The Challenges of Severe Combined Immunodeficiency Screening in a Two Screen State

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Maryland SCID screening background

- We use the **In situ TREC Assay** adapted from the CDC
  - Dried blood spot punches are washed in QIAGEN Solution 2
  - Quanta qPCR Toughmix is added to the washed punches
  - DNA present in the punch is used as template for qPCR
- Duplex Taqman qPCR reaction
  - **TREC (T cell receptor excision circles)**: a marker for T cells in the blood spot
  - **RNase P**: Internal positive control for qPCR reaction quality
- “Ct” based cutoffs used
  - Threshold cycle (Ct) is inversely proportional to the starting material of the qPCR reaction

*Higher Ct values indicate less starting material or a lower quality qPCR reaction*
Maryland SCID screening background

• Live screening started on April 1\textsuperscript{st}, 2016
  • All specimens received have been screened for SCID
  • 109,276 births (207,510 specimens)
• Two sample state (73,000 births per year)
  \textcolor{red}{NB} specimens collected < 7 days of life
  \textcolor{blue}{SUB} specimens collected ≥ 7 days of life
• Additional specimens from military bases through a contract with the Department of Defense
Follow up algorithm for Maryland SCID screening

- **Inconclusive**: Written report requests new specimen
- **Normal**: TREC Ct ≤ cutoff
- **Abnormal**: Written report with low GA instructions
- **TREC Assay**
  - RNase P Ct > cutoff
  - TREC Ct > 35
  - Two prior normal screens
- **CRITICAL Follow up**: Flow cytometry diagnostic testing
- **Abnormal** Follow up: Request repeat specimen
- **Abnormal** Follow up: Flow cytometry diagnostic testing

- **Gestational age + age ≥ 36 weeks**
- **No**
- **Yes**

- First abnormal screen
- Second abnormal screen
State of Maryland

Division of Newborn and Childhood Screening

The Challenges of SCID Screening in a Two Screen State

• April 1, 2016 – August 25, 2017
• 4 confirmed SCID cases in the first 109,276 births
• 1 in 27,319 births
• No known missed cases
• 100% accuracy in CDC proficiency testing program

SCID screening summary statistics

<table>
<thead>
<tr>
<th>Mutations found</th>
<th>Age at the time of initial report</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAG1</td>
<td>5 days old</td>
<td>Successful bone marrow transplant</td>
</tr>
<tr>
<td>ADA</td>
<td>8 days old</td>
<td>Successful gene therapy treatment</td>
</tr>
<tr>
<td>IL2RG (X-linked SCID)</td>
<td>6 days old</td>
<td>Pending</td>
</tr>
<tr>
<td>RMRP (cartilage-hair hypoplasia)</td>
<td>4 days old</td>
<td>Pending</td>
</tr>
</tbody>
</table>

Mutations found: RAG1, ADA, IL2RG, RMRP
Age at the time of initial report: 5 days old, 8 days old, 6 days old, 4 days old
Status: Successful bone marrow transplant, Successful gene therapy treatment, Pending

The Challenges of SCID Screening in a Two Screen State
Early screening results foreshadow problems to come

- The first month of screening prompted follow up calls in approximately 0.6% of babies
- Surprising ratio of specimen ages
  - 12 NB specimens (<7 days old)
  - 30 SUB specimens (≥7 days old)
The first month of screening showed differences between NB and SUB specimens.
Another month of screening indicated that the differences between NB and SUB populations were in flux.
Fluctuations in SUB TREC Ct values

Week 1

Requires follow up

Specimen TREC Ct values

Number of specimens

The Challenges of SCID Screening in a Two Screen State
A floating TREC cutoff for SUB specimens reduces the reporting differences

Daily median of TREC Ct values

Specimens reported per day

Floating SUB cutoffs calculated daily
Using fresh Solution 2 alters screening data

Daily median of TREC Ct values

New lot of Solution 2

The Challenges of SCID Screening in a Two Screen State
No further lot-to-lot variation when assuming a shorter shelf life of Solution 2
Overall lessons learned

- Careful data analyses are critical to limit the damage caused by unexpected science.
- Pressure to go live with testing before we were fully staffed exacerbated our reporting problems.

Begin screening with the minimum required staff → No time for data analysis → Corrective measures delayed → Increased false positive rate
Baby J story
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