Case Study: SCID/TREC/DBS/qPCR Monitoring

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“Newborn screening is an extraordinarily successful example of the application of advances in medical genetics to personal and public health benefit.”
Editorial, Nature Genetics, October 2010

April 25, 2016
APHL-CDC
**SCID: Severe Combined Immunodeficiency**

- Heterogeneous group of genetic disorders
- Defects of cellular and humoral immune responses
- Absence or very low T-cell counts
- Incidence: 1 / 40,000-75,000 newborns
- Clinical symptoms: first months of life (median age at Dx 4-7 months)
- Multiple severe viral, bacterial and fungal infections after birth
- Often fatal within first year of life
- Survival rate is 94% when treated with HSCT by 3.5 months of age
- **TREC (T-cell Receptor Excision Circles)** are DNA by-products of T-cell maturation in the thymus
- Low or absent TRECs in DBS likely indicates T-cell deficiency
Generation of excised DNA loops during gene rearrangement

Portion of gene being excised

(Rest of gene) — — — — — — (Rest of gene)

(Rest of gene) — — — — — — (Rest of gene)

Excised DNA fragment

Gene joins together to fill the excised gap

(Rest of gene) — — — — — — (Rest of gene)

Fragment ends join to form a DNA loop
δRec-ψJα TREC Is Ideal

- Created from the sequential rearrangements of the TCR α/δ locus
  - 70% of thymocytes that express α/β TCR will form this specific TREC

- Signal joint region of this TREC is flanked by a conserved region
  - Allows for universal primer design that will always detect this TREC

- Occurs late in maturation
  - Likely to generate a functional and diverse T cell repertoire
Real Time PCR

- Reporter/Quencher
- 5’ Nuclease Activity
- Probe Cleavage
- Sequence Specific
- Multiplex Capability
Cost-Effective and Scalable DNA Extraction Method from Dried Blood Spots.


Abstract

BACKGROUND: Dried blood spot (DBS) samples have been widely used in newborn screening (NBS) for the early identification of disease to facilitate the presymptomatic treatment of congenital diseases in newborns. As molecular genetics knowledge and technology progresses, there is an increased demand on NBS programs for molecular testing and a need to establish reliable, low-cost methods to perform those analyses. Here we report a flexible, cost-efficient, high-throughput DNA extraction method from DBS adaptable to small- and large-scale screening settings.

METHODS: Genomic DNA (g.DNA) was extracted from single 3-mm diameter DBS by the sequential use of red cell lysis, detergent-alkaline, and acid-neutralizing buffers routinely used in whole blood and plant tissue DNA extractions. We performed PCR amplification of several genomic regions using standard PCR conditions and detection methods (agarose gel, melting-curve analysis, TaqMan-based assays). Amplicons were confirmed by BigDye® Terminator cycle sequencing and compared with reference sequences.

RESULTS: High-quality g.DNA was extracted from hundreds of DBS, as proven by mutation detection of several human genes on multiple platforms. Manual and automated extraction protocols were validated. Quantification of g.DNA by Oligreen® fluorescent nucleic acid stain demonstrated a normal population distribution closely corresponding with white blood cell counts detected in newborn populations.

CONCLUSIONS: High-quality, amplifiable g.DNA is extractable from DBSs. Our method is adaptable, reliable, and scalable to low- and high-throughput NBS at low cost ($0.10/sample). This method is routinely used for molecular testing in the New York State NBS program.
SCID Screening Method

3.2mm punch → ~1000 DNA extractions/day → ~1200 RT-qPCR samples/day

Analysis
History of SCID Testing

- Automated assay developed and validated 12/2009-9/2010
- Submitted validation package to CLEP for approval on 9/08/2010
- CLEP and emergency regulation approved 9/27/2010
- SCID screening started 9/29/2010
- 1st “True SCID” baby detected 12/27/2010 (NICHD Support)
- Presumptive Positive (Borderline) category added 1/25/2011
- Commissioner of Health officially adds SCID to NSP panel 4/12/2011
Why Automate?

• Accommodate increased throughput

• Reduce repetitive stress injuries

• Address staffing shortages

• Increase reproducibility and consistency of results
Follow up of Infants ≥ 37 weeks Gestational Age with Positive NBS for SCID in NYS

Referral:
<150 TRECS/µL

Presumptive Positive/Borderline*:
151-199 TRECS/µL

Repeat NBS ASAP

< 200 TRECS/µL  ≥ 200 TRECS/µL

No further follow up

• NBS calls PMD & specialist @ Specialty Treatment Center (STC)
• Determine if child had cardiac surgery including thymectomy & if pre-thymectomy screen was negative

PMD contacts parent/guardian

• Child evaluated by PMD or specialist
• Check CBC, lymphocyte subsets, mitogens
• Repeat NBS card sent to Wadsworth Lab
• Specialist interprets tests & determines management plan as needed
Duplex Amplification Plots

