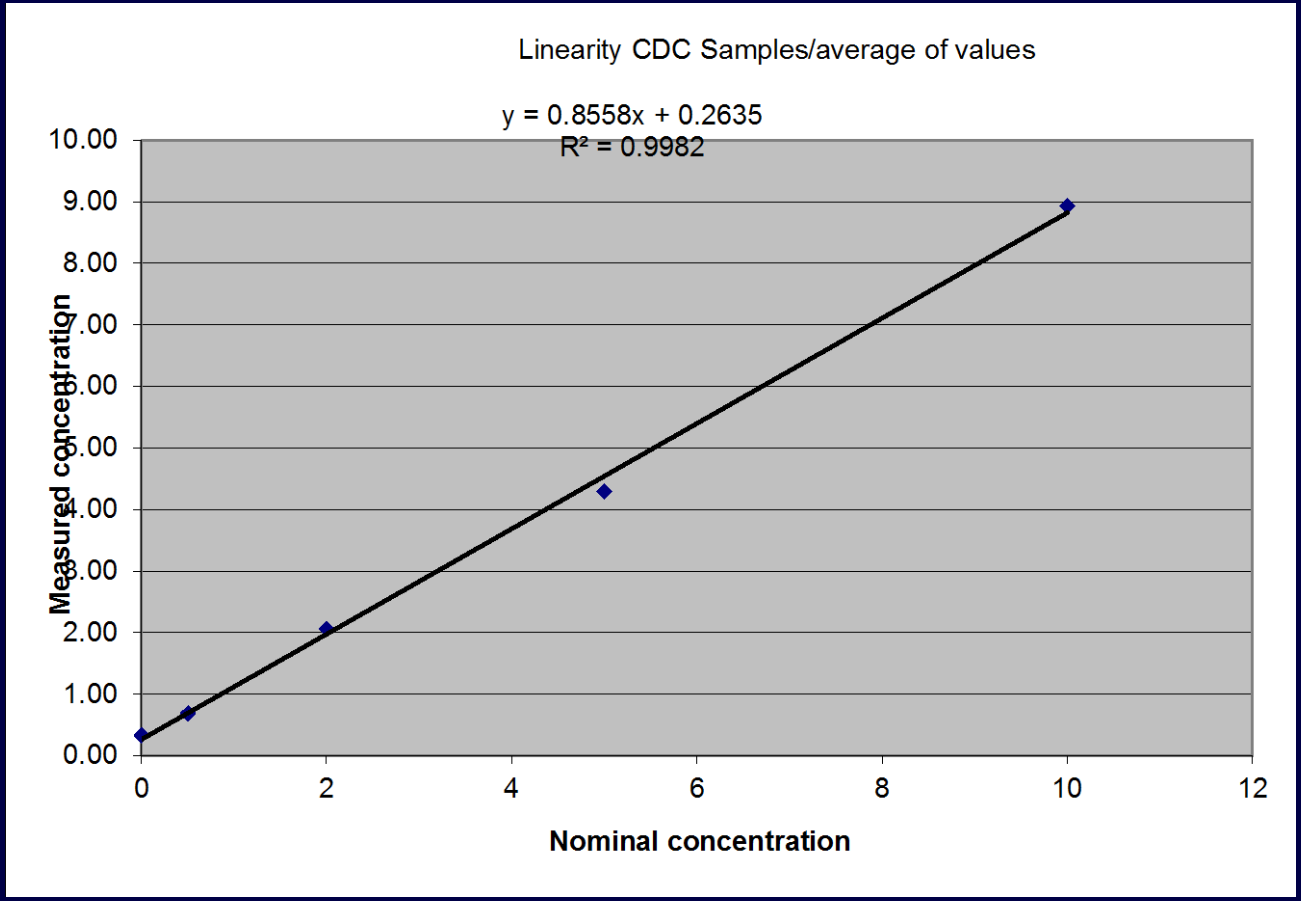
Follow screening algorithm

* Important to combine quickly with LSD extract.

