Connecticut Newborn Screening For X-Linked Adrenoleukodystrophy

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Katherine A. Kelley Public Health Laboratory
Rocky Hill, CT 06067
CT Newborn Screening

► CGS 19a-55 mandates screening of all CT newborns for select genetic and metabolic disorders
► The CT State Lab screens for 64 disorders including AA, OA, Urea Cycle, FAO, hemoglobin production, endocrine disorders, autoimmune & peroxisomal disorders

► 37,242 births in 2016
► 99.89% newborns screened
► CF Screening conducted at UCONN and Yale Laboratories
► DPH Family Health Section oversees hearing screening, CCHD screening and birth defect registry
# Connecticut NBS Timeline

<table>
<thead>
<tr>
<th>Year</th>
<th>Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>1964</td>
<td>PKU, CH, GALT</td>
</tr>
<tr>
<td>1979</td>
<td>MSUD, CAH, MSUD, HCY</td>
</tr>
<tr>
<td>1983</td>
<td>CPT1, GAII, CPTII, CACT, CUD</td>
</tr>
<tr>
<td>1995</td>
<td>PPA, MMA, IVA, HMG, 3MCC, MCD, GA I, ßKT</td>
</tr>
<tr>
<td>05/2004</td>
<td>ARG, CIT, ASA, OTC, SCAD, DE RED, MMA, HHH*, NKH*</td>
</tr>
<tr>
<td>09/2004</td>
<td>M/SCAD, IBG, EME, FIGLU, 2MBG, 2M3HBA, 3MGA, CPS, PC, RMD, PHE, BIOPT (REG), BIOPT (BS)</td>
</tr>
<tr>
<td>11/2004</td>
<td>SCID, T-Cell Lymphopenia</td>
</tr>
<tr>
<td>01/2005</td>
<td>X-ALD</td>
</tr>
<tr>
<td>09/2010</td>
<td></td>
</tr>
<tr>
<td>10/2011</td>
<td></td>
</tr>
<tr>
<td>07/2016</td>
<td>*removed 2016</td>
</tr>
</tbody>
</table>
CT Newborn Screening
Short Term Follow-Up and Tracking

Responsibilities:

► Using the NBS database, assuring that all infants are screened
► Reporting abnormal results and
  ► Requesting a repeat NBS specimen or
  ► Referring to a regional diagnostic/treatment center
► Following up through diagnosis or exclusion of a disorder
► Maintaining and reporting of statistics
► Educating stakeholders
► Maintaining and trouble shooting the NBS database
► Collaborating with and supporting hospital and birthing center staff, diagnostic/ treatment center staff, primary care providers and parents
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-lyosphosphatidylcholine (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.
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.

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

► 01/2013: SB 465, An Act Requiring Newborn Screening for X-ALD was proposed
► 07/2013: Public Act 13-242 was approved with language added regarding the development and validation of reliable methodology or an FDA cleared kit
► Commissioner of Public Health elected to delay the start of X-ALD screening until after the addition of X-ALD to the RUSP
► 08/2015: Advisory Committee on Heritable Disorders in Newborns and Children voted in favor of the addition of X-ALD to RUSP
► 09/2015: Validation of CDC negative-ion LC-MS/MS method for X-ALD screening began
► 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
► 07/01/2016: X-ALD Screening went live in CT
► All infants born as of October 1, 2015 screened for X-ALD
X-Linked Adrenoleukodystrophy (X-ALD) in Connecticut
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

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 31ºC 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/ acetonitrile 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
X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut
## X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

### Connecticut Precision Results:

#### C24:0-LPC Overall Instrument Precision

<table>
<thead>
<tr>
<th>QC ID</th>
<th>Batch Info</th>
<th>Mean (µmol/L)</th>
<th>Standard Deviation</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDCQC14101</td>
<td>Both Instruments Overall</td>
<td>0.0564</td>
<td>0.0106</td>
<td>18.84%</td>
</tr>
<tr>
<td>CDCQC14102</td>
<td>Both Instruments Overall</td>
<td>0.8226</td>
<td>0.170</td>
<td>20.63%</td>
</tr>
<tr>
<td>CDCQC14103</td>
<td>Both Instruments Overall</td>
<td>3.6476</td>
<td>0.599</td>
<td>16.42%</td>
</tr>
</tbody>
</table>