TREC Amplification plot

Rnase p amplification plot
Overall PPV = 18.7%
Classic SCID PPV = 2.1%

Table 2 Patients with Abnormal Flow Cytometry and/or CBC

<table>
<thead>
<tr>
<th>Number of Infants</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Classic SCID: 2 X-linked SCID; 2 ADA deficiency; 1 JAK3-related SCID; 1 IL7Ra; 1 T-B+NK+ SCID, etiology unknown</td>
</tr>
<tr>
<td>1</td>
<td>T-B+NK+ SCID, etiology unknown, multiple congenital anomalies, expired</td>
</tr>
<tr>
<td>20</td>
<td>Idiopathic T cell lymphopenia of the newborn</td>
</tr>
<tr>
<td>18</td>
<td>Non-SCID, syndrome with T cell impairment: (1 chromosome 6p deletion; 3 Down syndrome, 1 chromosome 10p13 deletion, 1 CHARGE syndrome, 11 DiGeorge syndrome, 1 trisomy 18)</td>
</tr>
<tr>
<td>7</td>
<td>Non-SCID, secondary T cell lymphopenia other than pre-term (1 leukemia, 1 hypoplastic left heart syndrome, 1 cardiac surgery with thymectomy, 1 gastroschisis, 1 congenital diaphragmatic hernia, 1 presumed metabolic disorder, 1 Dandy Walker syndrome)</td>
</tr>
<tr>
<td>6</td>
<td>Low CD19 of unknown etiology</td>
</tr>
<tr>
<td>4</td>
<td>Other: 2 neutropenia; 1 low absolute lymphocyte count, 1 hypogammaglobulinemia</td>
</tr>
</tbody>
</table>

New York State Newborn Screening Laboratory
SCID Algorithm

DNA Analysis for T-Cell Receptor Excision Circles (TRECs) and RNase P (control amplicon)

> 200 TRECs/μL blood and RNase P Cq < 35

≤ 200 TRECs/μL blood and RNase P Cq < 35*

Retest in Duplicate

≥ 125 and ≤ 200 TRECs/μL blood and RNase P Cq < 35 and Gestational Age ≥ 37 weeks

< 125 TRECs/μL blood and RNase P Cq < 35 and Gestational Age ≥ 37 weeks

≤ 200 TRECs/μL blood and RNase P Cq < 35 and Gestational Age < 37 weeks

TRECs/μL blood = 0 and RNase P Cq < 35 for All Gestational Ages

SCREEN NEGATIVE

BORDERLINE REPEAT REQUESTED

BORDERLINE REPEAT REQUESTED When gestational age is ≥ 37 weeks

SCREEN POSITIVE

*If RNase P Cq is ≥ 35, a repeat specimen is requested due to possible DNA extraction failure.
<table>
<thead>
<tr>
<th>Race/ethnicity</th>
<th>Proportion screen negative infants %</th>
<th>Proportion screen positive infants %</th>
<th>N infants&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean TREC copies/μl (95 % confidence interval)&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>White</td>
<td>48.4</td>
<td>29.6</td>
<td>31,315</td>
<td>1,885 (1,873–1,898)</td>
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<tr>
<td>Black</td>
<td>16.4</td>
<td>41.6</td>
<td>10,227</td>
<td>1,649 (1,628–1,669)</td>
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<tr>
<td>Hispanic</td>
<td>17.7</td>
<td>14.7</td>
<td>9,899</td>
<td>1,844 (1,821–1,866)</td>
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<tr>
<td>Asian</td>
<td>8.0</td>
<td>2.4</td>
<td>5,606</td>
<td>1,861 (1,833–1,888)</td>
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<tr>
<td>Other</td>
<td>9.5</td>
<td>11.3</td>
<td>6,512</td>
<td>1,819 (1,793–1,846)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Includes screen negative (not referred) and screen positive infants tested over a 3 month period
SEQUENCING FORWARD PRIMER
SEQUENCING REVERSE PRIMER
ASSAY FORWARD PRIMER
ASSAY REVERSE PRIMER
PROBE

GAAGAAGAAGGCTCTGTCATAGTGTAATAACATTTTGTATCTTATTCATTGTCTTCTCATCCCTGAATA
TACACTCTGCTCTCTCTATCTCTGCTCTGAAAGGCGAGAAAGAGGGCAGCCCTCTCCAAGGCAAAATG
GGGCTCCTGTGGGAACAGAGGGGTGCCCTGTCAACAAAGGTGATGCCACATCCCTTTCAACCATGC
TGACACCTCTCGTTTTTGTAAAGG
CCCACCTCTGTG/CACGGTATGCCATAGGACCTGCAACCCGT
GCCTAAACCCTGCAGCTGACGGGCACGGGCCCTGTCTGCTTCTCATCCCGTTCTCACGAGTTGCAGATAAA
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<th>Mutation</th>
<th>SNP</th>
<th>%</th>
<th>INITIAL AVE</th>
<th>QS AVE</th>
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<tr>
<td>c.999390</td>
<td>C</td>
<td>42.5%</td>
<td>1283</td>
<td>1409</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>41.5%</td>
<td>117</td>
<td>595</td>
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<tr>
<td></td>
<td>Y</td>
<td>8.0%</td>
<td>559</td>
<td>1220</td>
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<tr>
<td></td>
<td>?</td>
<td>5.0%</td>
<td>474</td>
<td>702</td>
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<tr>
<td>c.999396</td>
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<td>714</td>
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<td>52</td>
<td>646</td>
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<tr>
<td></td>
<td>Y</td>
<td>0.1%</td>
<td>58</td>
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<td></td>
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<td>DEL</td>
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<td>INS/DEL</td>
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<td>76</td>
<td>236</td>
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<tr>
<td>POSS INS/DEL</td>
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<td>11.0%</td>
<td>260</td>
<td>683</td>
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<td>FAILURES</td>
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<td>3.0%</td>
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<td>12</td>
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<tr>
<td>QUESTIONABLES</td>
<td></td>
<td>9.5%</td>
<td>336</td>
<td>537</td>
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<td>ALL 12 PLATES</td>
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<td>100.0%</td>
<td>665</td>
<td>984</td>
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<table>
<thead>
<tr>
<th>rs76132819 (%)</th>
<th>rs79211180 (%)</th>
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<tr>
<td>Race</td>
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<tr>
<td>White</td>
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<tr>
<td>Black</td>
<td>30.0</td>
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<tr>
<td>Hispanic</td>
<td>54.0</td>
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<tr>
<td>Asian</td>
<td>85.0</td>
</tr>
<tr>
<td>Other</td>
<td>24.0</td>
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</table>
Liquid Handling System

