An Evolution of Michigan’s SCID Algorithm

A qualitative approach for the T cell receptor excision circle (TREC) assay for the detection of primary immune deficiency syndromes (PIDS) demonstrates better sensitivity and specificity versus a quantitative approach.

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Real time polymerase chain reaction (PCR) of T cell receptor excision circles (TREC’s) and reference gene ($\beta$-actin).

TREC’s are small pieces of DNA that are formed during the differentiation of T cells in the thymus as a result of the rearrangement of the T cell receptor genes.
TREC Formation

This process doesn’t work properly in PID’s

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Overall Analysis Scheme

3.2 mm DBS is punched into a 96 well plate

Partially automated DNA extraction using an Eppendorf epMotion 5075

Automated set up of real time qPCR in a 384 well format using the epMotion 5075

Duplex qPCR amplification and analysis (TREC and β actin) on a 7900HT.
A Typical TREC Plasmid Standard Curve

![Graph showing a typical TREC Plasmid Standard Curve. The graph plots the Ct values against the quantity of the plasmid. The graph includes several data points at different concentrations: 5,000, 2,500, 1,250, 625, 156, 78, and 39. The Ct values for each concentration are indicated with black arrows and the corresponding concentration values are shown near the data points.]
Too much variability in the area of interest.

Last point on daily standard curve 10 copies.
**Current Qualitative Algorithm**

- **TREC CT < 35.00**
  - Yes: Normal
  - No: Repeat to confirm

- **TREC CT ≥ 36.3 and normal β actin CT ≤ 30.00**
  - Yes: Normal
  - No: Borderline positive; request another DBS

- **TREC CT ≥ 37.00**
  - Yes: Very Low
  - No: Send to clinic for CBC differential and flow cytometry

If there are 2 borderline positive or 3 inconclusive samples in a row, patient is referred for flow cytometry.

**We have never reported quantities on our reports!**

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Timeline of algorithms

A: Initial Michigan Method
   10/1/2011 to 2/29/2012

B: New and Improved Michigan Method
   3/1/2012 to 8/31/2012

C: 9/1/2012 to 10/2/2012

D: 10/3/2012 to 1/31/2013

E: 2/1/2013 to current

Initial Michigan Method
New and Improved Michigan Method
## Algorithm A (10/1/2011 to 2/29/2012)

<table>
<thead>
<tr>
<th>Normal and low birth weight babies</th>
<th>TREC quantity copies per µl of blood</th>
<th>B actin Quantity copies per µl of blood</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>≥4000</td>
<td></td>
<td>Strong positive</td>
</tr>
<tr>
<td>&lt;30</td>
<td>&lt;4000</td>
<td></td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>

- Time = ~5 months
- 32 cases per month
- N = 44,712
- Total PPV = 18.58%
- Total FPR = 0.21%
## Algorithm B (3/1/2012 to 8/31/2012)

<table>
<thead>
<tr>
<th>Condition</th>
<th>TREC quantity copies per μl of blood</th>
<th>B actin Quantity copies per μl of blood</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal birth weight ≥2500 grams</td>
<td>&lt;30</td>
<td>≥8000</td>
<td>Strong positive</td>
</tr>
<tr>
<td>Low birth weight &lt;2500 grams</td>
<td>&lt;20</td>
<td>≥8000</td>
<td>Strong positive</td>
</tr>
<tr>
<td>Low birth weight &lt;2500 grams</td>
<td>20 to &lt;30</td>
<td>≥8000</td>
<td>Borderline positive</td>
</tr>
<tr>
<td>All babies</td>
<td>&lt;30</td>
<td>&lt;8000</td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>

Time= ~6 months
36 cases per month
N= 57,023
Total PPV= 25.0%        Total FPR= 0.09%
Algorithm C (9/1/2012 to 10/2/2012)

<table>
<thead>
<tr>
<th>Normal and low birth weight babies</th>
<th>TREC quantity copies per µl of blood</th>
<th>B actin CT value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;20</td>
<td>≤30.00</td>
<td>Strong positive</td>
</tr>
<tr>
<td></td>
<td>20 to &lt;30</td>
<td>≤30.00</td>
<td>Borderline positive</td>
</tr>
<tr>
<td></td>
<td>&lt;30</td>
<td>&gt;30.00</td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>

Time = ~1 month

* 85 cases
N = 9,054
Total PPV = 10.59%  Total FPR = 0.83%

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### Algorithm D (10/3/2012 to 1/31/2013)

