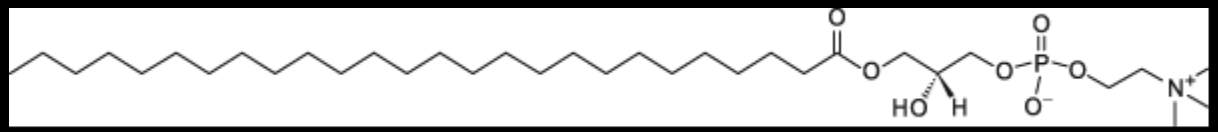
Screening for Leukodystrophies: Update on New York State's Experience

October 29, 2014



ALD screening in New York

ALD Screening

Sequence of Events 1 – Legislated

- Aidan Seeger, a 7 year old from Brooklyn passes 4/29/2012
- Mrs. Seeger called Dr. Caggana in May 2012 to discuss screening
- Family garnered support: NY politicians; website; billboards
- Bill submitted August 2012
- Approved by Health Finance Committee 02/28/2013
- Became law 03/31/2013; start 01/01/2014 (actual 12/30/2013)

Condition Information

- Causes damage to the myelin sheath; brain insulator
- Accumulation of saturated very long chain fatty acids (VLCFAs)
- Lack of a transporter protein that moves VLCFA into peroxisomes for degradation
- Affects predominantly males; females can have mild disease; rarely cerebral disease in females
- Frequency: 1/17,000 – 1/20,000 births
- Expect 12 to 15 cases annually in New York

Three Types of Adrenoleukodystrophy

- **Childhood cerebral form (4-8 years/45%): hyperactivity, vision problems, loss of verbal understanding, regression in school, handwriting, seizures, aphagia**

- **Adrenomyeloneuropathy (males in their 20's/35%): muscle weakness, difficulty thinking quickly, poor sight memory; uncontrolled urination**

- **Addison's disease: lack of steroid hormones (cortisol and aldosterone); decreased appetite, low blood pressure, increased pigmentation, muscle wasting, vomiting, coma**

New York State Assay
(Mod. Krabbe and ALD)

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

↓ 1 hour extraction

Remove 50 µL of extract and combine with LSD extract

↓

Analyze samples, 1.5 minutes per sample/Marker is C26:LPC

↓

Follow screening algorithm

Technical challenges in ALD Screening

- Interfering compounds – requires second tier HPLC-MS/MS to reduce positives
- Adding C26:0-LPC channel to LSD test: – lost GALC-IS signal (corrected)
- Adding ALD extract to GALC: Linearity of GALC affected - slope 1.5 (normally 1.1, corrected)
- Edge Effects on plates (evaporation, corrected)
- C26:0 LPC has low solubility relative to interferent

Population Statistics (12/30/13 – 10/21/14)

C26:0-LPC (uM)	
mean	0.23
StDev	0.066
max	2.78

ALD N = 2198833 samples		
C26:0	**Count**	**Yr-Count**
>0.35	10803	1228
>0.4	4339	493
>0.5	707	80
>0.6	215	24
Birthrate for NY = ~240,000 First tier cutoff = 0.4 μM		