Liquid Handling System is capable of 1200 DNA extractions per 8 hour day.
IN THE BEGINNING

- Extract DNA
- Run real time assay for TRECs
  - Use RNaseP to determine quality of extraction
- Calculate number of TRECs per µl of blood
- >200 TreCs/ µl of blood sample is WAL
- ≤200 TreCs/ µl of blood retest in duplicate
IN THE BEGINNING

- After retest:
  - Average of 3 tests > 200 Trecs = WAL
  - 2 retests are greater than 200, but average is less than 200 = WAL
  - ≤ 200 Trecs, infant is >37 weeks gestation age = Referral
  - ≤ 200 Trecs, infant is <37 weeks gestation age = Rptpre
    - As for a repeat specimen when infant has reached 37 weeks gestational age
First 3.5 months of screening

- Screened 63,565 infants
- 100 Rptpre
- 127 Referrals 36/month
- 0.19% Referral rate
100 Repeat Pre

- 88 individual infants
- 9 expired due to prematurity complications
- Received repeats on all others except 1
- All went WAL (some took multiple samples) except 3 referrals
  - All 3 diagnosed as No Disease
127 Referrals

- Received 85 (67%) repeats
  - 47 >200 on repeat
  - 38 ≤200 on repeat
- 73 (86%) closed as No Disease
- 3 DiGeorge syndrome
- 1 CHARGE syndrome
- 1 syndrome with T cell impairment
- 3 Idiopathic T cell lymphopenia
- 2 Secondary T cell lymphopenia
- 1 other
127 Referrals

- 4 expired with no diagnosis
- 2 lost to follow up
- 2 diagnosed with SCID
  - 1 was born in May diagnosed with SCID
    - Sent in sample for “confirmatory testing”
    - No detectable TREC
  - 1 infant had 3 samples sent to NBS
  - All 3 had no detectable TREC


2011 CHANGES

- Added a Presumptive Positive Category (PP) for full term infants
  - Starting in January samples with <150 TRECs became Referrals
  - Samples with $\geq 150 \leq 200$ TRECs became PP
    - Request repeat specimen ASAP
  - In July the PP category was increased to $\geq 125 \leq 200$ TRECs
2011

- Screened 243,183 infants
- 279 Rptpre
- 330 PP
- 201 Referrals 17/month
- 0.08% Referral rate
  - 0.21% without PP
2011 Repeat Pre

- 279 Repeat Pre
- 254 individual infants
- 22 expired due to prematurity complications
- Received repeats on all others except 6
- Most went WAL (some took multiple samples)
  - 10 samples became PP
    - 1 expired
    - 1 DiGeorge
    - 1 Lost to follow up
    - 7 No disease
2011 Repeat Pre

- 6 became Referrals
  - 2 closed as Idiopathic T cell Lymphopenia resolved
    - Left over from pre maturity
  - 3 No Disease
  - 1 SCID
    - Baby expired before confirmation
    - Baby born at 33 weeks
    - Autopsy showed thymic atrophy
    - Tested 18 known SCID genes post mortem, no mutations found
    - Flow results not available to me
    - No detectable TRECs for us 3 times
  - Changed algorithm
    - All infants with non detectable TRECs are referred regardless of gestational age
2011 PP SAMPLES

- 330 PP Samples
- 5 expired
- Received repeats on all others except 19
- Most went WAL ( > 200 TRECS )
- 22 samples became Referrals ( ≤ 200 )
  - 1 T Cell Lymphopenia resolved
  - 2 DiGeorge
  - 1 Idiopathic T cell lymphopenia
  - 1 Secondary T cell lymphopenia ( infant has dandy walker syndrome )
  - 17 No disease
2011 Referrals

- 201 Referrals
- 133 No disease
- 5 B cell deficiency
- 7 Idiopathic T cell lymphopenia
- 8 Secondary T cell lymphopenia
- 4 Syndrome with T cell Impairment
- 16 expired
- 6 lost to follow up
2011 Referrals

- 1 Down Syndrome
- 10 DiGeorge
- 1 “other”
- 3 SCID
  - ADA SCID
    - No detectable TRECsto on 2 samples
  - IL2RG
    - No detectable TRECsto on 2 samples
  - Gene unknown
    - No detectable TRECsto on 3 samples
    - This is the baby that was premature
2012 Referrals

- 249 Referrals
  - 182 No Disease
  - 5 SCID
  - 1 Leaky SCID
  - 15 Idiopathic T cell Lymphopenia
  - 8 Secondary T cell Lymphopenia
  - 2 Syndrome with T cell impairment
  - 10 DiGeorge
  - 3 Down Syndrome
  - 3 B cell Deficiency
  - 3 “other”
  - 9 expired
  - 1 parental refusal
  - 7 lost to follow up

