Current Testing Algorithm for SCID

T-cell receptor excision circle (TREC) assay

< 125 Avg TREC or
2 samples <200 Avg TREC

Refer to Specialist for Diagnostic Testing

Physician ordered tests

Molecular tests – gene panel

• Slow turn-around times
• Insurance denial
• Increased stress to families
Potential Benefits of Molecular Testing by the NBS Program:

- Shortened Turn-Around Time (TAT)
- Faster diagnosis; phenotype prediction
- Earlier decision on best treatment options – better outcomes
- Cost savings to family and health care system
- Less stress for families
APHL/CDC Cooperative Agreement - Specific Aims

- Develop and validate a 2nd tier multi-gene immunodeficiency panel
- Evaluate Utility of Targeted NGS for SCID in a Consented Study
  - Identify causative gene?
  - Shortened time to diagnosis?
  - Earlier, targeted treatment?
- Provide CDC with quality control/reference samples
NYS NBS 39-gene SCID panel

Gene Selection:
- Commercial SCID panels
- CLSI guideline
- Literature search and case studies

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
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<th>Gene</th>
<th>Gene</th>
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<th>Gene</th>
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<tbody>
<tr>
<td>ADA</td>
<td>AK2</td>
<td>ATM</td>
<td>BLNK</td>
<td>BTK</td>
<td>CD3D</td>
<td>CD3E</td>
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<tr>
<td>CD3G</td>
<td>CD247</td>
<td>CD40LG</td>
<td>PTPRC</td>
<td>CHD7</td>
<td>CORO1A</td>
<td>DCLRE1C</td>
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<tr>
<td>DKC1</td>
<td>DOCK2</td>
<td>DOCK8</td>
<td>FOXN1</td>
<td>GATA2</td>
<td>IGHM</td>
<td>IL2RG</td>
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<td>IL7R</td>
<td>JAK3</td>
<td>LIG4</td>
<td>MTHFD1</td>
<td>MTR</td>
<td>NHEJ1</td>
<td>NBN</td>
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<td>PNP</td>
<td>PRKDC</td>
<td>RAC2</td>
<td>RAG1</td>
<td>RAG2</td>
<td>RMRP</td>
<td>SLC46A1</td>
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<td>STAT5B</td>
<td>TBX1</td>
<td>WAS</td>
<td>ZAP70</td>
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</tbody>
</table>
NGS Technology

Illumina TruSeq Custom Amplicon Panel

Illumina MiSeq

In-house Developed Bioinformatics Pipeline
Validation

- NGS Sequence
  - 8 samples from infants diagnosed with SCID – genetic cause known
  - 13 additional samples from infants diagnosed with SCID or other immunodeficiency – genetic cause not reported to us
  - Reference Sample – NA12878 (NIST; GIAB) – published genotypes

- Sanger sequence
  - 8 samples Sanger sequenced for all 39 genes
    - 657 amplicons/sample = 5256 amplicons total
  - Sanger confirm all pathogenic/likely pathogenic/VOUS in remaining samples

- Assessed reproducibility through replicate testing within and between runs
# Accuracy – Concordance with Commercial Diagnostic Lab

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gene</th>
<th>Variant (cDNA)</th>
<th>Variant (aa)</th>
<th>Zygosity</th>
<th>Agreement with Dx Lab</th>
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</thead>
<tbody>
<tr>
<td>102</td>
<td>CHD7</td>
<td>c.434G&gt;A</td>
<td>p.Trp145Ter</td>
<td>Het</td>
<td>Yes</td>
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<tr>
<td>105</td>
<td>ADA</td>
<td>c.301C&gt;T</td>
<td>p.Arg101Trp</td>
<td>Hom</td>
<td>Yes</td>
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<tr>
<td>108</td>
<td>IL7R</td>
<td>c.83-2A&gt;T</td>
<td>-</td>
<td>Het</td>
<td>Yes</td>
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<tr>
<td></td>
<td>IL7R</td>
<td>c.353G&gt;A</td>
<td>p.Cys118Tyr</td>
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<td>110</td>
<td>IL2RG</td>
<td>c.982C&gt;T</td>
<td>p.Arg328Ter</td>
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<td>113</td>
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<td>p.Arg289Ter</td>
<td>Hom</td>
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<td>115</td>
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<td>c.301C&gt;T</td>
<td>p.Arg101Trp</td>
<td>Hom</td>
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<tr>
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<td>JAK3</td>
<td>c.2872G&gt;T</td>
<td>p.Glu958Ter</td>
<td>Het</td>
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<tr>
<td></td>
<td></td>
<td>c.1261delC</td>
<td>p.Leu421fs</td>
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<td></td>
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<td>120</td>
<td>RAG2</td>
<td>c.501A&gt;C</td>
<td>p.Arg167Ser</td>
<td>Het</td>
<td>Yes</td>
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<tr>
<td></td>
<td></td>
<td>c.283G&gt;A</td>
<td>p.Gly95Arg</td>
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<td></td>
</tr>
</tbody>
</table>

Validation Approved by NYS CLEP on 7/17/2018
Proposed Algorithm for SCID NGS Consented Study

T-cell receptor excision circle (TREC) assay

- < 125 Avg TRECs
- 2 samples <200 Avg TRECs

Refer to Specialist for Diagnostic Testing

CBC, Flow cytometry, Mitogen studies

Immunodeficiency identified; Obtain Informed Consent

39-gene SCID NGS panel
Consented Study

• IRB approval - 8/10 NYS SCID Specialty Care Centers
• 3 Part Consent
  1) Consent to SCID NGS panel testing
  2) Consent to participation in Immunodeficiency Registry for Long-Term Follow-up
  3) Consent to provide a blood sample for CDC to develop quality assurance materials
  