Positive Controls

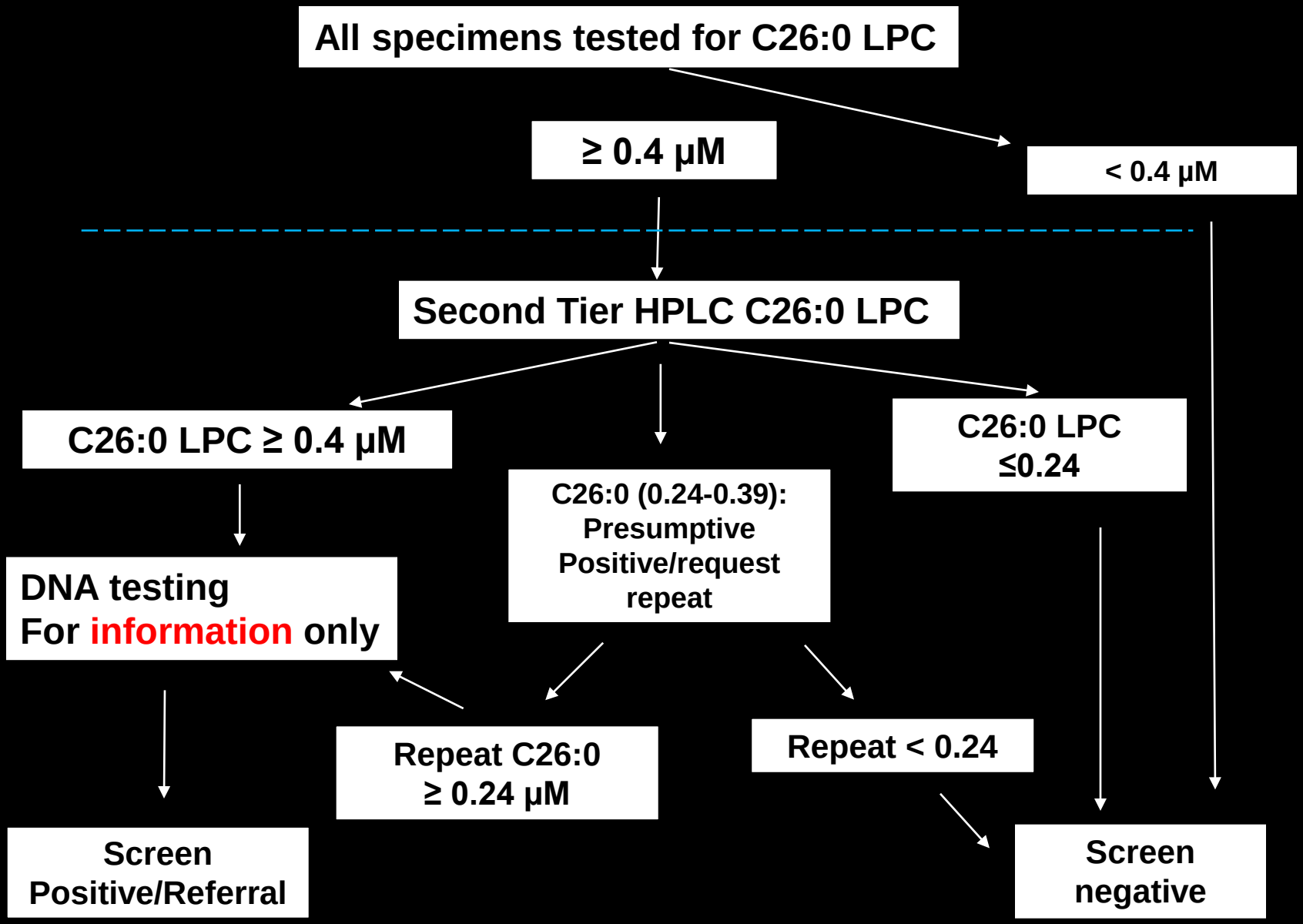
Positive controls, Tier 1 results

Sample ID	Accession #	Condition	C26:0 (µM)
ALD_1	20042872073	ALD	1.2
ALD_2	20131571625	Zellweger	1.75
ALD_3	20042091488	ALD	1.3
ALD_4	20070191816	Zellweger	1.53
ALD_5	20001511848	ALD	0.78
ALD_6	19991892305	ALD	1.08
ALD_7	20021191634	ALD	1.09
ALD_8	20100251314	Carrier	0.78
ALD_9	20041381090	ALD	1.19
ALD_10	20023531007	ALD	1.28

Mayo positive controls

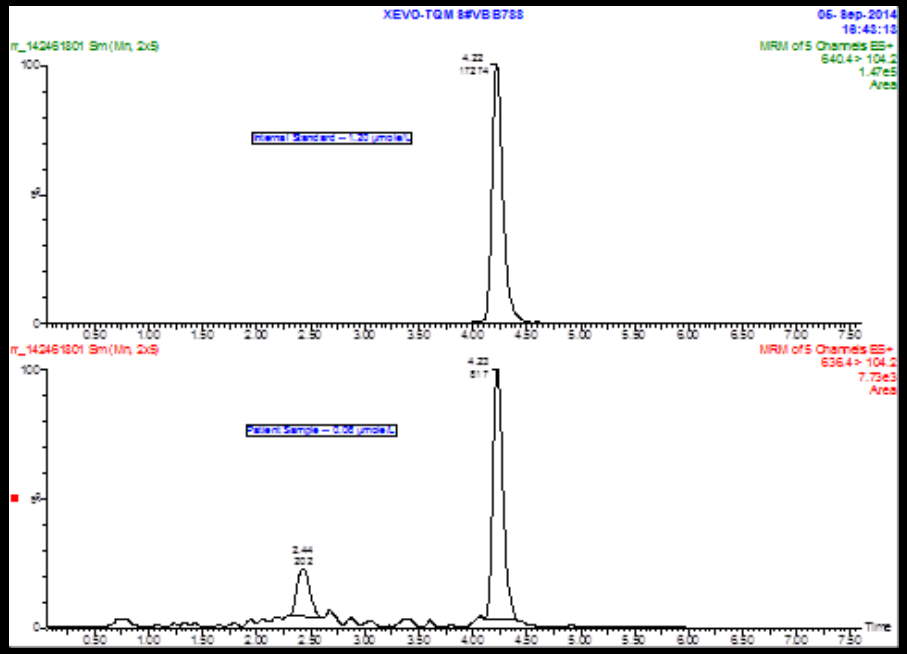
Sample ID	Patient information	C26:0 (µM)
PLSD 041614-04	XALD #67655 7.7 year old male	1.03
PLSD 041614-05	XALD #61933 7.8 year old male	0.48
PLSD 041614-06	XALD #67651 8.8 year old male	0.69