2013 Referrals

- 233 Referrals
  - 187 No Disease
  - 5 SCID
  - 1 Variant SCID
  - 12 Idiopathic T cell Lymphopenia
  - 7 Secondary T cell Lymphopenia
  - 2 Syndrome with T cell impairment
  - 8 DiGeorge
  - 1 Down Syndrome
  - 4 B cell Deficiency
  - 3 “other”
  - 1 expired
  - 1 parental refusal
  - 5 lost to follow up
SNPs and Moving the Lab

- Extensive sequencing showed SNPs in the TREC probe region
  - Sequenced 1079 Ref, PP and WAL samples
  - 41% were homozygous for c.999390 and 8% were heterozygous
- Learned we were moving the DAI
- Redesigned TREC probe
- New liquid handlers
- New Real Time Machines
1 YEAR OF SCREENING AT DAI WITH NEW PROBES/EQUIPMENT

- March 2014 to March 2015
- Screened 240,728 infants
- 277 Rptpre
- 182 PP
- 65 Referrals 5/month
- 0.03% Referral rate
  - 0.1% without PP
POST MOVE REPEAT PRE

277 Repeat Pre
- 240 individual infants
- 28 expired/no repeat
- 12 became PP
- 9 became Referrals
  - 3 No Disease
  - 3 Secondary T cell
  - 1 DiGeorge
  - 1 “other”
  - 1 expired
**POST MOVE PP**

- 182 PP
- 11 expired/no repeat
- 18 became Referrals
  - 9 No Disease
  - 1 Variant SCID
  - 1 Idiopathic T cell
  - 3 Secondary T cell
  - 2 DiGeorge
  - 1 B cell Deficiency
  - 1 “other”
POST MOVE REFERRALS

65 Referrals

- 26 expired/no repeat
- 25 No Disease
- 4 SCID
- 5 Idiopathic T cell Lymphopenia
- 2 Secondary T cell Lymphopenia
- 1 Syndrome with T cell impairment
- 9 DiGeorge
- 1 B cell Deficiency
- 1 “other”
- 7 expired
- 2 lost to follow up
<table>
<thead>
<tr>
<th></th>
<th># of infants screened</th>
<th># of Referrals</th>
<th># of PPs</th>
<th># of Rptpre</th>
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<tr>
<td>2011</td>
<td>243,183</td>
<td>201</td>
<td>330</td>
<td>279</td>
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<tr>
<td>2012</td>
<td>242,412</td>
<td>249</td>
<td>543</td>
<td>323</td>
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<td>2013</td>
<td>239,410</td>
<td>233</td>
<td>554</td>
<td>320</td>
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<tr>
<td>Post Move</td>
<td>240,728</td>
<td>65</td>
<td>182</td>
<td>277</td>
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## Yearly Averages

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<th>Move</th>
<th>Pre</th>
<th>Move</th>
<th>Post</th>
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<td>Referrals</td>
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<td>PPs</td>
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<td>Referral Rate</td>
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<td>PP Rate</td>
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<td>.07</td>
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<tr>
<td>Rptpre Rate</td>
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<td>.13</td>
<td></td>
<td>.11</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>2012</td>
<td>2013</td>
<td>Post move</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>No Disease</strong></td>
<td>133 (66%)</td>
<td>182 (73%)</td>
<td>187 (80%)</td>
<td>25 (38%)</td>
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<tr>
<td><strong>SCID</strong></td>
<td>3 (1.5%)</td>
<td>5 (2.5%)</td>
<td>5 (2.5%)</td>
<td>4 (6%)</td>
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<tr>
<td><strong>Leaky SCID</strong></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>Variant SCID</strong></td>
<td>0</td>
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<td>1</td>
<td>0</td>
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<tr>
<td><strong>DiGeorge</strong></td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>9</td>
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<tr>
<td><strong>Secondary T Cell</strong></td>
<td>8 (4%)</td>
<td>8 (3%)</td>
<td>7 (3%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td><strong>Idiopathic T Cell</strong></td>
<td>7 (3.5%)</td>
<td>15 (6%)</td>
<td>12 (5%)</td>
<td>5 (3%)</td>
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<tr>
<td><strong>Syndrome with T cell Impairment</strong></td>
<td>4</td>
<td>2</td>
<td>2</td>
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<td>5</td>
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<tr>
<td><strong>Down Syndrome</strong></td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0</td>
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NBSO6-A

Newborn Blood Spot Screening for Severe Combined Immunodeficiency by Measurement of T-cell Receptor Excision Circles; Approved Guideline
1. Typical SCID is characterized by < 300 autologous T-cells/µL of blood. Such infants would need emergent treatment by HCT, enzyme, or gene therapy.

2. Leaky SCID or Omenn syndrome (OS) is defined as hypomorphic defects in known SCID genes with 300–1500 autologous T-cells/µL with no evidence of maternal engraftment. These patients require HCT, enzyme, or gene therapy. NOTE: OS includes the following clinical and immunological features in addition to the hypomorphic gene defect: erythroderma, hepatosplenomegaly, eosinophilia, elevated immunoglobulin E (IgE), and restricted TCR diversity (oligocloneity) of T-cells even if there may be normal CD3 T-cell counts or T-cell lymphocytosis.

