Connecticut Newborn Screening For X-Linked Adrenoleukodystrophy

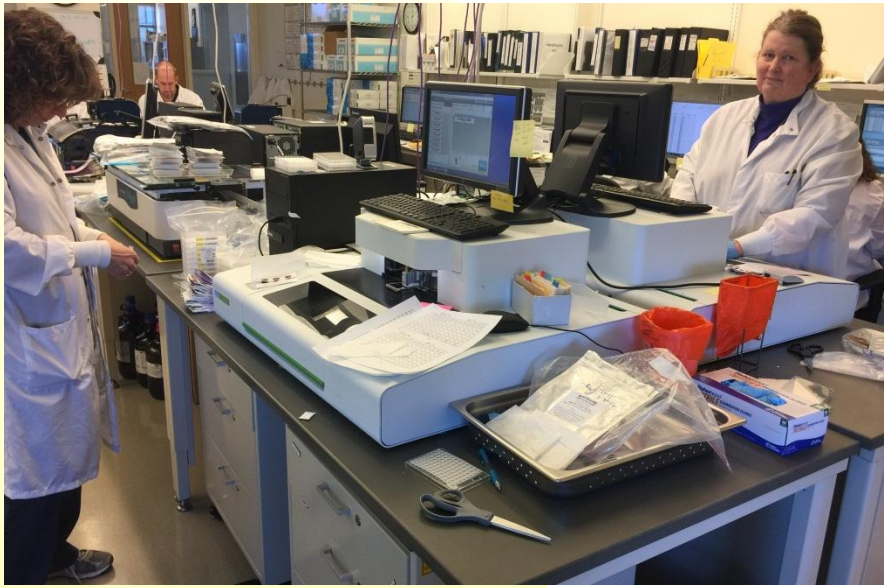
Adrienne Manning, Division Director, Newborn Screening
Connecticut Department of Public Health
Katherine A. Kelley Public Health Laboratory
Rocky Hill, CT

CT Newborn Screening

CGS 19a-55 mandates that all newborns in Connecticut be screened for selected genetic and metabolic disorders. The CT DPH NBS Laboratory carries out the identification of over 60 different metabolic disorders related to the digestion of various food compounds, steroid production and absorption, autoimmune development and blood hemoglobin production disorders. Left undetected and untreated these diseases often lead seizures, developmental disabilities, failure to thrive or death.

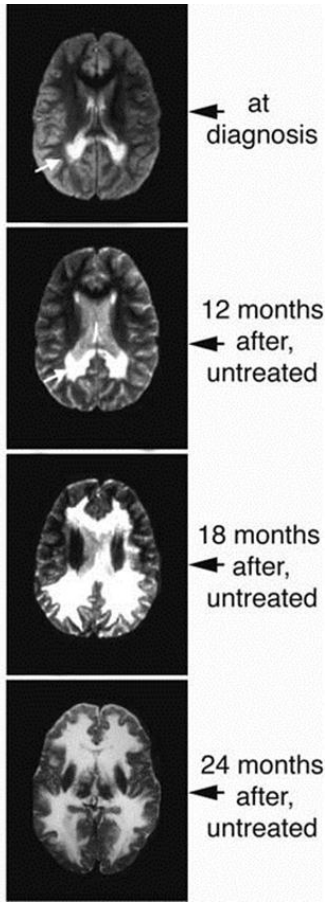
Testing of heel-stick specimens involves multiple technologies and procedures and covers a wide range of disorders which, if detected prior to the onset of symptoms of the diseases, can be treated with medication, diet and in the case of some disorders, stem cell transplants, which greatly improves the possibility of longer and more normal lives for affected individuals.

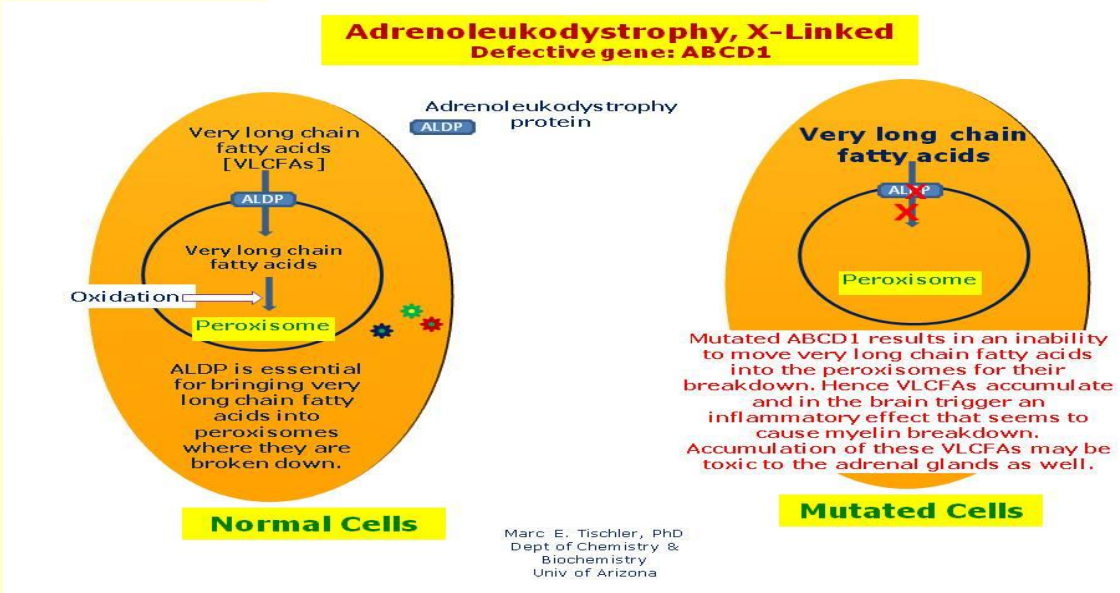
CT Newborn Screening



X-Linked Adrenoleukodystrophy (X-ALD)

X-ALD is the most common peroxisomal disorder with an estimated incidence of 1:17,000. This disorder is caused by mutations in the ALD peroxisomal transmembrane protein, ALDP, and the gene ABCD1. The severity of this mutation varies from childhood cerebral ALD (C-CALD), generally lethal with onset between ages 4 and 10, to adult-onset adrenomyeloneuropathy (AMN). Reduced activity of the peroxisomes for the breakdown of saturated very long-chain fatty acids (VLCFAs) causes increased levels of C26:0 VLCFA and accumulation of C26:0-lysophosphatidylcholine (C26:0-LPC), causing inflammatory demyelination of nerve cells within the brain and lesions that can be seen using an MRI. X-ALD often also causes the dysfunction of the adrenal gland, resulting in adrenal insufficiency or Addison's disease. The childhood form of the disease often leads to rapid degeneration, loss of cognitive ability, vegetative state and death. The milder adult-onset form, AMN**,** typically begins between ages 21 and 35. Symptoms include progressive stiffness, weakness or paralysis of the lower limbs and can also result in deterioration of brain function. About half the women who are X-ALD heterozygote will develop a milder form of AMN but will almost never develop symptoms seen in males with X-ALD. Limited therapy (BMT, Lorenzo's oil) is available for X-ALD patients, however, it has been demonstrated that successful treatment is critically dependent on pre-symptomatic initiation for any form of X-ALD therapy.