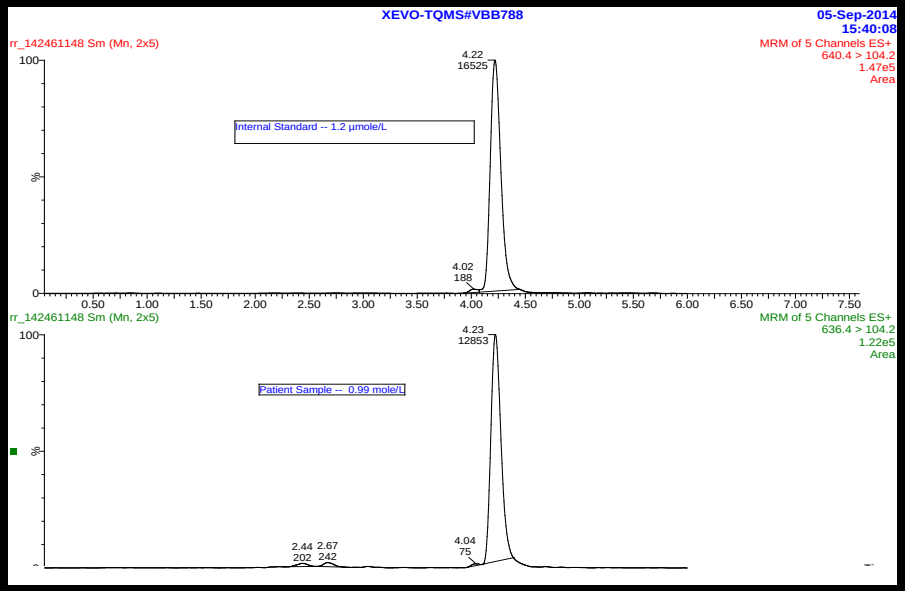
ALD Screening Algorithm



Second Tier: HPLC-MS/MS

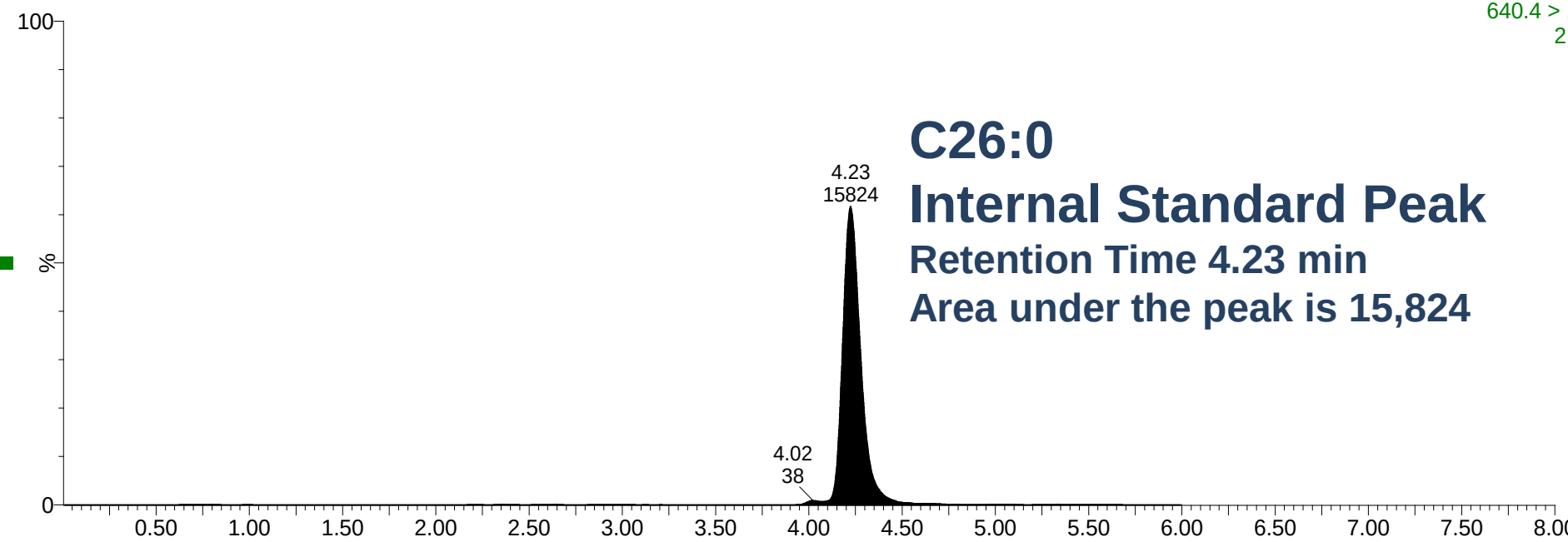
Second Tier:
1. Reduce interferents
2. Reduce false positives