3. Variant SCID is defined as lymphopenia, usually 300–1500 autologous T-cells/µL, functional T-cell impairment, and no known mutation. These patients may or may not need HCT. They are usually identified by NBS.

4. Syndromes with primary T-cell lymphopenia (CD3 T-cells ≤ 1500 cells/µL) or T-cell impairment include DGS, CHARGE syndrome (Coloboma of the eye, Heart anomalies, choanal Atresia, Retardation of growth and development, Genitourinary anomalies, Ear anomalies and deafness), Jacobsen, RAC2 defect, DOCK8 deficiency, and ataxia telangiectasia. Some of these clinical conditions (e.g., DGS) will require thymus transplant or HCT, while others may not.

5. Secondary T-cell lymphopenia (CD3 T-cells ≤ 1500 cells/µL) not due to prematurity alone includes patients with intestinal lymphangiectasia, anasarca, gastrochisis, third-spacing, gastrointestinal atresia, cardiac surgery with or without thymectomy, congenital heart defects, congenital infection by HIV, and neonatal leukemia.

6. Preterm infants may have T-cell lymphopenia (CD3 T-cells ≤ 1500 cells/µL) and no other recognizable disorder. T-cell lymphopenia associated with prematurity often resolves with age.

In newborns with typical SCID (category 1), TREC will generally be undetectable or clearly below the expected range for healthy term infants. Newborns with conditions in the remaining categories may also have low or undetectable TREC, but in some cases their TREC values may fall within the expected range for healthy term infants.
- SC001: Expired, no diagnosis
- SC010: Disease, SCID, gene not specified
- SC011: Disease, SCID, IL2Rγ (K-Linked) related
- SC012: Disease, SCID, ADA related
- SC013: Disease, SCID, IL7Rα related
- SC014: Disease, JAK3 related
- SC015: Disease, SCID, gene specified, not IL2Rγ, ADA, IL7Rα or JAK3
- SC020: Disease, Leaky SCID, Omenn syndrome, gene not specified
- SC021: Disease, Leaky SCID, Omenn syndrome, gene specified
- SC022: Disease, Non-SCID, Syndrome with T cell impairment
- SC023: Disease, Non-SCID, DiGeorge Syndrome/Chromosome 22q11 deletion...
- SC024: Disease, Non-SCID, Down Syndrome/Trisomy 21
- SC025: Disease, Non-SCID, CHARGE syndrome
- SC026: Disease, Non-SCID, Secondary T cell lymphopenia
- SC027: Disease, not detected by newborn screening
- SC028: Disease, Variant SCID
- SC029: Disease, not on NBS panel
- SC030: Possible disease
- SC040: No disease
- SC041: No disease, transient deficiency due to prematurity
- SC042: No disease, no longer a referral due to algorithm change
- SC043: No disease, idiopathic T cell lymphopenia - resolved
- SC050: Lost to follow-up
- SC054: Lost to follow-up, infant in care
- SC059: Lost to follow-up, parental refusal
- SC070: Other
- SC071: Other, B cell deficiency
TRUE SCID CASE

- Male
- Full Gestation
- Normal birthweight
- DOB 10/22/15
- Initial Sample returned no detectable TREC
- Second sample 1 week later returned no detectable TREC
- Third sample 3 weeks later returned no detectable TREC
NEWBORN SCREENING PROGRAM
NEW YORK STATE DEPARTMENT OF HEALTH
WADSWORTH CENTER, BIGGS LABORATORY, P.O. BOX 509
ALBANY, NY 12201-0509
PHONE: 518.473.7552 FAX: 518.474.0405
EMAIL: nbsinfo@health.state.ny.us
WEBSITE: http://www.wadsworth.org/newborn/

Newborn Information:
Name: [redacted]
DOB: 10/22/2014

SEVERE COMBINED IMMUNODEFICIENCY DIAGNOSTIC FORM

PLEASE INDICATE A DIAGNOSIS:
[ ] No evidence of immune dysfunction
[X] Severe combined immunodeficiency, specify gene and mutations, if Available
[ ] Idiopathic T cell lymphopenia
[ ] DiGeorge syndrome
[ ] Other, specify

[X] CONFIRMATORY TESTING IS ATTACHED

Physician Signature: [signature] Date: 11/18/2014

Print Name: Elizabeth Feuille, MD  Facility/practice: Mt. Sinai

SPECIALTY CARE CENTER
Charlotte Cunningham-Rundles, MD
Dept. Medicine - Allergy and Immunology
Mount Sinai Medical Center
5 East 98th Street, 11th Floor, Box 1089
New York, NY 10029
<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Range</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells</td>
<td>0</td>
<td>53-84</td>
<td>%</td>
</tr>
<tr>
<td>CD4 T cells</td>
<td>0</td>
<td>35-65</td>
<td>%</td>
</tr>
<tr>
<td>CD8 T cells</td>
<td>15</td>
<td>12-28</td>
<td>%</td>
</tr>
<tr>
<td>B cells</td>
<td>58</td>
<td>6-32</td>
<td>%</td>
</tr>
<tr>
<td>NK cells</td>
<td>40</td>
<td>4-18</td>
<td>%</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>98</td>
<td>0.7-3.5</td>
<td>cells/μL</td>
</tr>
<tr>
<td>CD4 T count</td>
<td>0</td>
<td>2500-5500</td>
<td>cells/μL</td>
</tr>
<tr>
<td>CD8 T count</td>
<td>10</td>
<td>1600-4000</td>
<td>cells/μL</td>
</tr>
<tr>
<td>B cell count</td>
<td>524</td>
<td>560-1700</td>
<td>cells/μL</td>
</tr>
<tr>
<td>NK count</td>
<td>358</td>
<td>720-2600</td>
<td>cells/μL</td>
</tr>
</tbody>
</table>

**Comments**

Immunogenetics Laboratory, New York Presbyterian Hospital - Columbia University Medical Center, Vanderbilt Clinic, 15-204 630 W 168th Street, New York, NY 10032
Phone: 212-305-3897, Electronic Report - Signed copy available in lab. Reviewed by Dr. George Vidal.