X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

ALD HISTORY AND ADVOCACY IN CONNECTICUT

June 26, 2008: 2-year old Joshua Florian died after a fever from undiagnosed Addison's disease. Later this was diagnosed as having a non-inherited type of X-ALD

Brian's Hope/The Kelley Family

At 6 Brian Kelley was diagnosed with X-ALD. Within six months of the diagnosis Brian, now 28, lost his mobility, speech, ability to eat and most of his vision and has been confined to a wheelchair. His parents Jean and Dr. Jack Kelley have been raising awareness for the importance of early detection of X-ALD through NBS and by speaking at various hearings and venues such as the Advisory Committee on Heritable Disorders in Newborns and Children meetings advocating for the addition of X-ALD to the RUSP

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

X-ALD HISTORY AND ADVOCACY IN CONNECTICUT

►**SB 465 was proposed in January 2013: An Act Requiring Newborn Screening for Adrenoleukodystrophy**

►**Public Act 13-242 Approved on July 2, 2013 with additional language to allow for development and validation of reliable methodology or an FDA cleared kit**

►**Commissioner of Public Health declined permission of the start of X-ALD screening until after the addition of X-ALD to the RUSP**

►**August 2015 Advisory Committee on Heritable Disorders in Newborns and Children voted in favor of the addition of X-ALD to RUSP**

► **September 2015 CT started validation of CDC negative-ion LC-MS/MS method for X-ALD screening**

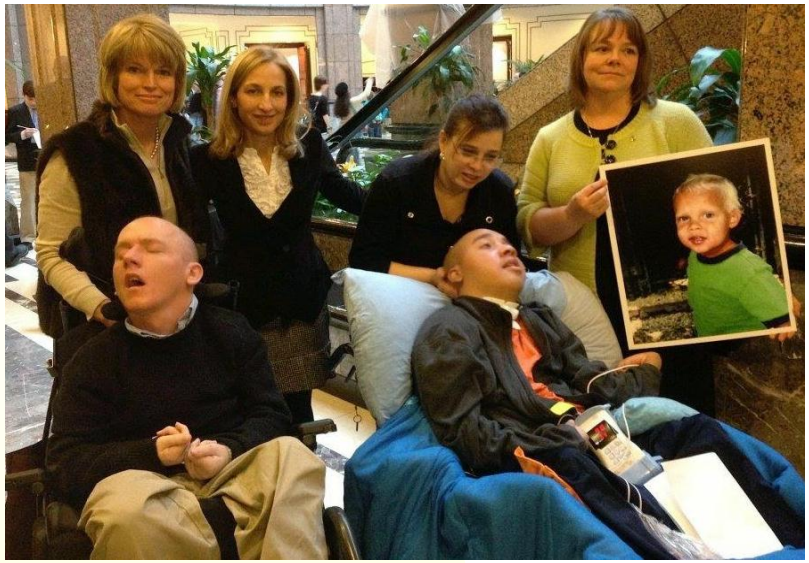
► **Non-patient sample analyses completed prior to patient sample analysis in order to assess the instrument and analysts' precisions and accuracy via coefficient of variation (% CV), % Bias, % Recovery, linearity, carryover, drift and analytical range calculations by using quality controls obtained from the CDC**

► **Patient analysis portion of the validation included over 27,000 newborn samples**

► **X-ALD Screening went live in CT on July 1, 2016**

► **All infants born as of October 1, 2015 screened for X-ALD**

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut



X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Clinica Chimica Acta 413 (2012) 1217–1221

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journal homepage: www.elsevier.com/locate/clinchim

Improved analysis of C26:0-lysophosphatidylcholine in dried-blood spots via negative ion mode HPLC-ESI-MS/MS for X-linked adrenoleukodystrophy newborn screening

Christopher A. Haynes *, Víctor R. De Jesús

Newborn Screening and Molecular Biology Branch, Centers for Disease Control and Prevention, 4770 Buford Hwy. NE, Atlanta, GA 30341, USA

Newborn Screening and Molecular Biology Branch			
Title: **Quantitation of Lysophosphatidylcholines**			
Document Number: **NSMB-C-METHOD.001**	Version: **01**	Effective Date: **June 3, 2015**	**Page 1 of 7**

1. PURPOSE

To provide a written standard operating procedure (SOP) for the quantitation of hexacosanoyl lysophosphatidylcholine (C26:0-LPC) and lignoceroyl lysophosphatidylcholine (C24:0-LPC) using high-performance liquid chromatography (HPLC) coupled to electrospray ionization (ESI) and tandem mass spectrometric (MS/MS) analysis.

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

SAMPLE PREPARATION PROCEDURE

►**Internal Standard (IS): 26:0-d4 Lyso PC 1-hexacosanoyl-d4-2-hydroxy-sn-glycero-3-phosphocholine, 5mg (Catalog# 860389P), Avanti Polar Lipids, Inc.**

►**Preparation of IS Stock Solution: Reconstitute 5mg IS material with 50mL Methanol—sonication of the solution is necessary to dissolve fully**

►**Dilute an aliquot of stock solution in 200mL Methanol to prepare Extraction Solution/IS Spiking Solution**

►**Punch 3.2mm blood spots into a 96-well plate**

►**Add 100µL IS Spiking Solution to each well containing a blood spot**

►**Shake for 30 minutes at 31°C and 650 rpm shaking speed**

►**Transfer extracts to a NUNC heat resistant polypropylene microtiter plate and cover plate with foil**

