Connecticut Newborn Screening
For Severe Combined Immunodeficiency (SCID)

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November 9, 2017
CT Newborn Screening

Screening of all CT newborns for select genetic and metabolic disorders is mandated

- Connecticut General Statutes (CGS 19a-55)

The CT State Lab screens for 64 disorders including AA, OA, Urea Cycle, FAO, hemoglobin production, endocrine, 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 Implementation Timeline

- PKU
- CH
- GALT
- MSUD
- CAH
- HCY
- BIO
- HGB S
- HGB SC
- Hgb C
- Hgb SD
- Hgb D
- Hgb SE
- Hgb E
- Hgb Bart's
- Hgb Sβ* Thal
- Variant Hg
- MSUD
- HCY
- MET
- TYR
- MCAD
- LCHAD
- VLCAD
- TFP
- CPT1
- GAII
- CPTII
- CACT
- CUD
- PPA
- MMA
- IVA
- HMG
- 3MCC
- MCD
- GA I
- ΒKT
- ARG
- CIT
- ASA
- OTC
- SCAD
- DE
- RED
- MMA
- HHH*
- NKH*
- M/SCAD
- IBG
- EME
- FIGLU
- 2MBG
- 2M3HBA
- 3MGA
- CPS
- PC
- RMD
- PHE
- BIOPT (REG)
- BIOPT (B5)
- SCID
- T-Cell Lymphopenia
- X-ALD

*removed 2016
CT Newborn Screening

Laboratory Responsibilities

- Receipt, login, sample quality evaluation
- Creating worklists, punching of samples into 96-well plates
- Sample preparation
- Instrument maintenance and analysis set-up
- Sample interpretation
- Reporting of sample results
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
Screening for Severe Combined Immunodeficiency (SCID) In Connecticut

Challenges For Implementation Of Molecular Screening Tests In A Newborn Screening Program

FUNDING    STAFFING    SPACE
SCID NBS Implementation in CT

Timeline

• 2008
  • National Level - Grant awarded to two laboratories for SCID testing (MA and WI)
  • Connecticut – Program had 8 Laboratory staff (12 in 2006-2007)
• Financial crisis—budget cuts and union concessions
• December 2009
  • Increased interest in SCID testing from laboratory management
  • Information was gathered from Massachusetts, Wisconsin and CDC
• Evaluation of available methods begins
• 2010 – Training Opportunities
  • February at the CDC
  • April at the New England Newborn Screening Laboratory
  • May at the Wisconsin Newborn Screening Laboratory
• Attempt made to acquire funds to implement SCID newborn screening in April 2010; no funding available
November 4, 2010

Dear Ms. Manning,

As you know, Health and Human Services Secretary Kathleen Sebelius, recently acted to add Severe Combined Immune Deficiency (SCID) to the core panel for universal screening of all newborns in the United States. The Jeffrey Modell Foundation has successfully implemented and helped fund this program, utilizing the TREG’s Assay as an initial screen and Bone Marrow Transplantation as an effective intervention.

We are pleased to report to you that 8 states are now screening all newborns for SCID and over 1.5 million newborns will be screened for this devastating disorder. There are 17 additional states currently developing programs to screen for SCID and related T-Cell Lymphopenia. Indeed, this program has already saved many babies’ lives!

Attached is a recently published article from the Journal of American Medical Association relating to this initiative. We have also included a brief “snapshot” of the Jeffrey Modell Foundation. We hope to pursue a working collaboration with the State of Connecticut pursuant to past communication. Please let us know how we can assist you in implementing this program and whether you require any further information.

With hope for our cause,

Vicki and Fred Modell
Co-Founders
Jeffrey Modell Foundation
747 Third Avenue
New York, New York 10017
T: 212.819.0200
F: 212.764.4180
www.info4pi.org

Best regards,

Vicki and Fred Modell
Co-Founders
Jeffrey Modell Foundation

Vicki and Fred Modell
Co-Founders
Jeffrey Modell Foundation
SCID NBS Implementation in CT

Timeline

2010
• National Level - ACHDNC Recommends SCID Screening to be added to the RUSP
• Connecticut - Mid-2010 to 2011: 6 laboratory staff

2011
• January: SB543 “An Act Providing Newborn Screening for Severe Combined Immunodeficiency Disease”
• July: SCID mandated to start October 1, 2011 via Section 38 of Public Act (PA) 11-48
• July: CDC In situ method chosen
• July: Equipment requisitions using agency funding for capital equipment procurement placed
• July: Method development and testing began July 2011
• August: Staff attend training at CDC for preparation of testing calibrator and control reference materials
• October: Validation began