**Collection time:** 2014-10-31 04:00  **Received time:** 2014-10-31 09:42  **Result time:** 2014-10-31 13:33  
**Last updated:** 2014-10-31 13:34  
**Status:** Final, Acnos: 21430490931, Performing Lab: NYP_CUMC

(Additional text and signature)
TRUE SCID

- Male
- Full Gestation
- Normal birthweight
- DOB 5/17/15
- Initial Sample returned no detectable TREC
- Second sample 2 week later returned no detectable TREC
- Third sample 1 weeks later returned no detectable TREC
ISSUES WITH CLOSING CASES

- No closing meetings/diagnosis not being checked by supervisors
- Incorrect diagnosis put into neometrics
  - Not what DX form states
  - When DX form states “other” choosing other (SCID70)diagnostic code
    - Should only be used when no other codes fit
- No continuity in closing cases
- No checking of the flow data
Flow data shows borderline T cells.
Reason unknown.

<table>
<thead>
<tr>
<th>Marker Name</th>
<th>Result</th>
<th>Unit</th>
<th>Normal Range</th>
<th>H/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROCESSED DATE &amp; TIME</td>
<td>1-7-15 1:30PM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% T CELLS (CD3)</td>
<td>15</td>
<td>%</td>
<td>60-85</td>
<td>L</td>
</tr>
<tr>
<td>ABSOLUTE T CELLS (CD3)</td>
<td>957</td>
<td>CELLSUL</td>
<td>2300-7000</td>
<td></td>
</tr>
<tr>
<td>% SUPPRESSOR (CD8)</td>
<td>4</td>
<td>%</td>
<td>9-23</td>
<td>L</td>
</tr>
<tr>
<td>ABSOLUTE SUPPRESSOR (CD8)</td>
<td>255</td>
<td>CELLSUL</td>
<td>400-1700</td>
<td></td>
</tr>
<tr>
<td>% HELPER (CD4)</td>
<td>11</td>
<td>%</td>
<td>41-68</td>
<td>L</td>
</tr>
<tr>
<td>ABSOLUTE HELPER (CD4)</td>
<td>702</td>
<td>CELLSUL</td>
<td>1700-5300</td>
<td></td>
</tr>
<tr>
<td>H/S RATIO</td>
<td>2.75</td>
<td>RATIO</td>
<td>1.3-6.3</td>
<td></td>
</tr>
<tr>
<td>% NK CELLS (CD16+56)</td>
<td>41</td>
<td>%</td>
<td>3-23</td>
<td>H</td>
</tr>
<tr>
<td>ABSOLUTE NK CELLS (CD16+56)</td>
<td>2616</td>
<td>CELLSUL</td>
<td>200-1400</td>
<td></td>
</tr>
<tr>
<td>% B CELLS (CD19)</td>
<td>42</td>
<td>%</td>
<td>4-26</td>
<td>H</td>
</tr>
<tr>
<td>ABSOLUTE B CELLS (CD19)</td>
<td>2680</td>
<td>CELLSUL</td>
<td>600-1900</td>
<td></td>
</tr>
</tbody>
</table>