Linearity LSDs



Linearity ALD



“Accuracy”: GALC/GAA

Tabulated CDC Activity Values:

QC Level	Activity	Calculated Activity (µmol/L/hr)	
		GALC	GAA
Low QC	CDC expected (95 %CL)	0.39 (0.32-0.46)	0.97 (0.56-1.38)
	NYS Observed	**0.52**	**0.83**
Med QC	CDC expected (95 %CL)	3.14 (2.60-3.69)	9.92 (8.02-11.82)
	NYS Observed	**3.29**	**6.24**
High QC	CDC expected (95 %CL)	6.04 (5.04-7.03)	19.99 (16.26-23.72)
	NYS Observed	**6.40**	**11.90**

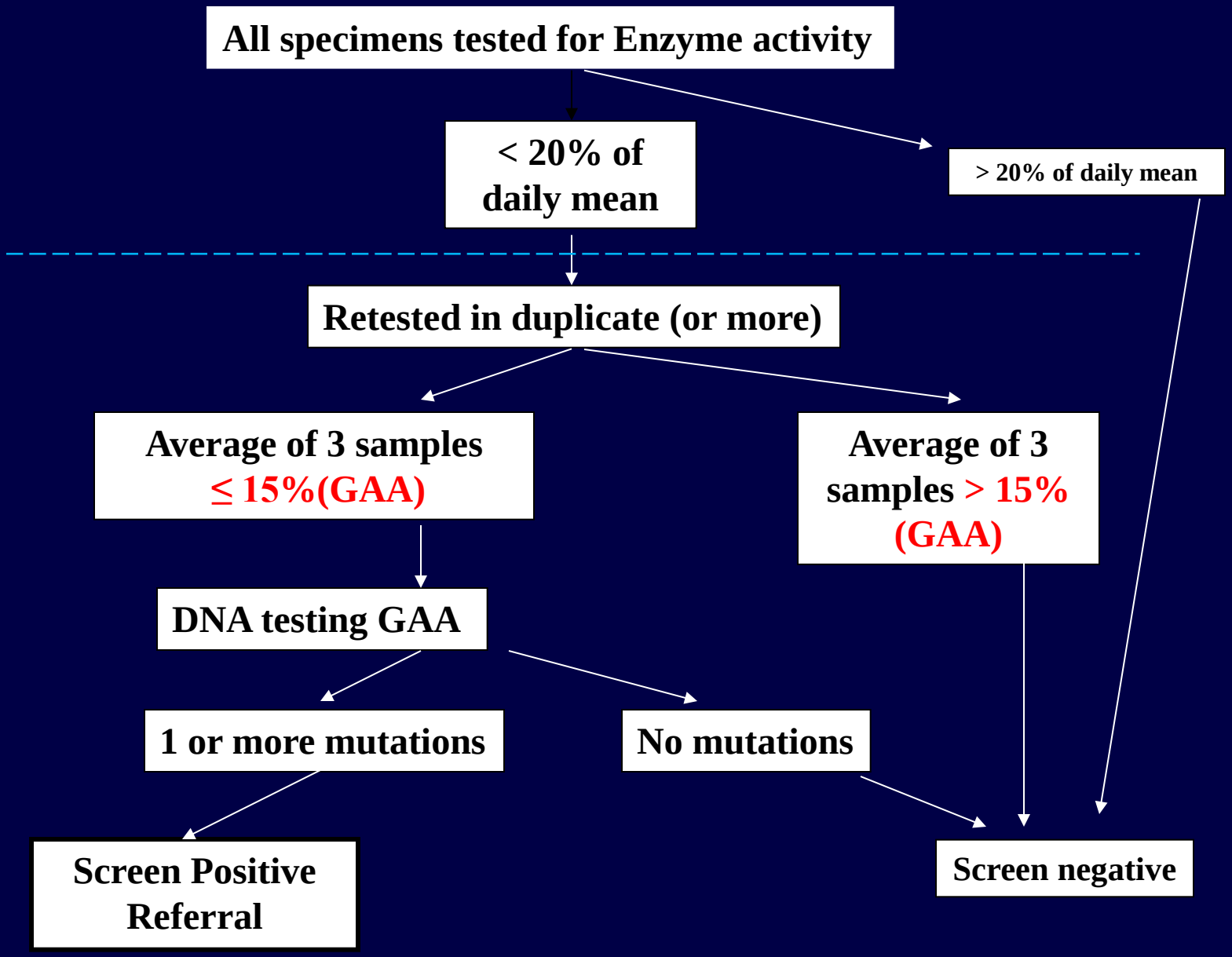
Table 4: Comparison of NY measured activities to CDC measured activities for each level of control sample

Limit of detection: GALC/GAA

Limit of Detection defined as Std Dev *3

	Low QC			
	P/IS Ratio		Activity (µmol/L/hr)	
	GALC	GAA	GALC	GAA
Mean	0.19	0.31	0.52	0.83
Median	0.20	0.31	0.53	0.83
Min	0.14	0.21	0.38	0.57
Max	0.23	0.39	0.62	1.06
Std Dev	0.030	0.055	0.079	0.150
LOD	0.09	0.16	0.24	0.45

Cutoffs and Testing Algorithm



Population Studies: Missouri Positive Controls Blinded study, 38 samples.