All infants born as of 1st October 2011 were screened for SCID with official start date of January 1, 2012.
SCID NBS Implementation in CT

Selection of Method – CDC In Situ Method

FUNDING

COST:
• ~$80,000 in instrument costs and ~$10,000 in ancillary costs
• QC and reference materials prepared at CDC during method training

STAFFING

MINIMAL STAFFING/SPECIALIZED STAFFING REQUIRED:
• Only 6 existing NBS staff
• No staff familiar with molecular biology/PCR methodology experience available
• Master’s student intern available from UCONN
• No DNA extraction required—easier method

SPACE

MINIMAL SPACE REQUIRED:
• No DNA extraction required—less space required, however no space available within the NBS laboratory: NEEDED TO BE CREATIVE
• Space was initially provided in another (serology) laboratory: a STORAGE CLOSET was emptied and converted to sample preparation area (dead air box used for preparation of primers, probes and mastermix), this area contained all pre-PCR steps/equipment
• ~4-feet of bench top space in the serology laboratory marked off for Stratagene PCR equipment
• Also were able to share Stratagene PCR equipment from another laboratory to decrease analysis time
8-point DBS B-TREC calibration curve

- Prepared using T lymphocyte depleted blood with aliquots of a human EBV (Epstein Barr virus)-transformed B-cell line that contain a single copy of TREC per cell for final a nominal concentration of TREC/µL of blood where a known number of cells have been added.*

- Quantitative and qualitative QC reference materials
- PerfCta Multiplex RT (2.5X) reaction cocktail for PCR amplification
- Qiagen DNA Purification Solution 1 and DNA Elution Solution 2
- Primers and Probes for TREC and RNase P

SCID NBS Implementation in CT

Method Summary

- Punch one 2.0 mm discs from DBS specimen into PCR tubes
- Wash with 125 µl of DNA purification solution S1 (shake for 15 minutes at room temp)
- Wash with 125 µl of DNA elution solution S2 (shake for 5 minutes at room temp)
SCID NBS Implementation in CT

Method Summary

- Discard S2 wash buffer and add 15 μl of qPCR Master Mix
- Run qPCR in Stratagene MX3000p
  - UNG Activation (5' @ 45°)
  - Denaturation (20' @ 95°)
  - Amplification [45 cycles: 15"@ 95° / 1' @ 60°]
- Analyze qPCR Data, Check QC Results and Report NBS Results

\[ y = -3.1409x + 36.607 \]
\[ R^2 = 0.963 \]
\[ E = 108\% \]
SCID NBS Implementation in CT

- Intern from UCONN assisted with method validation process due to major staffing shortages
- Pre-patient analysis meeting held with state clinical immunologist (information about who could fulfill this role obtained through discussions with CDC and Dr. Lisa Kobrynski) to set guidelines for follow-up for possible true abnormal findings; set a lower limit action level for TREC recovery
- Patient sample population analysis commenced following accuracy and precision study (samples received 10/3/11 to 11/15/11, >4400 samples analyzed)
- Massachusetts (New England Newborn Screening) program assisted with second analysis of potentially abnormal results using their well-established and validated method
- Guidance available through Massachusetts, CDC and Wisconsin during the validation process
SCID NBS Implementation in CT

Validation Results

<table>
<thead>
<tr>
<th>Validation Patient Analysis Results</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Analyzed</td>
<td>4457</td>
</tr>
<tr>
<td>% Repeated Total (Full Term and Preterm)</td>
<td>105 (2.36%)</td>
</tr>
<tr>
<td>1st Unsatisfactory</td>
<td>17 (0.38%)</td>
</tr>
<tr>
<td>Not Tested (waiver, expired)</td>
<td>7 (0.167%)</td>
</tr>
<tr>
<td>1st Sample Abnormal Retest Specimens Tested</td>
<td>13 Preterm</td>
</tr>
<tr>
<td>Sample Results</td>
<td></td>
</tr>
<tr>
<td>Median (50th Percentile)</td>
<td>252</td>
</tr>
<tr>
<td>10% Median (5th Percentile)</td>
<td>25.2</td>
</tr>
<tr>
<td>Mean (Average)</td>
<td>281</td>
</tr>
<tr>
<td>10% Mean</td>
<td>28.1</td>
</tr>
<tr>
<td>Analysis Acceptance Criteria</td>
<td></td>
</tr>
<tr>
<td>Efficiency</td>
<td>85-115%</td>
</tr>
<tr>
<td>R-Squared</td>
<td>&gt;=0.93</td>
</tr>
<tr>
<td>Validation Cutoff</td>
<td></td>
</tr>
<tr>
<td>EGA All</td>
<td>55</td>
</tr>
<tr>
<td>Post Validation Initial Cutoff</td>
<td></td>
</tr>
<tr>
<td>EGA &gt;= 37 weeks</td>
<td>40</td>
</tr>
<tr>
<td>EGA &lt;37 weeks</td>
<td>25</td>
</tr>
<tr>
<td>Current Cutoff</td>
<td></td>
</tr>
<tr>
<td>EGA &gt;= 37 weeks</td>
<td>30</td>
</tr>
<tr>
<td>EGA &lt;37 weeks</td>
<td>25</td>
</tr>
</tbody>
</table>