We have not established institutional age-adjusted reference values for pediatric peripheral blood lymphocyte subsets; rather we refer to ranges published in the literature. Source of the pediatric reference range:
| SCID10 | Disease, SCID, gene not specified | The baby has SCID and we don’t know what gene/mutation is the cause. Use this code if we don’t know if the baby was tested for mutations or if the baby was tested but we don’t know what mutations they have. | - SCID  
- SCID, gene unknown  
- SCID, genetics not done |
|--------|----------------------------------|------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| SCID11 | Disease, SCID, IL2RG (X-Linked)-related | Baby has SCID because of an IL2RG (interleukin 2 receptor gamma) mutation. | - Interleukin 2 receptor gamma deficiency/mutation  
- IL2RG deficiency/mutation  
- IL2RG-SCID |
| SCID12 | Disease, SCID, ADA-related | Baby has SCID because of an ADA (adenosine deaminase) mutation. | - ADA deficiency/mutation  
- Adenosine deaminase deficiency/mutation  
- ADA-SCID |
| SCID13 | Disease, SCID, IL7Ra-related | Baby has SCID because of an IL7Ra (interleukin 7 receptor alpha) mutation. | - IL7Ra deficiency/mutation  
- Interleukin 7 receptor alpha deficiency/mutation  
- IL7Ra-SCID |
| SCID14 | Disease, JAK3-related | Baby has SCID because of a JAK3 (Janus kinase 3) mutation. | - JAK3 deficiency/mutation  
- Janus kinase 3 deficiency/mutation  
- JAK3-SCID |
| SCID15 | Disease, SCID, gene specified, not IL2RG, ADA, IL7Ra or JAK3 | The baby has SCID and was tested for mutations in IL2RG, ADA, IL7Ra and JAK3. No mutations in any of these 4 genes (IL2RG, ADA, IL7Ra and JAK3) was found. | - Gene is specified but is not one that we have a diagnosis code for |
| SCID20 | Disease, Leaky SCID, Omenn syndrome, gene not specified | Baby has Omenn syndrome, but we don’t know what mutation the baby has. | - Leaky SCID  
- Omenn syndrome  
- Absolute T cells (ABS T cells (CD3)) are 300-1500, mitogens were tested and are abnormal. |
| SCID21 | Disease, Leaky SCID, Omenn syndrome, gene specified | Baby has Omenn syndrome and the mutation causing the syndrome is known. Omenn syndrome is often due to mutations in: | - Leaky SCID  
- Omenn syndrome  
- Omenn syndrome with RAG1 deficiency/mutation  
- Omenn syndrome with RAG2 deficiency/mutation  
- Omenn syndrome with DCLRE1C/Artemis deficiency/mutation  
- Absolute T cells (ABS T cells (CD3)) are 300-1500, mitogens were done and are abnormal |
| SCID22 | Disease, Non-SCID, Syndrome with T cell impairment | Infant has low T-cells and a syndrome other than DiGeorge (SCID23), Down’s (SCID24), CHARGE (SCID25), or Omenn (SCID20, SCID21) syndrome. | - VACTERL or VATER  
- Opitz syndrome/GBB  
- Trisomy 13/Patau syndrome  
- Trisomy 18/Edwards syndrome  
- Cartilage hair hypoplasia  
- Ring chromosome 17  
- 6p deletion syndrome  
- 17q duplication syndrome  
- Ataxia telangiectasia  
- Jacobsen syndrome  
- Multiple genetic defects/multiple congenital anomalies/MCA/cytogenetic abnormalities (consult supervisor) |
<table>
<thead>
<tr>
<th>Absolute T cell count</th>
<th>&gt;1500</th>
<th>300-1500</th>
<th>&lt;300</th>
<th>ABS CD3; Absolute CD3; ABS CD3+; Absolute CD3+</th>
<th>The total number of T cells per cubic millimeter of blood (mm³), measured using flow cytometry.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitogens – phytohaemagglutinin test (“PHA”)</td>
<td>Varies by lab</td>
<td>Varies by lab</td>
<td>Varies by lab</td>
<td>PHA</td>
<td>Each baby needs at least 3 different mitogens tested. If 2 out of the 3 mitogen tests are in the abnormal range, the mitogens are considered to be abnormal.</td>
</tr>
<tr>
<td>Mitogens – pokeweeds mitogen test (“PWM”)</td>
<td>Varies by lab</td>
<td>Varies by lab</td>
<td>Varies by lab</td>
<td>PWM</td>
<td>Mitogen tests help determine whether the baby’s immune system is functional/can work properly.</td>
</tr>
<tr>
<td>Mitogens – Candida test (“CA”)</td>
<td>Varies by lab</td>
<td>Varies by lab</td>
<td>Varies by lab</td>
<td>CA</td>
<td></td>
</tr>
<tr>
<td>Mitogens – tetanus toxin test (“TT”)</td>
<td>Varies by lab</td>
<td>Varies by lab</td>
<td>Varies by lab</td>
<td>TT</td>
<td></td>
</tr>
<tr>
<td>Mitogens – concanavalin A test (“CONA”)</td>
<td>Varies by lab</td>
<td>Varies by lab</td>
<td>Varies by lab</td>
<td>CON; CONA</td>
<td></td>
</tr>
</tbody>
</table>

If there are questions or something is confusing – ask a supervisor.
Doctor stated they believed baby to have SCID
DX form sent in stating idiopathic T cell lymphopenia
Cases closed as secondary T cell lymphopenia

In April 2015 additional sample was sent in for testing because baby was a candidate for transplant and wanted TREC values
Sample had no detectable TREC
In August began having case review meetings.
Noticed DX form and neometrics had different diagnosis.
Noticed Dr initially thought SCID
Because idiopathic was checked and flow was borderline, decided to call and try to get additional information
Flow performed in March showed ABS T cells of 138, well within the SCID category.

Baby has confirmed Purine Nucleoside Phosphorylase (PNP) deficiency
- Metabolic disorder which can cause SCID
- Extremely rare, <50 cases known worldwide
- Doctor stated that it is pretty similar to ADA SCID
- Baby was preparing to undergo transplant

Case closed as SCID15 (known gene)

Concern over >200 TREC value?
Referral 868

- DOB 5/19/14
- Initial sample taken 6/21/14  Average of 49 TREC's (0,57,91) REF
- Repeat Specimen taken 6/2 UNS
- 6/9 received 2 sets of flow data with a DX of SCID
  - 6/4 ABS T 74
  - 6/5 ABS T 75
Repeat taken 6/23  Average of 167 TREC$s
RPTREF
  • Trec values :  228,307,0,224,78