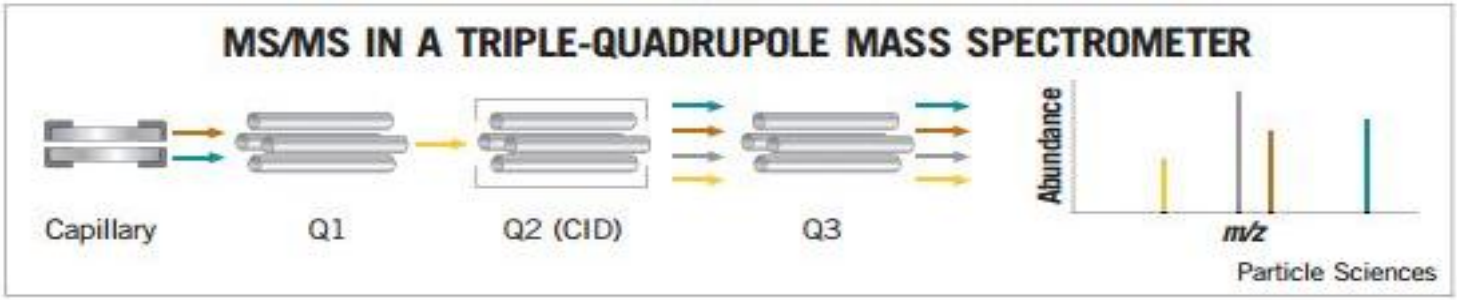
ANALYSIS PROCEDURE

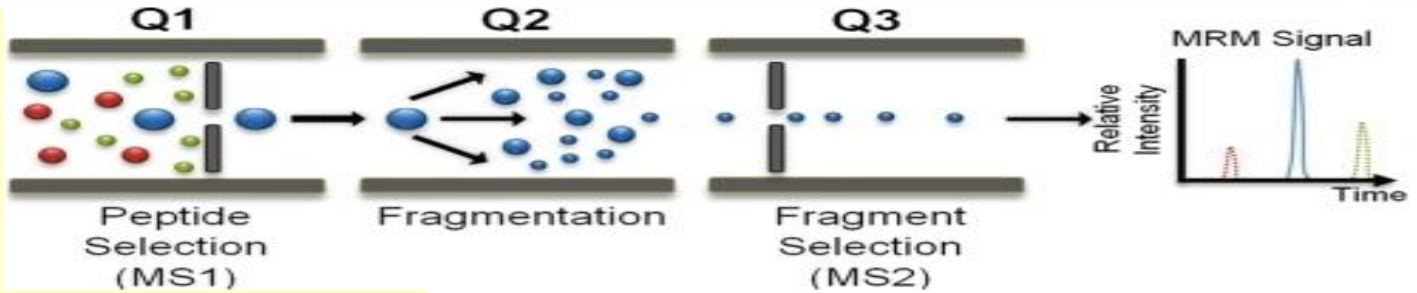
►**Analyze extracts using a Triple Quadrupole LC-MS/MS instrument with Turbo Spray Ion Source in negative ionization mode**

►**LC isocratic flow of 50:50 methanol/ water with 5mM Ammonium Acetate at 0.45mL/min, Waters XTerra MS C8 Column, 125Å, 2.5 µm, 2.1 mm X 50 mm**

►**20µL Sample Injection Volume, Total run time: 1.11 min**

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut





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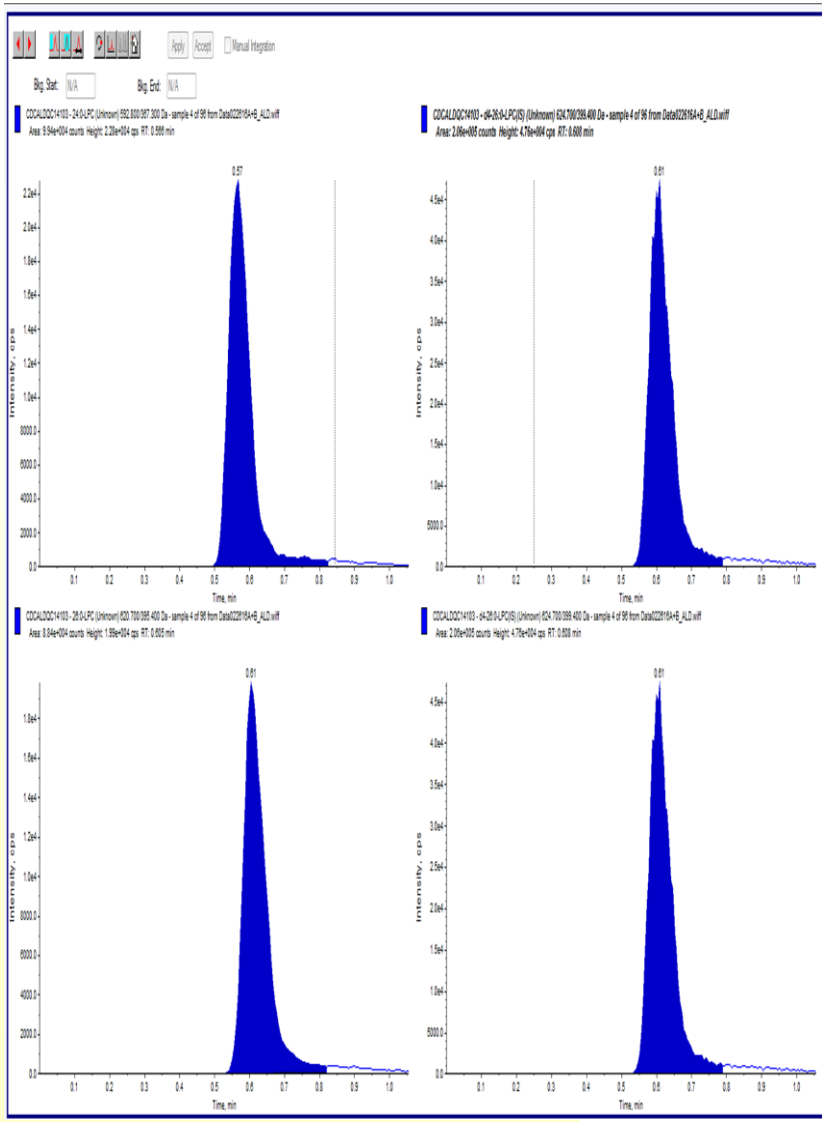
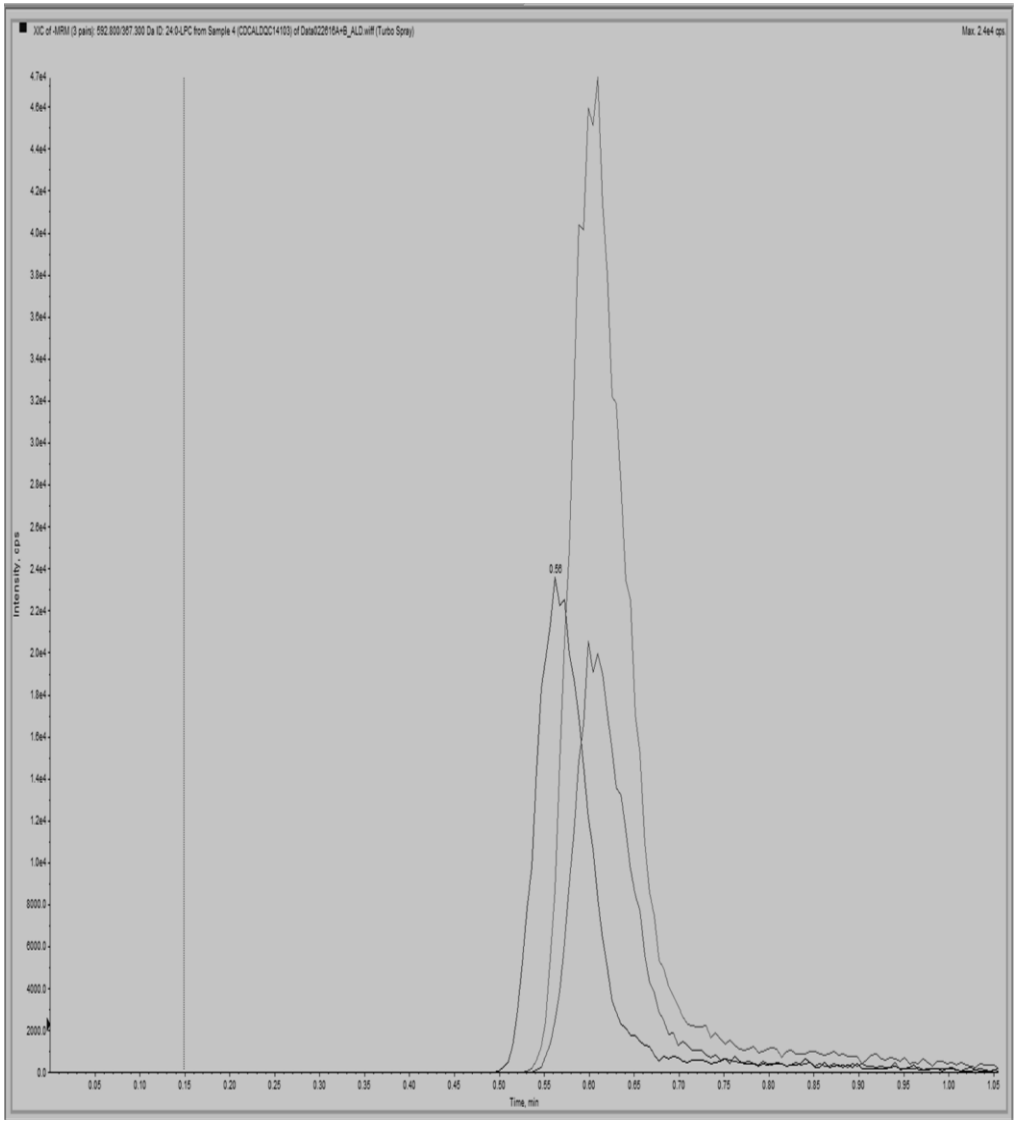