5 Full Term Patient samples sent to Massachusetts for analysis during validation patient population study (4 normal), 1 CONFIRMED SCID during validation
## SCID Newborn Screening in CT

**Current Testing Information**

CT Algorithm for reporting sample results:

<table>
<thead>
<tr>
<th>Gestation Age</th>
<th>TREC (copies/µL)</th>
<th>RNase P (Cq)</th>
<th>Action</th>
<th>Final result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>≥ 30</td>
<td>&lt;28</td>
<td>NA</td>
<td>Normal</td>
</tr>
<tr>
<td>&lt;37</td>
<td>≥ 25</td>
<td>&lt;28</td>
<td>NA</td>
<td>Normal</td>
</tr>
<tr>
<td>Any</td>
<td>Any</td>
<td>≥28</td>
<td>Repeat sample x 2</td>
<td>Invalid, Request Repeat Specimen 2X</td>
</tr>
<tr>
<td>≥37</td>
<td>≥10, &lt;30</td>
<td>&lt;28</td>
<td>Repeat sample x 2</td>
<td>Abnormal, Request Repeat Specimen 1X</td>
</tr>
<tr>
<td>Any</td>
<td>&lt;10</td>
<td>&lt;28</td>
<td>Repeat sample x 2</td>
<td>Abnormal, Immediate Referral</td>
</tr>
<tr>
<td>Any</td>
<td>=No Ct</td>
<td>&lt;28</td>
<td>Repeat sample x 2</td>
<td>Abnormal, Immediate Referral</td>
</tr>
<tr>
<td>&lt;37</td>
<td>≥10, &lt;25</td>
<td>&lt;28</td>
<td>Repeat sample x 2</td>
<td>Abnormal, Request Repeat Specimen 2X</td>
</tr>
</tbody>
</table>
SCID Newborn Screening in CT
Current Testing Information

CT NICU Algorithm:

Connecticut newborn screening algorithm for congenital T cell lymphopenia (SCID) in NICUs

1st Dried Blood Spot

- Breast Milk O.K. §
- Standard PID precautions
- Repeat NBS
- Flow Cytometry® for pts with abnormal TREC x2

GA ≥ 37wks
TREC < 30
O8
GA < 37
TREC > 25

GA ≥ 37wks
TREC 10-25
- Non-syndromic
- Opportunistic infections
- No PID family hx
- No erythroderma

GA < 37
TREC 10-30

TREC < 10

Immediate Flow cytometry
- No Breast Milk until mother proven CMV seronegative
- Standard PID precautions AND reverse isolation
- Consult w/ clinical immunologist