  Participants can consent to 1, 2, all 3, or none of the parts
Consented Study

- Since receiving approval to offer the SCID NGS test (7/17/2018):
  - 5 infants consented into the study

<table>
<thead>
<tr>
<th>Consent Part</th>
<th># Consented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part I: Consent to SCID multi-gene NGS analysis</td>
<td>5</td>
</tr>
<tr>
<td>Part II: Consent to long term follow-up registry</td>
<td>4</td>
</tr>
<tr>
<td>Part III: Consent to blood sample for CDC QA materials</td>
<td>1</td>
</tr>
</tbody>
</table>
Case # 1

- Day 2 of life: TREC = 0
- Day 12 of life: TREC = 0
- Flow cytometry: Lymphopenia
- Day 18 of life: Consent for SCID NGS
- Variants detected:
  - IL2RG p.Cys182Ser (c.545G>C) – hemizygous – Likely Pathogenic
  - MTHFD p.Leu521= (c.1561T>C) – heterozygous – Uncertain Significance
- Diagnosis: X-linked SCID
- Day 88 of life: Stem cell transplant
- Last office visit: “Very well appearing happy child”; “Continues to do well overall”; “Stable engraftment”; followed monthly
Case # 2

• Day 2 of life: TREC = 30
• Day 7 of life: TREC = 2
• Flow cytometry: Lymphopenia
• Day 22 of life: Consent for SCID NGS
• Variant detected:
  – \textit{DOCK8} \textit{p.Ala324Asp} (c.971C>A) – heterozygous – Uncertain Significance
• Patient moved to NC – being followed at Duke
• SCID panel (86-gene) performed at a commercial diagnostic laboratory confirmed our results. Also identified heterozygous VOUS in \textit{TAPBP} (not on our panel)
• Patient doing well, but continuing to be followed; low T-cell counts, functionally normal
Case # 3

- Day 21 of life: TREC = 190 (borderline – request repeat)
- Day 105 of life: TREC = 166 (borderline - referral)
- Day 115 of life: Consent for SCID NGS
- Flow cytometry: Not available at time of consent
- Variants detected:
  - NONE
- Day 135 of life: TREC = 398 (within acceptable limits)
- Case Closed as “No evidence of immune dysfunction”
Case # 4

- Day 2 of life: TREC = 164 (borderline – request repeat)
- Day 15 of life: TREC = 103 (referral)
- Day 29 of life: Consent for SCID NGS
- Flow cytometry: Not available at time of consent
- Variant detected: 
  - \textit{LIG4} p.Arg580Gln (c.1739G>A) – heterozygous – Uncertain Significance
- Day 30 of life: TREC = 1285 (within acceptable limits)
- Flow cytometry results: Normal
- Case Closed as “No evidence of immune dysfunction”
Case # 5

• Day 3 of life: TREC = 0
• Flow cytometry: “Unusual lymphocyte pattern” per physician
• Day 25 of life: Consent for SCID NGS
• Variants detected:
  – $RAG1$ p.Cys176Phe (c.527G>T) – homozygous – Likely Pathogenic
  – $IL7R$ p.Met10Val (c.28A>G) – heterozygous – Uncertain Significance
• Awaiting diagnosis and treatment plan from physician
## Turn-Around Time (TAT)

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>SCID NGS Panel TAT (listed on lab website)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneDx</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Invitae</td>
<td>10-21 days</td>
</tr>
<tr>
<td>Blueprint Genetics</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Cincinnati’s</td>
<td>42 days</td>
</tr>
<tr>
<td>Prevention Genetics</td>
<td>20-26 days</td>
</tr>
<tr>
<td>NYS Newborn Screening Lab</td>
<td>9 – 21 business days (average 14 days)</td>
</tr>
</tbody>
</table>

*Initial goal was to return NGS results in 7 calendar days*
Barriers to Faster TAT

- **NGS**
  - Day 1: DNA extraction/quantification
  - Day 2 – 3: Library preps
  - Day 4: Sequencing (run time ~24 hours)
  - Day 5: Bioinformatic analysis of sequence data
- **Sanger sequencing (starts Day 6)**
  - Confirmation of Pathogenic, Likely Pathogenic, and VOUS
  - NGS low coverage regions (<20X coverage)
- **Variant classification**
  - Database searching
  - Literature review
  - ACMG criteria
### Sanger Sequencing

<table>
<thead>
<tr>
<th>Metric</th>
<th>Count</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Variants Identified per Sample</td>
<td>62</td>
<td>(50-78)</td>
</tr>
<tr>
<td># of Variants Requiring Sanger Confirmation</td>
<td>5</td>
<td>(2-10)</td>
</tr>
<tr>
<td># of Regions with Low NGS Coverage (&lt;20X) Requiring Sanger Sequencing</td>
<td>29</td>
<td>(28-30)</td>
</tr>
</tbody>
</table>

Exploring ways to decrease TAT for Sanger Sequencing:
- NextGen PCR instrument – decreases 2 hour amplification to 20 minutes
- Reagents for faster PCR amplicon clean-up
Summary

• A likely causative genetic variant was identified for 2 of 5 infants tested using a 39-gene SCID NGS panel; a VOUS was identified in a 3rd infant

• Turn-around time for NGS results was comparable or faster than the TAT listed by several commercial diagnostic labs

• Enrollment into the consented SCID NGS study has been slow

• A meeting with the Specialty Care Center Directors and participating families is planned for September 2019
  – Discuss experience with the consent process
  – Discuss benefits of identification of genetic cause of immunodeficiency
  – Discuss benefits of genetic testing being performed by Newborn Screening laboratory
Acknowledgments:

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SCID Specialty Care Centers
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Women’s and Children’s Buffalo
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Mount Sinai
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Westchester Medical Center
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