X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Precision Results:

C24:0-LPC Overall Instrument Precision				
QC ID	**Batch Info**	**Mean (µmol/L)**	**Standard Deviation**	**% CV**
CDCQC14101	Both Instruments Overall	0.0564	0.0106	18.84%
CDCQC14102	Both Instruments Overall	0.8226	0.170	20.63%
CDCQC14103	Both Instruments Overall	3.6476	0.599	16.42%

C26:0-LPC Overall Instrument Precision				
QC ID	**Batch Info**	**Mean (µmol/L)**	**Standard Deviation**	**% CV**
CDCQC14101	Both Instruments Overall	0.0252	0.0046	18.16%
CDCQC14102	Both Instruments Overall	0.8013	0.122	15.20%
CDCQC14103	Both Instruments Overall	3.8146	0.573	15.03%

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

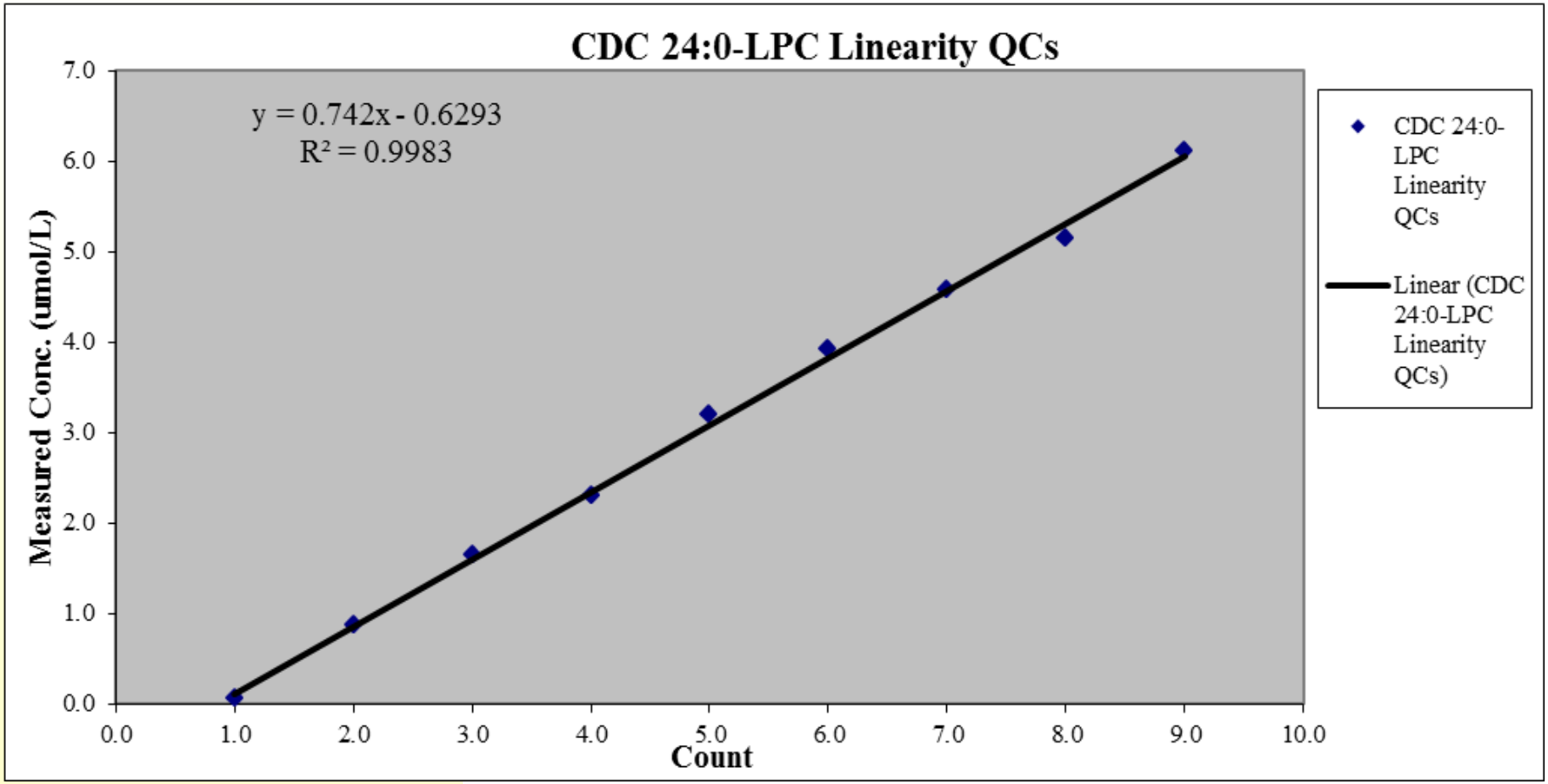
Connecticut Accuracy Results:

C24:0-LPC Overall Instrument Accuracy					
QC ID	**Batch Info**	**Nominal Concentration (µmol/L)**	**Mean (µmol/L)**	**% Bias**	**% Recovery**
CDCQC14101	Both Instruments Overall	0.000	0.056	NA	NA
CDCQC14102	Both Instruments Overall	1.00	0.823	17.74%	76.62%
CDCQC14103	Both Instruments Overall	5.00	3.648	27.05%	71.82%

C26:0-LPC Overall Instrument Accuracy					
QC ID	**Batch Info**	**Nominal Concentration (µmol/L)**	**Mean (µmol/L)**	**% Bias**	**% Recovery**
CDCQC14101	Both Instruments Overall	0.000	0.025	NA	NA
CDCQC14102	Both Instruments Overall	1.00	0.801	19.87%	77.61%
CDCQC14103	Both Instruments Overall	5.00	3.815	23.71%	75.79%

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Linearity Results:



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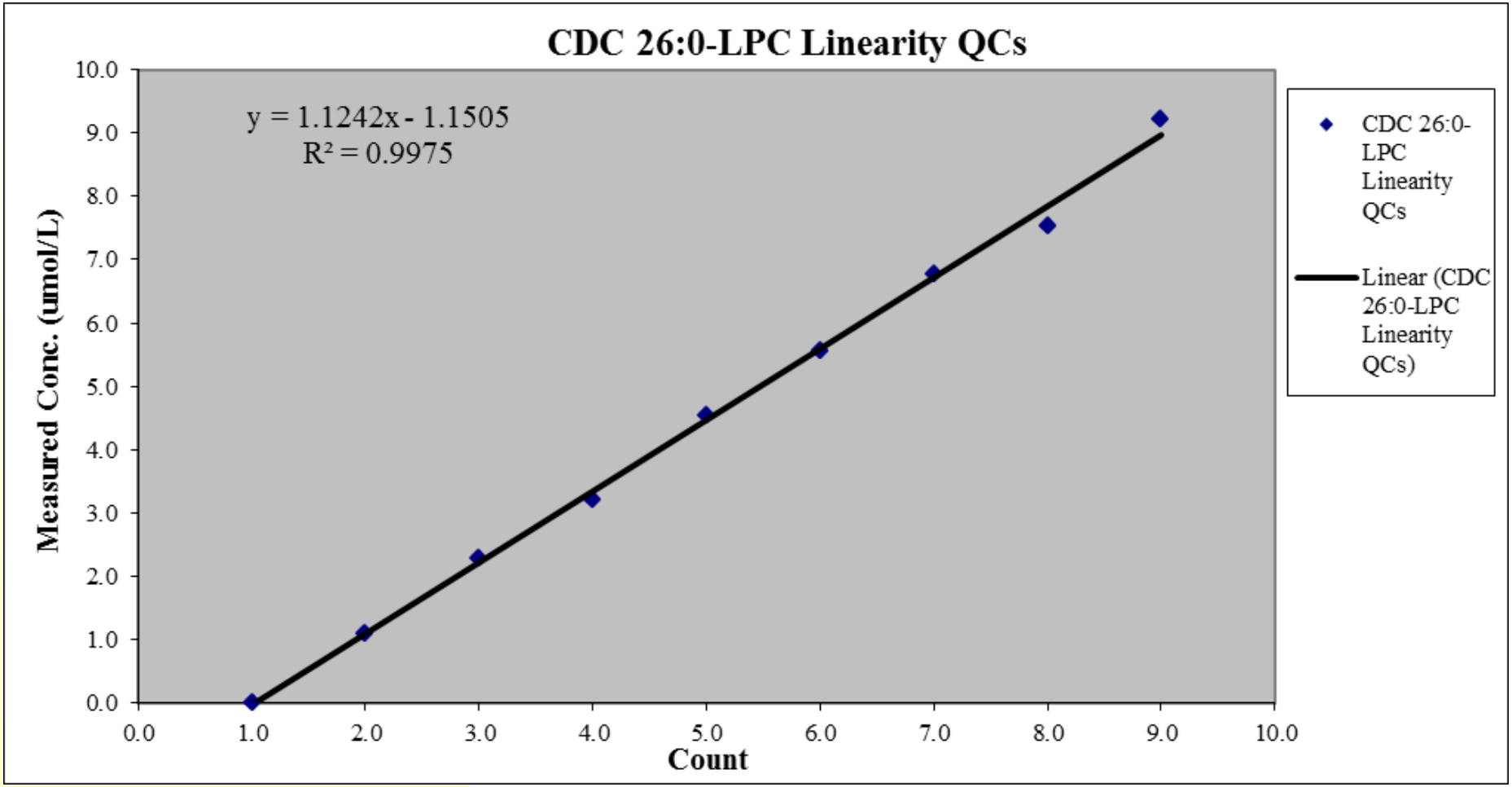
Connecticut Linearity Results:



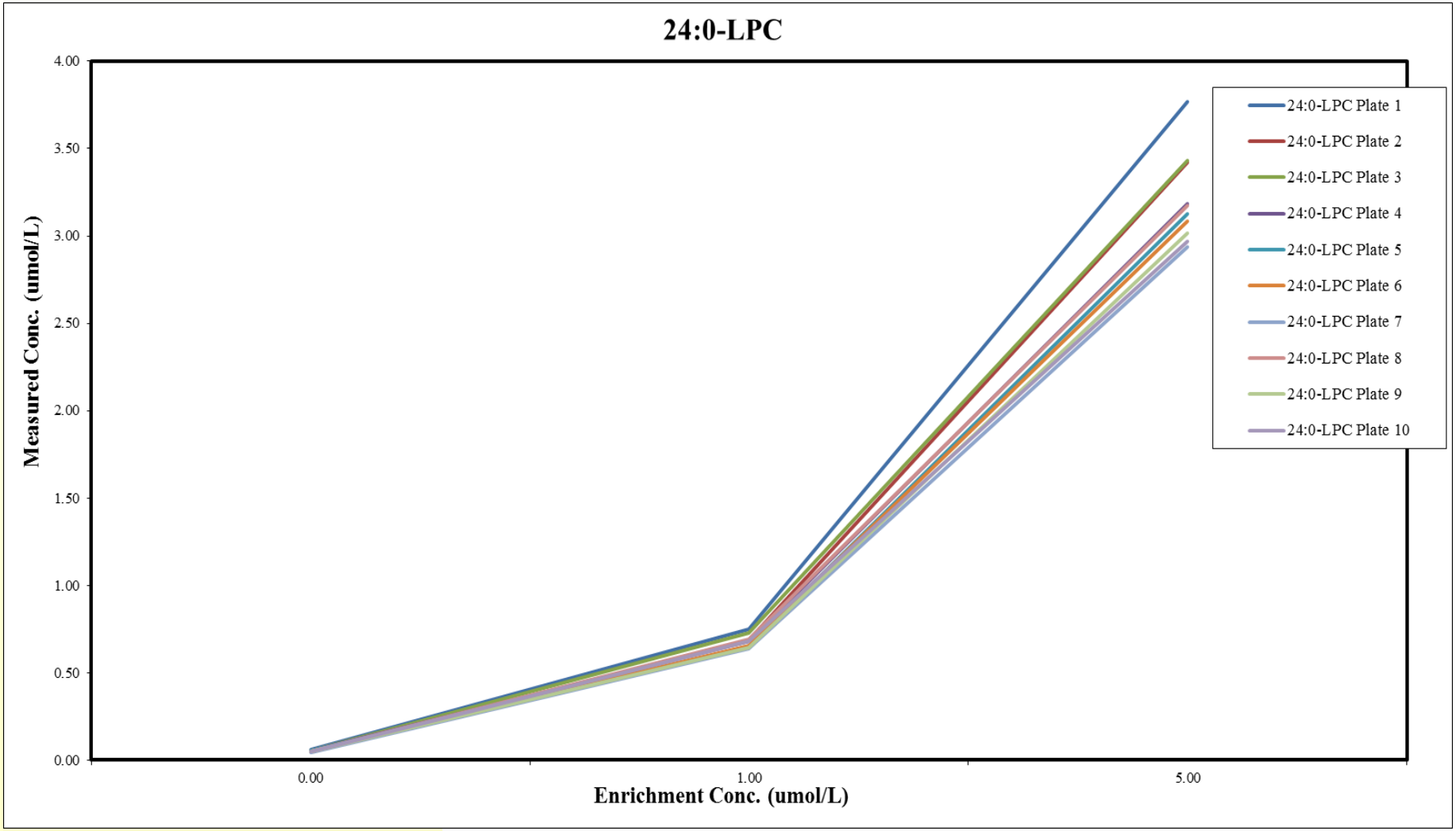
X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Carryover Results:

	C24:0-LPC			C26:0-LPC		
Parameters Evaluated	**MS 1 (µmol/L)**	**MS 2 (µmol/L)**	**Overall MS (µmol/L)**	**MS 1 (µmol/L)**	**MS 2 (µmol/L)**	**Overall MS (µmol/L)**
First Set CDC14101 Mean	0.0597	0.0577	0.0587	0.0267	0.0245	0.0256
Second Set CDC14101 Mean	0.0611	0.0608	0.0609	0.0296	0.0282	0.0289
% Difference (1st set vs 2nd set)	-2.35%	-5.28%	3.79%	10.78%	15.11%	12.85%
2-tailed TTest	0.536	0.288	0.219	0.035	0.002	0.001
if p > 0.05 differences are not significant	OK	OK	OK	Flag	Flag	Flag
Patient Cutoff	0.15	0.15	0.15	0.16	0.16	0.16
Potential Carryover (Patient Cutoff * % Mean Difference)	NA	NA	NA	0.0173	0.0242	0.0206
Instrument Potential False Positive Lower Limit Threshold from Carryover	NA	NA	NA	0.1427	0.1358	0.1394

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Drift Results:



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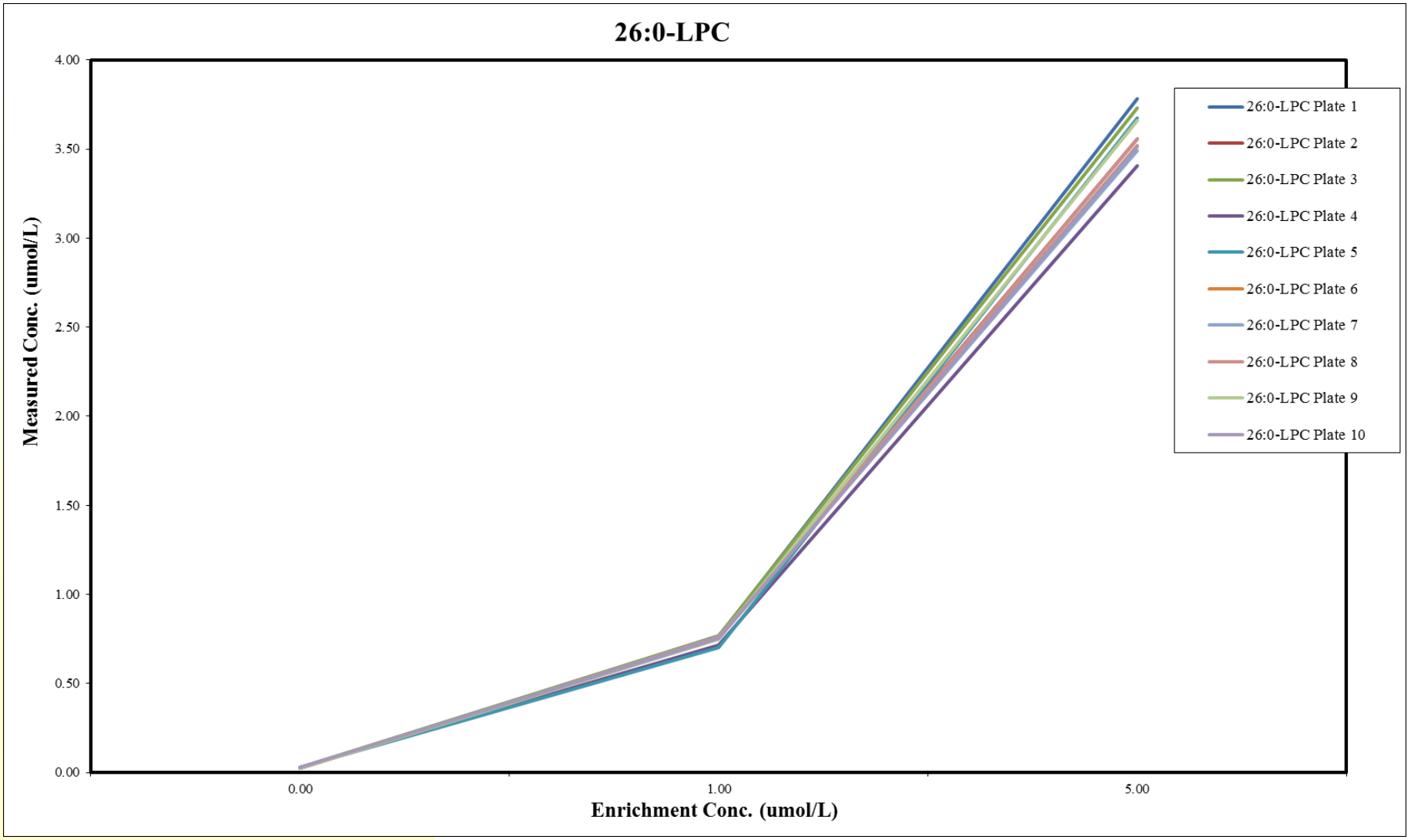
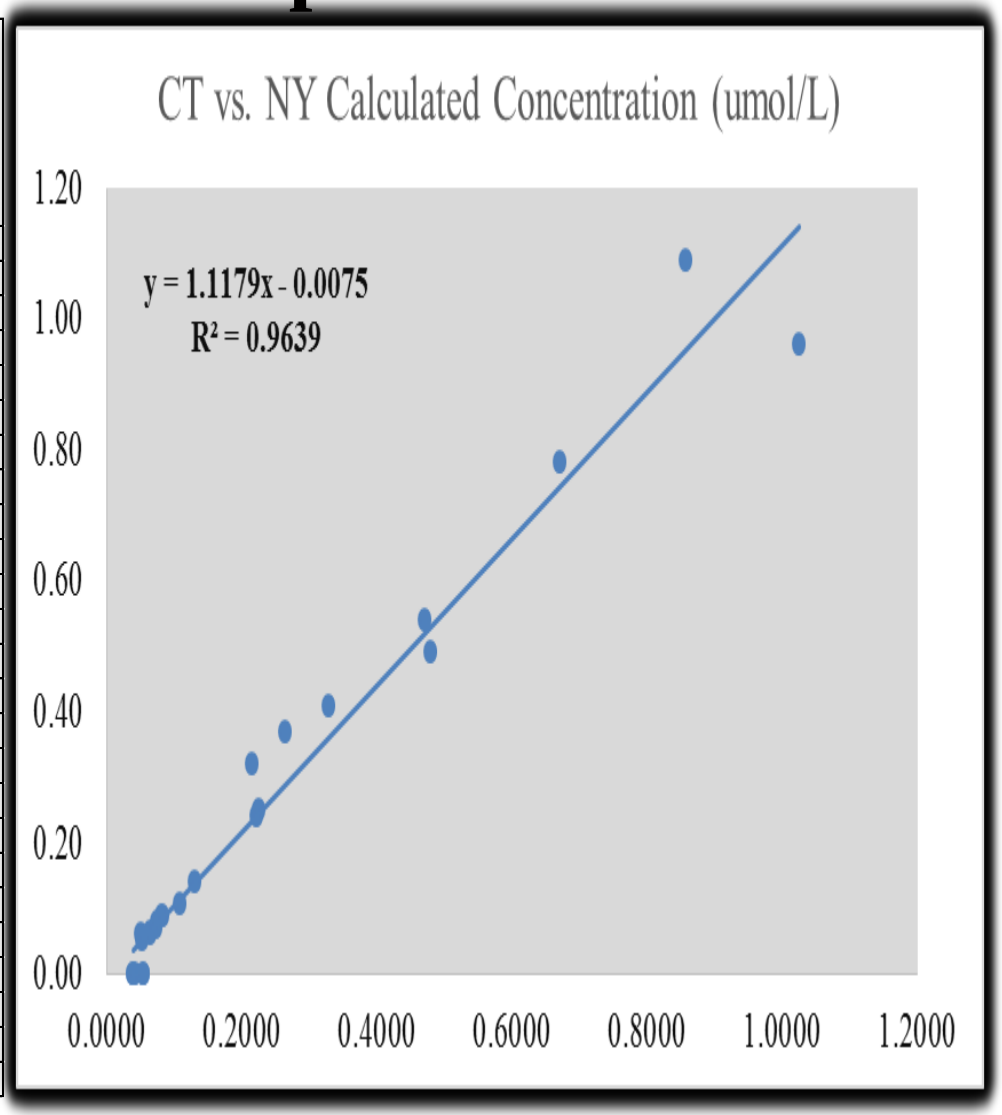
Connecticut Drift Results:



X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Blinded NY Sample Results:

Patient ID	CT Calculated Concentration (µmol/L)	Tier 2: NY Calculated Concentration (µmol/L)	Absolute % Difference Calculations	Analyte	Sample Diagnosis UNBLINDED
NY001	0.2647	0.3700	28.47%	26:0-LPC	Borderline
NY002	1.0261	0.9600	6.88%	26:0-LPC	ALD Boy
NY003	0.2219	0.2400	7.56%	26:0-LPC	Borderline
NY004	0.4803	0.4900	1.99%	26:0-LPC	ALD Boy
NY005	0.1304	0.1400	6.87%	26:0-LPC	Normal
NY006	0.0514	0.0600	14.28%	26:0-LPC	Normal
NY007	0.0636	0.0600	6.06%	26:0-LPC	Normal
NY008	0.4712	0.5400	12.73%	26:0-LPC	ALD Boy
NY009	0.0730	0.0700	4.22%	26:0-LPC	Normal
NY010	0.0836	0.0900	7.06%	26:0-LPC	Normal
NY011	0.6708	0.7800	14.00%	26:0-LPC	Zellweger
NY012	0.0549	N/A	NA	26:0-LPC	Normal
NY013	0.0388	N/A	NA	26:0-LPC	Normal
NY014	0.3280	0.4100	20.00%	26:0-LPC	ALD Boy (lowest)
NY015	0.0390	N/A	NA	26:0-LPC	Normal
NY016	0.0812	0.0900	9.83%	26:0-LPC	Normal
NY017	0.0427	N/A	NA	26:0-LPC	Normal
NY018	0.0548	N/A	NA	26:0-LPC	Normal
NY019	0.2253	0.2500	9.88%	26:0-LPC	Borderline
NY020	0.1077	0.1077	0.00%	26:0-LPC	Normal
NY021	0.8571	1.0900	21.37%	26:0-LPC	ALD Boy
NY022	0.0642	0.0642	0.00%	26:0-LPC	Normal
NY023	0.0521	0.0521	0.00%	26:0-LPC	Normal
NY024	0.2146	0.3200	32.94%	26:0-LPC	Borderline
NY025	0.0737	0.0800	7.87%	26:0-LPC	Normal