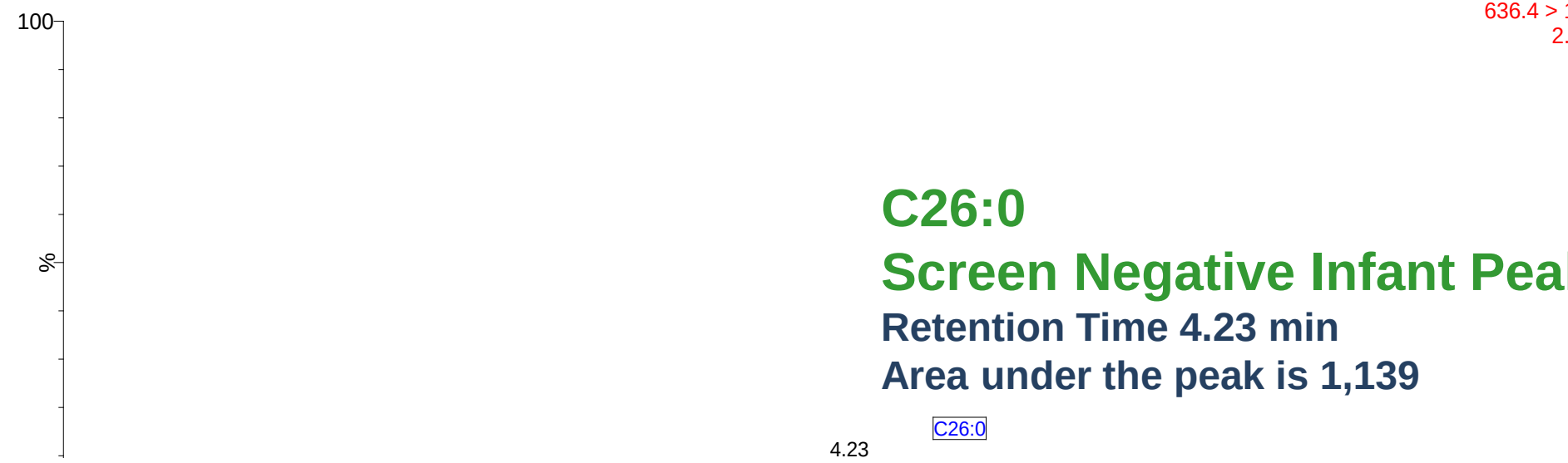
Sample ID	Sample Result (µMole/L) 1st Tier	2nd Tier
NB_3223	0.40	0.12
NB_3231	0.41	0.08
NB_3251	0.48	0.11
NB_3286	0.40	0.09
NB_3288	0.42	0.08
NB_3337	0.40	0.07
NB_3364	0.42	0.06
NB_3387	0.40	0.08
NB_3542	0.41	0.08
NB_3544	0.92	0.08
NB_3556	1.09	0.10
NB_3669	0.43	0.07
NB_3948	0.45	0.11
NB_5395	0.41	0.09
NB_5997	0.55	0.10
NB_6009	0.42	0.08
NB_6251	0.42	0.09
NB_6253	0.40	0.07
NB_6771	0.43	0.09
NB_6772	0.53	0.08
NB_6919	0.41	0.05
NB_6981	0.53	0.36
NB_8321	2.44	0.14
NB_8322	1.62	0.07
NB_8326	0.42	0.08
NB_8327	0.64	0.11
NB_8424	1.81	0.08
NB_8439	0.58	0.09
NB_8451	0.51	0.08
NB_8511	0.40	0.09
NB_8599	0.97	0.08
NB_8600	0.50	0.09
NB_8601	0.43	0.09
NB_8846	0.61	0.36
NB_8872	0.41	0.08
NB_8895	0.40	0.14
NB_9001	1.02	0.87
NB_9148	1.06	0.08
NB_9160	0.95	0.10
NB_9172	0.41	0.07
NB_9220	0.43	0.08
NB_9480	0.41	0.08
NB_9589	0.42	0.10
NB_9663	0.40	0.14
NB_9669	0.45	0.09