On 7/2 received additional flow data

6/9  ABS T 191
6/23 ABT T 428
Closed as SCID
Last flow above SCID range, multiple TREC values above 200
Figure 1. Simultaneous detection of TREC (blue), KREC (orange) and RNaseP (green) in a Triplex Assay from 348 DBS-derived normal controls (A) and 8 DBS-derived positive controls (B). RNaseP was used as an amplification control. All qPCR reactions were run on a QuantStudio 12K Flex system (Life Technologies). Taqpath Master Mix with Mustang Purple as the passive reference dye was utilized with our custom Taqman Probes: FAM-TREC-MGBNFQ, VIC-KREC-QSY and ABY2.0-RNaseP-QSY (Life Technologies).
<table>
<thead>
<tr>
<th>Gene</th>
<th>HGNC Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA</td>
<td>ADENOSINE DEAMINASE</td>
</tr>
<tr>
<td>AK2</td>
<td>ADENYLATE KINASE 2</td>
</tr>
<tr>
<td>ATM</td>
<td>ATM SERINE/THREONINE KINASE</td>
</tr>
<tr>
<td>BLNK</td>
<td>B-CELL LINKER</td>
</tr>
<tr>
<td>BTK</td>
<td>BRUTON AGAMMAGLOBULINEMIA TYROSINE KINASE</td>
</tr>
<tr>
<td>CD3D</td>
<td>CD3d MOLECULE, DELTA (CD3-TCR complex)</td>
</tr>
<tr>
<td>CD3E</td>
<td>CD3e MOLECULE, EPSILON (CD3-TCR complex)</td>
</tr>
<tr>
<td>CD3G</td>
<td>CD3g MOLECULE, GAMMA (CD3-TCR complex)</td>
</tr>
<tr>
<td>CD247</td>
<td>CD247 MOLECULE</td>
</tr>
<tr>
<td>CD40LG</td>
<td>CD40 LIGAND</td>
</tr>
<tr>
<td>PTPRC</td>
<td>PROTEIN-TYROSINE PHOSPHATASE, RECEPTOR-TYPE, C</td>
</tr>
<tr>
<td>CHD7</td>
<td>CHROMODOMAIN HELICASE DNA BINDING PROTEIN 7</td>
</tr>
<tr>
<td>CORO1A</td>
<td>CORONIN ACTIN BINDING PROTEIN 1A</td>
</tr>
<tr>
<td>DCLRE1C</td>
<td>DNA CROSS LINK REPAIR 1C</td>
</tr>
<tr>
<td>DKC1</td>
<td>DYSKERA TOSIS CONGENITA 1, DYSKERIN</td>
</tr>
<tr>
<td>DOCK2</td>
<td>DEDICATOR OF CYTOKINESIS 2</td>
</tr>
<tr>
<td>DOCK8</td>
<td>DEDICATOR OF CYTOKINESIS 8</td>
</tr>
<tr>
<td>FOXN1</td>
<td>FORKHEAD BOX N1</td>
</tr>
<tr>
<td>GATA2</td>
<td>GATA BINDING PROTEIN 2</td>
</tr>
<tr>
<td>IGHM</td>
<td>IMMUNOGLOBULIN HEAVY CHAIN CONSTANT MU</td>
</tr>
<tr>
<td>IL2RG</td>
<td>INTERLEUKIN 2 RECEPTOR, GAMMA</td>
</tr>
<tr>
<td>IL7R</td>
<td>INTERLEUKIN 7 RECEPTOR</td>
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<tr>
<td>JAK3</td>
<td>JANUS KINASE 3</td>
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<tr>
<td>LIG4</td>
<td>LIGASE IV DNA ATP-DEPENDENT</td>
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<tr>
<td>MTHFD1</td>
<td>METHYLENETETRAHYDROFOLATE DEHYDROGENASE-1</td>
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<td>MTR</td>
<td>5-METHYLTETRAHYDROFOLATE HOMOCYSTEINE METHYLTRANSFERASE</td>
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<td>NHEJ1</td>
<td>NONHOMOLOGOUS END JOINING FACTOR 1</td>
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<tr>
<td>NBN</td>
<td>NIBRIN</td>
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<tr>
<td>PNP</td>
<td>PURINE NUCLEOSIDE PHOSPHORYLASE</td>
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<tr>
<td>PRKDC</td>
<td>PROTEIN KINASE DNA ACTIVATED CATALYTIC POLYPEPTIDE</td>
</tr>
<tr>
<td>RAC2</td>
<td>RAS-RELATED C3 BOTULINUM TOXIN SUBSTRATE 2</td>
</tr>
<tr>
<td>RAG1</td>
<td>RECOMBINATION ACTIVATING GENE 1</td>
</tr>
<tr>
<td>RAG2</td>
<td>RECOMBINATION ACTIVATING GENE 2</td>
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<tr>
<td>RMRP</td>
<td>RNA COMPONENT OF MITOCHONDRIAN RNA PROCESSING ENDORIBONUCLEASE</td>
</tr>
<tr>
<td>SLC46A1</td>
<td>SOLUTE CARRIER FAMILY 46 (folate transporter), MEMBER 1</td>
</tr>
<tr>
<td>STAT5B</td>
<td>SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 5B</td>
</tr>
<tr>
<td>TBX1</td>
<td>T-BOX 1</td>
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<tr>
<td>WAS</td>
<td>WISKOTT-ALDRICH SYNDROME</td>
</tr>
<tr>
<td>ZAP70</td>
<td>ZETA CHAIN (TCR) ASSOCIATED PROTEIN KINASE 70kDa</td>
</tr>
</tbody>
</table>