X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Validation Sample Results:

0.24	NY 26:0-LPC cutoff (lower)	**Request for another sample**	**Proposed Connecticut Reporting Algorithm**
32.94%	Largest % Difference NY vs CT (CT values lower than NY)		
0.0791	(% Difference CT vs NY) * NY cutoff		
0.16	**CT Calculated lower cutoff (NY cutoff -((% Difference CT vs NY) * NY cutoff))**		
0.39	NY 26:0-LPC cutoff (upper)	**Refer child for followup testing**	
32.94%	Largest % Difference NY vs CT (CT values lower than NY)		
0.128	(% Difference CT vs NY) * NY cutoff		
0.26	**CT Calculated upper cutoff (NY cutoff -((% Difference CT vs NY) * NY cutoff))**		

	24:0-LPC (µmol/L)	26:0-LPC (µmol/L)
Mean	0.0654	0.0606
Median	0.0633	0.0593
25th Percentile	0.0528	0.0503
75th Percentile	0.0757	0.0690
99th Percentile	0.1185	0.1011
Borderline Cutoff (99.9th percentile)	NA	0.157
Presumptive Positive Cutoff	0.157 (99.9th)	0.257 (99.98th)
Range	0.0118 to 0.3917	0.0143 to 0.9098
Number Analyzed during the validation	27495	27495

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Initial Laboratory ID	Accession #	DOB	NBS Initial Sample Result	NBS Repeat Sample Result	Final Result
562036001	74733553	1/1/2016	Borderline ABN, repeat sample requested	ABNORMAL	X-ALD
565567001	74474057	1/14/2016	Borderline ABN, repeat sample requested	NORMAL	NORMAL
565276001	74726136	1/16/2016	Borderline ABN, repeat sample requested	NORMAL	NORMAL
565562001	74294451	1/18/2016	Borderline ABN, repeat sample requested	NORMAL	NORMAL
587503001	74419936	4/12/2016	ABNORMAL REFERRAL	ABNORMAL	X-ALD
595530001	75410142	5/11/2016	ABNORMAL REFERRAL	ABNORMAL	X-ALD

brian's hope

About ALD Events

First Baby with ALD Identified in CT

March 1, 2016 by Brian's Hope — Leave a Comment

It is bittersweet but good to know the process for ALD newborn screening is working in CT. In January, our first CT baby to have ALD was identified. The child is in the care of specialists and will receive the appropriate monitoring and treatments, which if given in the early phase, dramatically improve the outcome of the disease.

This is the statement from the parents, Autumn and Samuel:

"We are so very thankful that ALD is now part of the newborn screening. It has changed what could have been a terminal diagnosis later on, into a diagnosis where our boys have a chance. Because ALD is a genetic disease, our other little boy (2 years) has been tested and is positive for ALD as well. We would not have had an idea of the chance of him having ALD without his little brothers screening until it was too late. One newborn screening has saved both of our boys."

http://brianshope.org/brians-hope-news/first-baby-with-ald-identified-in-ct/

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

DIAGNOSTIC/TREATMENT CENTER FOLLOW UP

Diagnostics:

► **VLCFA—Kennedy Krieger Lab**
► **ABCD1 Sequence Analysis—Baylor Lab**

Confirmed X-ALD:

► **Testing of siblings, other family members**
► **Females: Seen once in clinic for counseling then followed by PCP**
► **Males: Seen in clinic for consultation by Endocrinology, Neurology and Hematology (if considering stem-cell transplant). Ongoing follow-up with specialty providers.**

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Answers to APHL New Disorders questions

CT definition of an abnormal screen? All results ≥ 0.157µmol/L for either C24:0-LPC or C26:0-LPC.

How is that different during the pilot vs. population screening phases? Previous reporting algorithms were to report C26:0-LPC as a primary analyte with C24:0-LPC not reported alone. During Minnesota's X-ALD validation they sent potential abnormal and true abnormal samples to CT for a second look. One known confirmed patient only had C24:0-LPC elevations.

How do you establish cut-offs? How is this different during the pilot vs. when you implement? Cutoffs were established using population percentile calculations combined with comparison of results obtained through confirmed patient sample analysis with state that supplied samples to determine if there was an overlap despite methodology differences.

What are your repeat rates for screening positive/borderline results? On average 1-3 samples/week repeat for borderline samples.

What changes did you have to make to the laboratory to prepare for screening? No changes were made to the laboratory.

What changes did you have to make to your workflow? Very little change was made to workflow since method is quick and so many analysts are cross-trained for LC-MS/MS analysis/usage.

What changes did you make to your personnel/staffing? No changes, existing staff were trained for method and instrumentation.

What came up that you did not think about? Instrument maintenance required more frequently due to "stickiness" of compounds. Preventative steps added to instrument routine/daily maintenance.

What solution did you come up with? Rail bake method analyzed once a week overnight, divert valve included in method.

Who did you reach out to for support/guidance? Sciex service engineers offered assistance and provided rail bake method as well as refresher training for cleaning Q0 of MS/MS instruments.

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Updated Sample Results:

Number of infants analyzed as of 06/01/17 (10/1/2015-06/01/2017)	61341
Total Screen Positive	17
Samples reported with 2nd request	7
Samples normal on second sample analysis	5
False Positive 2016	1
Pending 2017	3
Confirmed ALD diagnosis newborn infant results	9 (4 male, 5 female)
Siblings Identified (and confirmed at Treatment Center) with ALD	2 (1 male, 1 female)
Total ALD Confirmed (10/1/2015-06/01/2017; siblings included)	11
Other	1 Zellweger 2017
Incidence Overall	~1:6815

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

ACKNOWLEDGEMENTS

- **Christopher Haynes, CDC, for method, technical assistance and Control Materials**
- **Joseph Orsini, New York Newborn Screening Program for technical assistance, confirmed patient sample blinded testing, data and graph displayed in slide, secondary screening of potential abnormal sample results during validation population analysis**
- **Mark Morrissey, New York Newborn Screening Program for assistance with secondary screening of potential abnormal sample results during validation population analysis**
- **Michele Caggana, New York Newborn Screening Program for technical assistance, guidance and advice**
- **Silvia Tortorelli, Mayo Clinic for technical assistance, original SOP methodology information**
- **Jean Kelley and Brian's Hope for encouragement and endless support of the CT NBS Program and for updates and pictures of the infants/families identified**
- **Marie Burlette, Supervising Nurse Consultant for Connecticut Newborn Screening Tracking and Short-Term Follow-Up for slides and diligently following up on reported abnormal NBS results**
- **CT NBS Program (and Melissa from CT Horizon IT) for the long hours, dedication and effort in launching the successful screening program for X-ALD in Connecticut**

Thank You!