r_142462659 Sm (Mn, 2x5)
MRM of 5 Channels ES
640.4 > 104
2.50
Ar

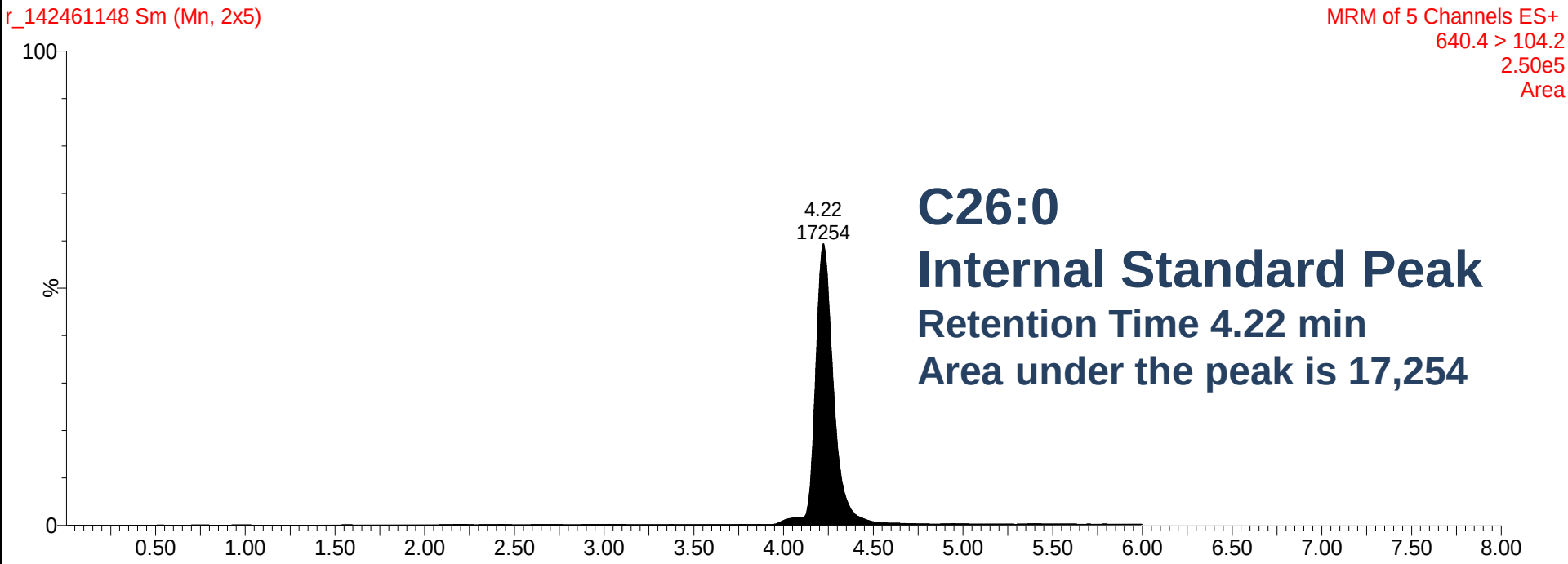


C26:0
Internal Standard Peak
Retention Time 4.23 min
Area under the peak is 15,824

r_142462659 Sm (Mn, 2x5)
MRM of 5 Channels ES
636.4 > 104
2.50
Ar



C26:0
Screen Negative Infant Peak
Retention Time 4.23 min
Area under the peak is 1,139