#### C26:0-LPC Overall Instrument Precision

<table>
<thead>
<tr>
<th>QC ID</th>
<th>Batch Info</th>
<th>Mean (µmol/L)</th>
<th>Standard Deviation</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDCQC14101</td>
<td>Both Instruments Overall</td>
<td>0.0252</td>
<td>0.0046</td>
<td>18.16%</td>
</tr>
<tr>
<td>CDCQC14102</td>
<td>Both Instruments Overall</td>
<td>0.8013</td>
<td>0.122</td>
<td>15.20%</td>
</tr>
<tr>
<td>CDCQC14103</td>
<td>Both Instruments Overall</td>
<td>3.8146</td>
<td>0.573</td>
<td>15.03%</td>
</tr>
</tbody>
</table>
X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Accuracy Results:

<table>
<thead>
<tr>
<th>QC ID</th>
<th>Batch Info</th>
<th>Nominal Concentration (µmol/L)</th>
<th>Mean (µmol/L)</th>
<th>% Bias</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDCQC14101</td>
<td>Both Instruments Overall</td>
<td>0.000</td>
<td>0.056</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CDCQC14102</td>
<td>Both Instruments Overall</td>
<td>1.00</td>
<td>0.823</td>
<td>17.74%</td>
<td>76.62%</td>
</tr>
<tr>
<td>CDCQC14103</td>
<td>Both Instruments Overall</td>
<td>5.00</td>
<td>3.648</td>
<td>27.05%</td>
<td>71.82%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>QC ID</th>
<th>Batch Info</th>
<th>Nominal Concentration (µmol/L)</th>
<th>Mean (µmol/L)</th>
<th>% Bias</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDCQC14101</td>
<td>Both Instruments Overall</td>
<td>0.000</td>
<td>0.025</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CDCQC14102</td>
<td>Both Instruments Overall</td>
<td>1.00</td>
<td>0.801</td>
<td>19.87%</td>
<td>77.61%</td>
</tr>
<tr>
<td>CDCQC14103</td>
<td>Both Instruments Overall</td>
<td>5.00</td>
<td>3.815</td>
<td>23.71%</td>
<td>75.79%</td>
</tr>
</tbody>
</table>
X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Linearity Results:

\[
y = 0.742x - 0.6293 \\
R^2 = 0.9983
\]
X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Linearity Results:

\[ y = 1.1242x - 1.1505 \]
\[ R^2 = 0.9975 \]
### X-Linked Adrenoleukodystrophy (X-ALD) in Connecticut

**Connecticut Carryover Results:**

<table>
<thead>
<tr>
<th>Parameters Evaluated</th>
<th>C24:0-LPC</th>
<th>C26:0-LPC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS 1 (µmol/L)</td>
<td>MS 2 (µmol/L)</td>
</tr>
<tr>
<td>First Set CDC14101 Mean</td>
<td>0.0597</td>
<td>0.0577</td>
</tr>
<tr>
<td>Second Set CDC14101 Mean</td>
<td>0.0611</td>
<td>0.0608</td>
</tr>
<tr>
<td>% Difference (1st set vs 2nd set)</td>
<td>-2.35%</td>
<td>-5.28%</td>
</tr>
<tr>
<td>2-tailed TTest</td>
<td>0.536</td>
<td>0.288</td>
</tr>
<tr>
<td>if p &gt; 0.05 differences are not significant</td>
<td>OK</td>
<td>OK</td>
</tr>
<tr>
<td>Patient Cutoff</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Potential Carryover (Patient Cutoff * % Mean Difference)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Instrument Potential False Positive Lower Limit Threshold from Carryover</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Connecticut Drift Results:
X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut
Connecticut Drift Results:
## X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

### Connecticut Blinded NY Sample Results:

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

### CT vs. NY Calculated Concentration (µmol/L)

![Graph showing the relationship between CT and NY calculated concentrations with a line of best fit and R² value of 0.9639](graph.png)

\[ y = 1.1179x - 0.0075 \]

R² = 0.9639
## X-Linked Adrenoleukodystrophy (X-ALD) in Connecticut

### Connecticut Validation Sample Results:

<table>
<thead>
<tr>
<th></th>
<th>24:0-LPC (µmol/L)</th>
<th>26:0-LPC (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>0.0654</td>
<td>0.0606</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>0.0633</td>
<td>0.0593</td>
</tr>
<tr>
<td><strong>25th Percentile</strong></td>
<td>0.0528</td>
<td>0.0503</td>
</tr>
<tr>
<td><strong>75th Percentile</strong></td>
<td>0.0757</td>
<td>0.0690</td>
</tr>
<tr>
<td><strong>99th Percentile</strong></td>
<td>0.1185</td>
<td>0.1011</td>
</tr>
<tr>
<td><strong>Borderline Cutoff (99.9th percentile)</strong></td>
<td>NA</td>
<td>0.157</td>
</tr>
<tr>
<td><strong>Presumptive Positive Cutoff Range</strong></td>
<td>0.157 (99.9th)</td>
<td>0.257 (99.98th)</td>
</tr>
<tr>
<td><strong>Number Analyzed during the validation</strong></td>
<td>27495</td>
<td>27495</td>
</tr>
</tbody>
</table>

