FACTORS TO CONSIDER IN THE NEWBORN SCREENING ALGORITHM FOR SEVERE COMBINED IMMUNODEFICIENCY

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SCREENING FOR SCID IN TEXAS

- Screen for absence of T-cell Receptor Excision Circles (TREC)
- Multiplex Real-Time PCR based assay
  - Automated DNA extraction followed by Real-Time PCR
  - Each SCID reaction amplifies two targets of interest:
    - T-cell receptor excision circle (TREC)
    - RNaseP reference gene extraction control
- TX is a 2-screen state. Testing specimens: All newborns, follow-ups, requested repeats
INITIAL SCREENING ALGORITHM

- **Multiplex TREC / RNaseP on DBS**
  - **TREC > 200 copies/μL**
    - Normal
  - **TREC ≤ 200 copies/μL**
    - Retest in duplicate from the same specimen
RETEST SCREEN ALGORITHM

Retest in duplicate from the same specimen

- TREC ≤ 110 copies/µL and RNaseP Ct ≤ 28.5:
  - TREC > 150 copies/µL: Normal
  - Abnormal (Undetectable or Low)

- 110 < TREC ≤ 150 copies/µL and RNaseP Ct ≤ 28.5:
  - No: LBW (<2000g)
  - Yes: Borderline

- TREC ≤ 150 copies/µL and RNaseP Ct > 28.5: uFC
Abnormal Result Code:
- Very low number of T-cell receptor excision circles (TREC). Please follow recommendations received from the DSHS newborn screening Clinical Care Coordination team.

Borderline Result Code:
- Borderline low number of T-cell receptor excision circles (TREC). Please repeat the newborn screen within 7 days.

uFC (SCID unsat) Result Code:
- Unsatisfactory - Please resubmit within 7 days: Specimen inadequate for accurate detection of TREC (T-cell receptor excision circles).
## SCID SCREENING DATA

### December 2012 – June 2015

<table>
<thead>
<tr>
<th>Category</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Specimens Screened</td>
<td>1,952,400</td>
</tr>
<tr>
<td># of 1st Screen Specimens (~ # of Newborns)</td>
<td>1,010,063</td>
</tr>
<tr>
<td># of Abnormal/Borderline Specimens</td>
<td>4,954 (0.25%)</td>
</tr>
<tr>
<td># of Newborns Referred</td>
<td>681 (0.07%)</td>
</tr>
<tr>
<td># of Diagnosed SCID Cases</td>
<td>25 (1:40,403)</td>
</tr>
<tr>
<td># of Secondary Diagnosed Cases **</td>
<td>322 (1:3,137)</td>
</tr>
</tbody>
</table>

**Does not include Preterm alone**
### Sensitivity, Specificity, False Positive Rate, False Negative Rate, and Positive Predictive Value—SCID Diagnosed

<table>
<thead>
<tr>
<th></th>
<th>Screen Positive</th>
<th>Screen Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID Diagnosed</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Cleared</td>
<td>656</td>
<td>1,009,382</td>
<td>1,010,038</td>
</tr>
<tr>
<td>Total</td>
<td>681</td>
<td>1,009,382</td>
<td>1,010,063</td>
</tr>
</tbody>
</table>

- Sensitivity = 100%
- Specificity = 99.9%
- False Positive Rate = 0.065%
- False Negative Rate = 0%
- Positive Predictive Value = 3.7%
POSITIVE PREDICTIVE VALUE—SCID & SECONDARY CONDITIONS, REFERRED

<table>
<thead>
<tr>
<th></th>
<th>Screen Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID and Secondary Conditions</td>
<td>316</td>
</tr>
<tr>
<td>Cleared</td>
<td>365</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>681</strong></td>
</tr>
</tbody>
</table>

Positive Predictive Value = 46.4%
LOW BIRTH WEIGHT ONLY

**Graph:**
- **X-axis:** TREC copies/µl whole blood
- **Y-axis:** Frequency

The graph shows the distribution of TREC copies per µl of whole blood for subjects in the low birth weight group, with a particular emphasis on frequency counts across different TREC copy ranges.
Presumptive Positive Rate: Normal birth weight 0.14%
Low birth weight 4%
BORDERLINE

- Low birthweight babies (<2000g) with TREC quantities between 110 TREC copies/µL and 150 TREC copies/µL
- 23 Secondary diagnosed cases with one or more Borderline result
  - 1 with Chromosomal Defects
  - 4 T-cell Syndrome
  - 4 Congenital Heart Defects
  - 1 Gastrointestinal disorder
  - 1 Lymphatic imbalance
  - 4 Multiple congenital anomalies with T-cell defect
  - 3 Pulmonary disorders
  - 2 Preterm and no other recognizable disorder
  - 3 Preterm with complications
- No SCID cases with a Borderline result
AGE AT SPECIMEN COLLECTION

The graph shows the relationship between TREC Copy # and Age at Collection (Days). The data points are scattered across the graph, with a trend line indicating a negative correlation between the two variables. As the age at collection increases, the TREC Copy # tends to decrease.
Presumptive Positive Rate: Normal status 0.09%
Sick w/o transfusion 1.3%
Sick w/ transfusion 13.3%
AGE OF SPECIMENS

Average TREC Quantity

Mean TREC Quantity (TRECs/μL Whole Blood)

Days after Collection

- Average TREC Quantity
- TREC cutoff
- Newborn TREC Quantity Mean During the Study Period
Average Median TREC Quantity: 1073 ± 173
Average Median TREC (copies/µL): 33.859 ± 0.273
Texas Newborn Screening Laboratory tests all 2nd screens even if the 1st screen was normal.

- Median RNaseP Ct for 1st screens is 0.75-1 lower than 2nd screens
- Median TREC quantity for 1st screens is ~15% lower than 2nd screens

During 12/1/2012 – 4/30/2014, 737 newborns had 1st Normal/2nd Non-normal.
- Majority (89.8%) were cleared by additional screens.
- 10.2% had secondary diagnoses.

Since then, 2 SCID cases have been identified with 1st Normal and 2nd Non-normal
**CASE #1 – SCID WITH UNKNOWN MUTATION**

<table>
<thead>
<tr>
<th>Screen</th>
<th>Age of Collection</th>
<th>SCID Screening Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>1 day</td>
<td>Normal</td>
</tr>
<tr>
<td>Second</td>
<td>7 days</td>
<td>Unsatisfactory</td>
</tr>
<tr>
<td>Third</td>
<td>15 days</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Fourth</td>
<td>36 days</td>
<td>Abnormal</td>
</tr>
</tbody>
</table>
**CASE #2 – ZAP70**

<table>
<thead>
<tr>
<th>Screen</th>
<th>Age of Collection</th>
<th>SCID Screening Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>First*</td>
<td>~3 days</td>
<td>Normal</td>
</tr>
<tr>
<td>Second</td>
<td>159 days</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Third</td>
<td>180 days</td>
<td>Abnormal</td>
</tr>
</tbody>
</table>

* Collected by birthing hospital and tested in newborn screening laboratory out of Texas
low birth weight and transfusion status have an effect on SCID screening results.

The median TREC value seems to decrease with age; however, statistical analysis performed indicated there was no significant difference.

DBS specimens are stable for SCID screening at least 1 year after collection.
- Alternative source of TREC calibrator
- Reagent lot variability
- Evaluate an alternate approach to determining our cutoff (e.g. MoM, floating)
- SCID cases can have 1st screen normal results.
- 2nd screens can aid in identifying certain types of SCID and secondary T-cell lymphopenia
THANK YOU!