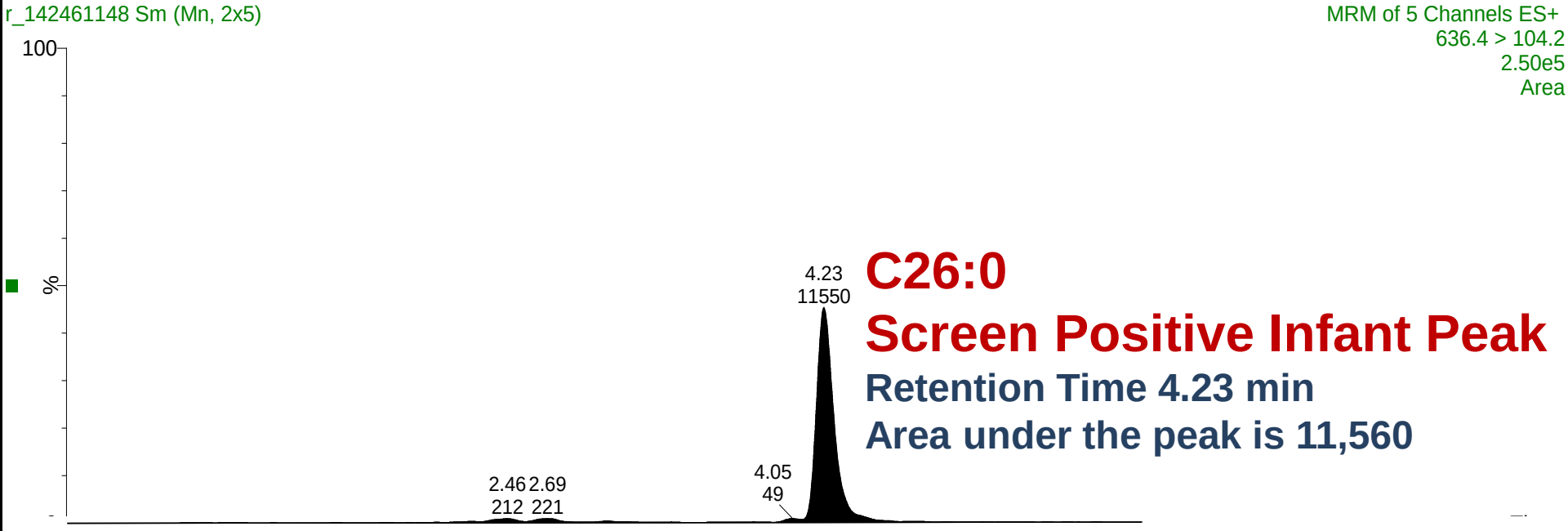


C26:0

Internal Standard Peak

Retention Time 4.22 min

Area under the peak is 17,254



C26:0

Screen Positive Infant Peak

Retention Time 4.23 min

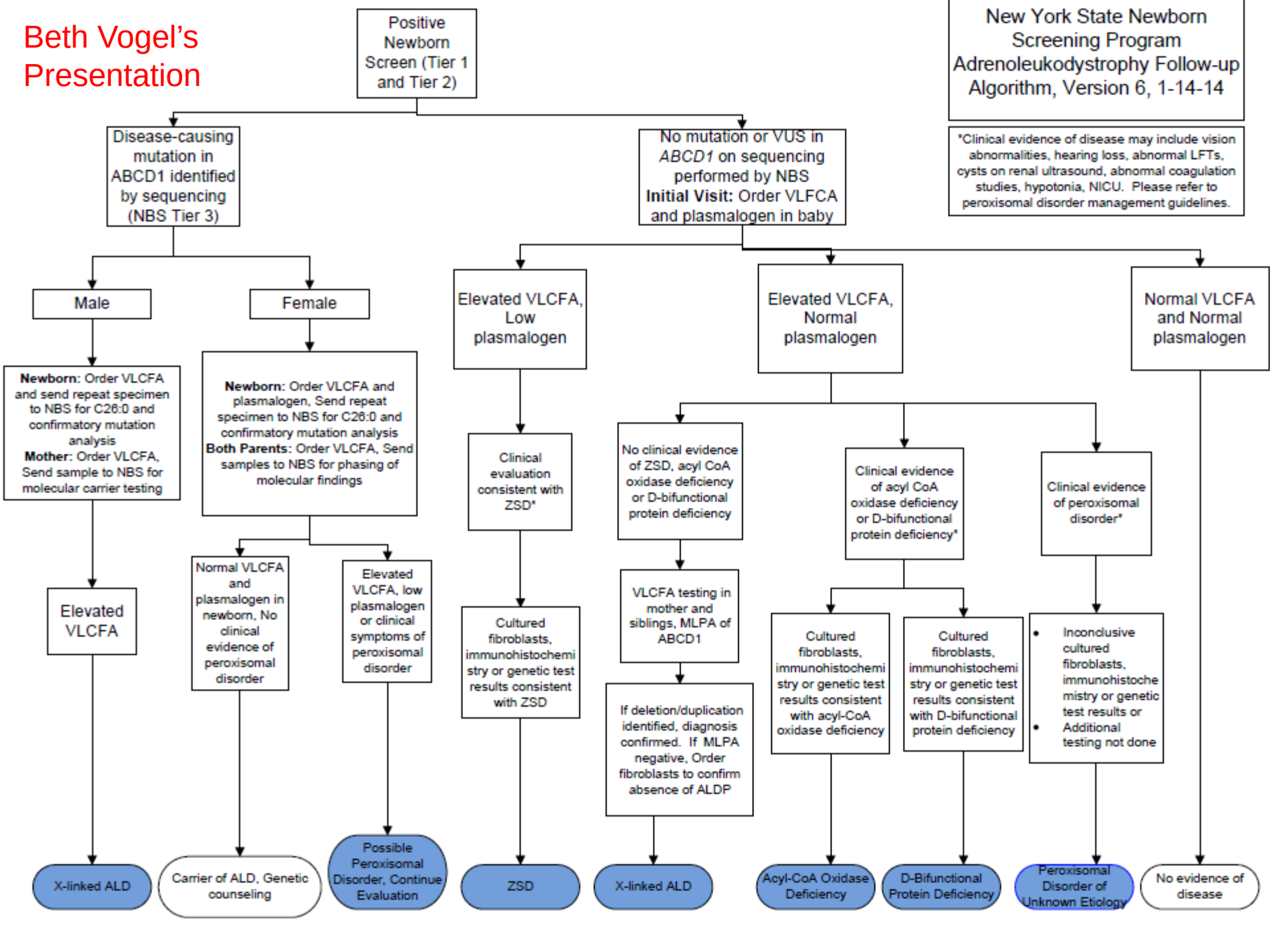
Area under the peak is 11,560

Third Tier: DNA Sequencing