### Proposed Connecticut Reporting Algorithm

<table>
<thead>
<tr>
<th></th>
<th><strong>Request for another sample</strong></th>
<th><strong>Proposed Connecticut Reporting Algorithm</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.24</td>
<td>NY 26:0-LPC cutoff (lower)</td>
<td></td>
</tr>
<tr>
<td>32.94%</td>
<td>Largest % Difference NY vs CT (CT values lower than NY)</td>
<td></td>
</tr>
<tr>
<td>0.0791</td>
<td>(% Difference CT vs NY) * NY cutoff</td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td><strong>CT Calculated lower cutoff (NY cutoff -((% Difference CT vs NY) * NY cutoff))</strong></td>
<td><strong>Refer child for followup testing</strong></td>
</tr>
<tr>
<td>0.39</td>
<td>NY 26:0-LPC cutoff (upper)</td>
<td></td>
</tr>
<tr>
<td>32.94%</td>
<td>Largest % Difference NY vs CT (CT values lower than NY)</td>
<td></td>
</tr>
<tr>
<td>0.128</td>
<td>(% Difference CT vs NY) * NY cutoff</td>
<td></td>
</tr>
<tr>
<td>0.26</td>
<td><strong>CT Calculated upper cutoff (NY cutoff -((% Difference CT vs NY) * NY cutoff))</strong></td>
<td><strong>Refer child for followup testing</strong></td>
</tr>
</tbody>
</table>

### Request for another sample

- **Presumptive Positive Cutoff**
  - 0.157 (99.9th)
  - 0.257 (99.98th)

### Proposed Connecticut Reporting Algorithm

- **Request for another sample**
- **Proposed Connecticut Reporting Algorithm**

<table>
<thead>
<tr>
<th></th>
<th><strong>Range</strong></th>
<th><strong>Proposed Connecticut Reporting Algorithm</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number Analyzed during the validation</strong></td>
<td>27495</td>
<td></td>
</tr>
</tbody>
</table>
**X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut**

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

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.”

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

SHORT TERM FOLLOW-UP AND TRACKING

- Communicate with Hospital and/or PCP regarding need for a repeat NBS specimen or referral to diagnostic/treatment center
- Referral to Dr. Michelle Manzon, Yale School of Medicine, Department of Genetics, when appropriate
- Obtain names, DOBs and gender of siblings and communicating this information to the NBS Lab and Yale Genetics
- Follow up through diagnosis or exclusion of X-ALD
- Educating stakeholders about X-ALD
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

CT X-ALD FAQ:

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:

<table>
<thead>
<tr>
<th>Description</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infants analyzed as of 08/01/17 (10/1/2015-08/01/2017)</td>
<td>67694</td>
</tr>
<tr>
<td>Total Screen Positive</td>
<td>24</td>
</tr>
<tr>
<td>Samples reported with 2nd request</td>
<td>12</td>
</tr>
<tr>
<td>Samples normal on second sample analysis</td>
<td>10</td>
</tr>
<tr>
<td>False Positive 2016</td>
<td>1</td>
</tr>
<tr>
<td>False Positive 2017</td>
<td>2</td>
</tr>
<tr>
<td>Confirmed ALD diagnosis newborn infant results</td>
<td>10 (5 male, 5 female)</td>
</tr>
<tr>
<td>Siblings Identified (and confirmed at Treatment Center) with ALD</td>
<td>2 (1 male, 1 female)</td>
</tr>
<tr>
<td>Other</td>
<td>1 Zellweger 2017</td>
</tr>
</tbody>
</table>

**Number Reported Abnormal PPV (Positive Predictive Value (PPV))**

Number of infants with an out-of-range result from a first or subsequent dried blood spot specimen requiring clinical diagnostic workup by an appropriate medical professional and with a confirmed diagnosis of the disorder by an appropriate medical professional, divided by the number of infants with an out-of-range result from the dried blood spot screen requiring a repeat specimen or a clinical diagnostic workup by an appropriate medical professional, reported by disorder category.; Note* Zellweger case included in confirmed case total)