Positive samples	NY activity	NY % of mean	Diagnosis
MO_23	0.28	1.8	Pompe - classical infantile
MO_8	0.31	2	Pompe - classical infantile
MO_11	0.68	4.5	Pompe - nonclassical infantile
MO_6	0.72	4.8	Pompe - late onset
MO_36	0.78	5.1	Pompe - classical infantile
MO_17	0.82	5.4	Pompe - late onset
MO_35	1.39	9.2	Pompe - late onset
MO_12	1.42	9.4	Pompe - late onset
MO_27	1.62	10.7	Pompe - late onset
MO_33	1.62	10.7	Carrier
MO_3	1.65	10.9	Genotype of unknown significance
MO_25	1.79	11.8	Pompe - late onset
MO_9	1.89	12.5	Pompe - late onset
MO_20	2.06	13.6	Genotype of unknown significance
MO_13	2.26	14.9	Genotype of unknown significance
MO-22	2.66	17.6	Pseudo deficiency
MO-30	3.19	21.1	Pseudo deficiency
MO-16	3.46	22.8	carrier
MO-38	3.66	24.2	Pseudo deficiency
MO-29	4.13	27.3	Pseudo deficiency

Thanks to Patrick Hopkins and Tracy Klug for sharing

Population Studies Statistics: 10/1/14 – 4/14/15

GAA N= 133809		(N = 250,000/year)
% of mean	**Count**	**Count**
<7	4	7
<8	4	7
<9	6	11
<10	9	17
<11	12	22
<12	17	32
<13	21	39
<14	31	58
<15	43	80
<20	154	288
After Repeat Data DNA/Referrals		
To DNA(<15)	43	80
DNA Tested	21	39
Polymorph	1	367
Normal Variant	0	0
Awaiting DNA	1	NA
Total Referrals	19	35

Referral 20 on Thursday, looks like a late onset case based on genotype

Only one/20 with Poly (pseudo-deficiency allele) only.

20 referred cases ~ 120,000 births

Referral #	Diagnosis	% Daily mean
1	Carrier of Pompe Disease	12.1%
2	Pompe Disease, Late Onset	7.2%
3	Not Seen, refussal (likely carrier)	11.7%
4	Pompe Disease, Late Onset	6.7%
5	Carrier of Pompe Disease	14.9%
6	Carrier of Pompe Disease	14.7%
7	Carrier of Pompe Disease	14.7%
8	Carrier of Pompe Disease	14.5%
9	Carrier of Pompe Disease	8.8%
10	Late onset with VOUS, further eval.	10.6%
11	Carrier of Pompe Disease	11.0%
12	Late onset	10.7%
13	Carrier of Pompe Disease	13.9%
14	Likely Carrier of Pompe Disease	10.8%
15	Likely Carrier of Pompe Disease	13.8%
16	Likely Carrier of Pompe Disease	13.0%
17	Likely Carrier of Pompe Disease	13.6%
18	Likely Late Onset Pompe	10.0%
19	Likely Carrier of Pompe Disease	10.3%
20	Likely Late Onset Pompe	10.3%

20 Referred Cases/1 pseudo

1. Four (5?) late onset (7.2%, 6.7%, 10.7%, 10.0%, 10.3%) (1:30,000)

2. Six confirmed to be carriers* (12.1%, 14.9%, 14.7%, 14.5%, 8.8%, 11.0%, 13.9%,

3. One patient, parents refused to bring child into for follow-up (11.7%, likely carrier).

4. Seven awaiting follow-up diagnostic testing (Likely one more late onset)

No infantile cases to date

* Carriers often have pseudodeficiency allele in trans

20 referred cases ~ 120,000 births

1. Current referral rate: 1:6000 (0.017%)

2. Potential late onset incidence: 5 late onset cases per 120,000 infants screened: 1/24,000*

3. 0.013% (15/120,000)

4. PPV: 25%

Conservative cutoff, if used 12% would have 11 referrals and still detected all potential late onset cases (PPV = 45%).

* Assumes all apparent carriers will develop symptoms. Big challenge is predicting severity of symptoms/age of onset

Thank you
Questions?

Acknowledgements:

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