Normal

CT Patient Results: Total Infants Screened 221,554 from 2011-2017

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Moderate T-cell Lymphopenia</td>
</tr>
<tr>
<td>Patient 2</td>
<td>SCID</td>
</tr>
<tr>
<td>Patient 3</td>
<td>22q11; partial DiGeorge</td>
</tr>
<tr>
<td>Patient 4</td>
<td>SCID</td>
</tr>
<tr>
<td>Patient 5</td>
<td>SCID</td>
</tr>
<tr>
<td>Patient 6</td>
<td>T &amp; B-cell lymphopenia</td>
</tr>
<tr>
<td>Patient 7</td>
<td>T-cell Lymphopenia</td>
</tr>
<tr>
<td>Patient 8</td>
<td>T-cell Lymphopenia</td>
</tr>
<tr>
<td>Patient 9</td>
<td>DiGeorge Syndrome</td>
</tr>
<tr>
<td>Patient 10</td>
<td>CLOVES Syndrome</td>
</tr>
<tr>
<td>Patient 11</td>
<td>T-cell Lymphopenia</td>
</tr>
<tr>
<td>Patient 12</td>
<td>T-cell Lymphopenia</td>
</tr>
<tr>
<td>Patient 13</td>
<td>T-cell Lymphopenia</td>
</tr>
<tr>
<td>Patient 14</td>
<td>T and B cell lymphopenia</td>
</tr>
<tr>
<td>Patient 15</td>
<td>T-cell Lymphopenia; 7q32 deletion including TCR beta gene</td>
</tr>
<tr>
<td>Patient 16</td>
<td>Moderate T-cell Lymphopenia</td>
</tr>
<tr>
<td>Patient 17</td>
<td>T-cell Lymphopenia</td>
</tr>
<tr>
<td>Patient 18</td>
<td>Sepsis, prematurity</td>
</tr>
<tr>
<td>Patient 19</td>
<td>Chronic Lymphopenia</td>
</tr>
<tr>
<td>Patient 20</td>
<td>T-cell Lymphopenia due to prematurity</td>
</tr>
<tr>
<td>Patient 21</td>
<td>T-cell Lymphopenia due gastrochisis and prematurity</td>
</tr>
<tr>
<td>Patient 22</td>
<td>DiGeorge Syndrome</td>
</tr>
<tr>
<td>Patient 23</td>
<td>DiGeorge Syndrome</td>
</tr>
<tr>
<td>Patient 24</td>
<td>T-cell Lymphopenia due to prematurity. Bronchopulmonary dysplasia, chromosomal abnormalities with duplication at 19q13.33 and 8q13.3</td>
</tr>
<tr>
<td>Patient 25</td>
<td>DiGeorge Syndrome with CCHD</td>
</tr>
<tr>
<td>Patient 26</td>
<td>Lost to f/u</td>
</tr>
<tr>
<td>Patient 27</td>
<td>Sepsis, prematurity</td>
</tr>
</tbody>
</table>
SCID Newborn Screening in CT

Original Investigation

Newborn Screening for Severe Combined Immunodeficiency in 11 Screening Programs in the United States

Antonia Kwan, PhD, MICPCH, Roshini S. Abraham, PhD, Robert Cornier, PhD, Amy Brower, PhD, Karen Androszeski, BS, Jordan K. Albrett, MD, Mer Baker, MD, Male Ballew, MD, Louis E. Bartholomew, MD, Francisco A. Bonilla, MD, PhD, Charles Berloppo, DPhil, Edward Brooks, MD, Michelle Caligiana, ScD, Jonathan Caplin, MD, Joseph A. Creti, MD, Anne Marie Cusack, PhD, James A. Connolly, MD, Morton J. Cowan, MD, Michael C. Curran, PhD, Alejandra Delerrio, MD, PhD, Matt Dallin, MD, Hilary E. Darby, MD, Scott K. Davis, MD, Joel N. DiLullo, MD, Loisa Fuhrer, MD, Debra Freedberg, MD, Ernest W. Gerflanc, MD, James E. Hale, BS, T. Colleen Hansen, MD, Beverly N. Hay, MD, Diane Hsu, MD, Anthony Jaffe, MD, PhD, Daisy Johnson, RN, Neima Kalavera, MD, Danna M. Ke, PhD, Donald H. Soeh, MD, Rachel Lee, PhD, Heather Lehman, MD, Zsili Le, PhD, Fred Loosy, PhD, Ali Abdel-Magied, MD, MBA, Achilleas Manning, BS, Swati Mocherla, MD, Theodore B. Moore, MD, Stanley J. Nadel, MD, Luigi D. Noritangadi, MD, Jordan S. O’Donnell, MD, Sung-Hyun Park, MD, Matthew Portnoy, MD, PhD, Kay Rodriguez, MD, JD, MPH, MPA, Neil Rosenberg, MD, John Rozic, MD, Mary Rulke, MD, Arvy Rubenstein, MD, Carlos A. Sazuwa-Muto, MD, Ginge Scott, RN, Patricia M. Scott, MD, Elizabeth Secord, MD, Christine Serontoy, MD, William F. Shearer, MD, PhD, Sathiyapriya Sivagn, MD, Mary E. Silver, MD, E. Richard Skelton, MD, Robert W. Sugerman, MD, David B. Tabor, MD, Farah Tavirani, MD, Stephen R. Veer, MD, Eric Viscido, MD, Jordan N. Wahl, MD, PhD, Richard L. Weiseman, MD, PhD, Michael S. Watson, MS, PhD, Geoffrey A. Weinberg, MD, Leonard B. Weiner, MD, Heather Wood, MD, Anne B. Yates, MD, Jennifer M. Zuck, MD.
SCID Newborn Screening in CT
Mid-2014 SCID Assay Troubleshooting