**Number Referred PPV (total confirmed with disease/total referred)**

(total confirmed with disease/total referred; Note* Zellweger case included in confirmed case total)

<table>
<thead>
<tr>
<th>Description</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence Overall</td>
<td>~1:6,769</td>
</tr>
<tr>
<td>Incidence Male</td>
<td>~1:13,539</td>
</tr>
<tr>
<td>PPV Abnormal ALD ONLY</td>
<td>41.67%</td>
</tr>
<tr>
<td>PPV Referred ALD ONLY</td>
<td>71.43%</td>
</tr>
<tr>
<td>PPV Referred ALD ONLY</td>
<td>78.57%</td>
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<tr>
<td>Number Reported Abnormal PPV</td>
<td>45.83%</td>
</tr>
<tr>
<td>Number Referred PPV</td>
<td>78.57%</td>
</tr>
</tbody>
</table>
## X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

<table>
<thead>
<tr>
<th>#</th>
<th>ID #</th>
<th>Gender</th>
<th>Interpretation/Action</th>
<th>Outcome</th>
<th>Notes</th>
<th>Birth Year</th>
<th>Spec Collected (age/days)</th>
<th>Spec Received (age/days)</th>
<th>24:0-LPC Abn</th>
<th>26:0-LPC Abn</th>
<th>BW, EGA</th>
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</thead>
<tbody>
<tr>
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<td>74733553</td>
<td>M</td>
<td>1st NBS Bdl/Repeat</td>
<td>ALD Confirmed</td>
<td>screened + during validation; older sibling also confirmed +</td>
<td>2016</td>
<td>1</td>
<td>4</td>
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<td>3515g, 38w</td>
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<tr>
<td>2</td>
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<td>Repeat NBS WNL</td>
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<td>older sibling also confirmed +</td>
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<td>5</td>
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<td>yes</td>
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<td>2185g, 34w</td>
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</tbody>
</table>
X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut
The State of Connecticut Newborn Screening Program

Make Sure Your Baby is Healthy

Newborn Screening (NBS) is important!

- Babies with some health problems may not look sick when they are born, but they may have trouble eating, gaining weight or have slower brain growth. They can also become very sick and sometimes die. Newborn Screening (NBS) helps find babies with certain health problems, so treatment can start early. Early treatment can help prevent serious illness and death.

Why does my baby need NBS?
- Without NBS, you cannot know if your baby has certain health problems.
- Connecticut NBS tests for over 60 health problems.
- If one of these health problems is not treated, your baby may:
  - become very sick
  - grow poorly
  - have physical disability
  - have brain damage
  - die
- With early treatment many problems can be prevented.

Who should get the test?
- Every newborn baby should be tested.

When is the test done?
- One to three days after birth.

How is the test done?
- The test is a simple blood sample. Blood is taken from your baby's heel.
- The blood is tested at the State Public Health Laboratory in Rocky Hill.

Can I say "no" to this test?
- You can say "no" to the test for religious reasons.
- You will be asked to sign a form that says you do not want your baby to be tested.

How do I get the test results?
- Ask your baby's doctor for the results.

CT NBS Parent Information

What does an abnormal result mean?
- It does not always mean that your baby is sick.
- There are many things that can cause an abnormal result.

An abnormal result can happen:
- if you took certain medications while pregnant
- if your baby was born early
- if your baby is born sooner than expected
- if your baby was born before the hospital
- if many other reasons

If your baby has an abnormal NBS test your doctor may:
- examine your baby
- ask about foods in your family
- repeat the NBS test
- order a different test
- talk to a genetic counselor

If my baby does have one of those medical problems, what will happen?
- Your doctor may:
  - give your baby a special diet
  - give your baby by mouth medicine
  - start other treatments
  - have your baby see a special doctor

What does Connecticut NBS test for?
- A number of conditions that may affect your baby's health.
- The conditions include:
  - metabolic disorders
  - genetic disorders
  - infectious diseases

What childhood diseases are caused by NBS?
- NBS can detect diseases that occur in later life as well as those that occur at birth.

Can NBS find all diseases?
- NBS can detect only certain conditions that are caused by genes.

Can NBS detect all problems?
- NBS is not designed to detect all problems.

Can NBS help prevent health problems?
- NBS can help prevent many health problems.

Can NBS detect all conditions?
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Can NBS find all diseases?
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
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Thank You!