1. Full sequencing of ABCD1 gene
2. Not intended to reduce referrals
3. Helps to Determine
    a. if females are ALD carriers
    b. if males have mutation
    c. if no mutation, consider other PGD

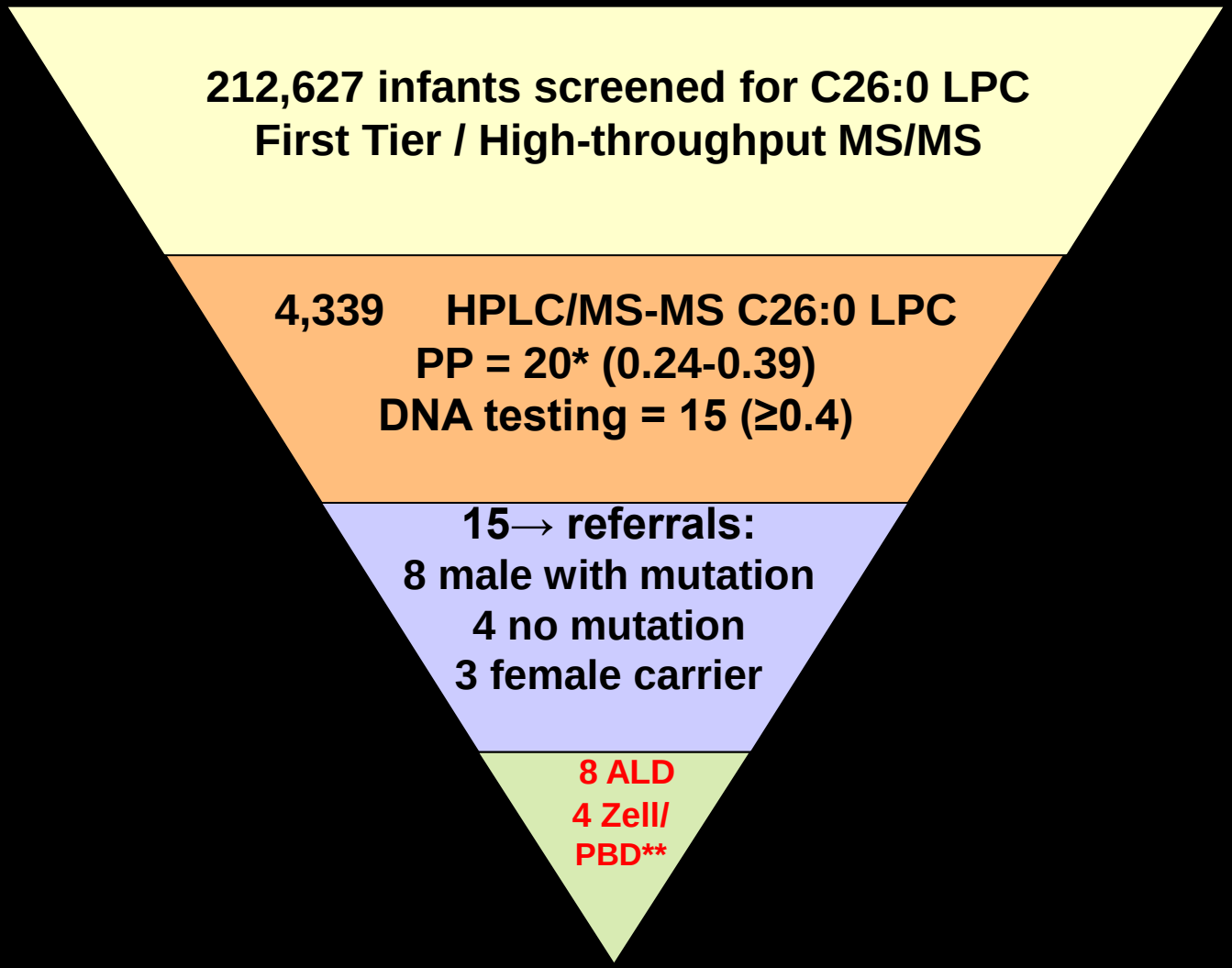
4. Genotype does not correlate with phenotype