What the amplification plots should look like for TREC

What the amplification plots actually looked like for TREC
SCID Newborn Screening in CT
Mid-2014 SCID Assay Troubleshooting

PROBLEM

• Multiple plate analysis failures
• 14 days of sample analysis backlog

TROUBLESHOOTING

• Contacted and collaborated with CDC Newborn Screening and Molecular Biology Branch: (Dr. Francis Lee, Dr. Jennifer Taylor and Golriz Yazdanpanah)
• Identified and eliminated potential causes

CULPRIT

• PCR Instrument
• Mastermix
• Primers/Probes
• S1 & S2 Reagents
• Calibration Reference Material

SOLUTION
SCID Newborn Screening in CT

FURTHER IMPROVEMENTS
• New Laboratory Space (as of 2012)
• Additional Instrumentation
• Additional Staff

MOLECULAR ASSESSMENT PROGRAM
• Reconfiguration of laboratory SCID testing setup/space
Implementation of SCID NBS in CT

Summary

Connecticut SCID NBS launch was successful (6th state in the country to start screening for SCID), however it was not without CHALLENGES

METHOD CHOICE
- In 2011, limited choices and no commercial method available, choice was between DNA extraction methods or In situ method
- Currently both commercial kits and LDTs are available for laboratories to choose from: MORE CHOICES enable laboratories to choose between FDA approved kits or LDTs based upon technical expertise, convenience, etc.
- In 2011 CT had no expertise with PCR, least complicated method was chosen, has worked very well

STAFFING
- No experience with PCR methods, but lots of SUPPORT/HELP available and given by other NBS laboratories (Massachusetts and Wisconsin) and the CDC to assist CT to start SCID testing
- Immunologist identified through assistance given by an immunologist in another state—one with contacts around the country
- Critically low staffing at time of mandate, however, methodology used was easier and required very little time to complete (~30 minutes sample preparation, 2 hours analysis)

SPACE
- Necessary to be CREATIVE/INNOVATIVE to identify and set up the minimal amount of space (Pre-PCR, Post-PCR) for carrying out the procedure (initially we used a storage closet and ~4 feet of bench space in another laboratory)

FUNDING
- For the types of assays available (commercial kits or LDTs), LDTs generally are less expensive
- Sharing of equipment with another laboratory reduced the initial amount of $$ needed to start SCID testing
# ACKNOWLEDGEMENTS

<table>
<thead>
<tr>
<th>Connecticut Newborn Screening</th>
<th>Molecular Assessment Program</th>
</tr>
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<tbody>
<tr>
<td>Joseph Ubaiké</td>
<td>Suzanne Cordovado (CDC Molecular Quality Improvement Program)</td>
</tr>
<tr>
<td>Corina Boluk</td>
<td>Christopher Greene (CDC Molecular Quality Improvement Program)</td>
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<tr>
<td>Mary Jo Guiliano</td>
<td>Rachel Lee (Biochemistry and Genetics Branch Manager, Texas DSHS)</td>
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<tr>
<td>Debra Studwell</td>
<td>Tim Davis (Lead Microbiologist, Hemoglobin and Molecular, Washington DOH)</td>
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<tr>
<td>Leslie Mills</td>
<td>Guisou Zarbalian (Senior Specialist, Newborn Screening and Genetics, Association of Public Health Laboratories)</td>
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<thead>
<tr>
<th>CDC Newborn Screening and Molecular Biology Branch</th>
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<tbody>
<tr>
<td>Edith Zimmermann</td>
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<tr>
<td>Kathryn Holden</td>
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<tr>
<td>Agnieszka Bouthot</td>
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<tr>
<td>Anna Filipkowska</td>
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<td>Barbara Szupryczynski</td>
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<td>Julie Riccio</td>
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<td>Ryan Richard</td>
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<td>Marie Burlette</td>
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<td>Rose Marie Mitchell</td>
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<td>Linda Bailey</td>
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<table>
<thead>
<tr>
<th>Yale New Haven Hospital</th>
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<tbody>
<tr>
<td>Neil Romberg</td>
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<tr>
<td>Odelya Pagovich</td>
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<td>Jason Catanzaro</td>
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<tr>
<td>Jennifer Taylor (RTI International)</td>
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<tr>
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<table>
<thead>
<tr>
<th>University of Connecticut</th>
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<tbody>
<tr>
<td>Lia Ribustello</td>
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<table>
<thead>
<tr>
<th>CCMC Pediatric Infectious Diseases and Immunology</th>
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<tr>
<td>Nicholas Bennett</td>
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Thank You!