Beth Vogel's Presentation



New York State Newborn Screening for X-ALD

December 30, 2013 to October 21, 2014



Status of ALD Referrals in New York

8 Adrenoleukodystrophy Cases Detected

1. 1.30, 1.14; p.E302K (*de novo* in child, known childhood onset)
2. 1.03, 0.84; p.W601X (known; cerebral adult onset)
3. 0.51, 0.40; p.P534S (phenotype unknown; different aa changes-adult onset)
4. 0.40, 0.26; p.R163H (known; symptomatic carrier -- sibling identified)
5. 1.21, 1.09; p.R189W (known; adult AMN; Addison's)
6. 0.67, 0.34; p.S572P (novel)
7. 1.24, 0.96; g.E6-10del (known; AMN in ex. 7-10 deletion)
8. 0.61, 0.49; p.G92R (novel) and p.R324C (novel)

Status of ALD Referrals in New York

7 Other Outcomes To Date

0.96, 0.89; (NICU; possible Zellweger; LTFU; BG)

1.29, 1.33; (NICU; peroxisomal biogenesis defect??)

1.79, 1.70; (NICU; peroxisomal biogenesis defect??)

2.56, 3.48; (NICU; Zellweger – two previous ZD siblings

0.58, 0.40; p.V583M (novel; BG carrier)

0.65, 0.50; p.E272del (reported; BG carrier)

0.62, 0.50; p.Q47Rfs*21 (novel; BG carrier)

ALD by the Numbers (190,368 births)

- **Referral rate: 1 in 12,691 or 0.008% of infants screened**
- **Incidence of ALD*: 1 in 23,796 births**
- **Incidence of ALD*: 1 in 11,898 males**
- **Incidence of PGDs: 1 in 47,592 births**

Too early for stable incidence rates – prediction is 1 in 17,000 to 1 in 20,000 births

* ***Assumption that all with Muts will become symptomatic.***

Challenges in ALD Screening

- **Genetic diversity –** ***(novel variants?; VOUS)***
- **Incomplete genotyping –** ***(undetected variants?)***
- **Later onset condition –** ***(boys and AMN)***
- **Potential for carriers to be symptomatic**
- **Assay doesn’t identify all carriers**
- **Potential for Dad to have AMN**
- **Lack of genotype:phenotype correlation**
- **Lack of correlation of C26 concentration to severity of disease**

Acknowledgements

- Monica Martin: first tier method development
- Mark Morrissey/Cathy Lubowski: second tier method development
- Dieter Matern and Staff: SOP for assay
- Chris Haynes for technical assistance and control samples
- Ann Moser for technical assistance and control samples
- Michele Caggana for many slides