#### Normal and low birth weight babies

<table>
<thead>
<tr>
<th>TREC quantity copies per µl of blood</th>
<th>B actin CT value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10</td>
<td>≤30.00</td>
<td>Strong positive</td>
</tr>
<tr>
<td>11 to 20</td>
<td>≤30.00</td>
<td>Borderline positive</td>
</tr>
<tr>
<td>≤20</td>
<td>&gt;30.00</td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>

- **Time**: ~3 months
- **53 cases per month**
- **N**: 36,728
- **Total PPV**: 8.81%
- **Total FPR**: 0.39%
## Algorithm E (2/1/2013 to current)

### Normal and low birth weight babies

<table>
<thead>
<tr>
<th>TREC CT value</th>
<th>B actin CT value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥37.00</td>
<td>≤30.00</td>
<td>Strong positive</td>
</tr>
<tr>
<td>36.30-36.99</td>
<td>≤30.00</td>
<td>Borderline positive</td>
</tr>
<tr>
<td>≥36.30</td>
<td>&gt;30.00</td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>

<p>| TREC quantity based on idealized standard curve |</p>
<table>
<thead>
<tr>
<th>CT value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>35.00</td>
<td>27</td>
</tr>
<tr>
<td>36.30</td>
<td>11</td>
</tr>
<tr>
<td>36.99</td>
<td>7</td>
</tr>
<tr>
<td>37.00</td>
<td>7</td>
</tr>
</tbody>
</table>

- **Time**: ~17 months
- **4 – 5 cases per month**
- **N= 178,682**
- **Total PPV= 28.57%**
- **Total FPR= 0.03%**

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Positive Predictive Value (PPV) for Borderline and Strong positives

- **Algorithm A**: PPV (Borderline) = 0.00; PPV (Strong) = 18.58
- **Algorithm B**: PPV (Borderline) = 0.00; PPV (Strong) = 32.73
- **Algorithm C**: PPV (Borderline) = 4.26; PPV (Strong) = 18.42
- **Algorithm D**: PPV (Borderline) = 0.82; PPV (Strong) = 35.14
- **Algorithm E**: PPV (Borderline) = 7.14; PPV (Strong) = 54.29

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False Positive Rate (FPR) for Borderline and Strong positives

- A: Borderline 0.00, Strong 0.21
- B: Borderline 0.03, Strong 0.06
- C: Borderline 0.49, Strong 0.34
- D: Borderline 0.07, Strong 0.33
- E: Borderline 0.02, Strong 0.01

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How Many Michigan Babies Have Been Screened For PID’s?

• 326,041 Michigan babies have been screened from 10/1/2011 to 8/31/2014

• Using clinical categories from Region 4
  ❖ 2 SCID
  ❖ 3 Leaky SCID
  ❖ 18 Syndromes with T cell impairment
  ❖ 21 Non-preterm secondary T cell lymphopenia
  ❖ 29 Lymphopenia
Acknowledgements

- Michigan Newborn Screening Program
- This work was partially funded by a research cooperative agreement from CDC (Grant # 01EH000936) and does not represent the official view of CDC.
## TREC Plasmid Curve

<table>
<thead>
<tr>
<th>TREC Ct values</th>
<th>S8=5000</th>
<th>S7=2500</th>
<th>S6=1250</th>
<th>S5=625</th>
<th>S4=156</th>
<th>S3=78</th>
<th>S2=39</th>
<th>S1=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1051</td>
<td>1058</td>
<td>1057</td>
<td>1060</td>
<td>1060</td>
<td>1047</td>
<td>1010</td>
<td>886</td>
</tr>
<tr>
<td>Mean</td>
<td>26.88</td>
<td>27.94</td>
<td>28.94</td>
<td>29.96</td>
<td>31.91</td>
<td>33.01</td>
<td>33.94</td>
<td>35.97</td>
</tr>
<tr>
<td>SD</td>
<td>0.41</td>
<td>0.41</td>
<td>0.44</td>
<td>0.43</td>
<td>0.46</td>
<td>0.48</td>
<td>0.51</td>
<td>0.69</td>
</tr>
<tr>
<td>-2SD</td>
<td>26.05</td>
<td>27.13</td>
<td>28.06</td>
<td>29.09</td>
<td>30.99</td>
<td>32.04</td>
<td>32.93</td>
<td>34.58</td>
</tr>
<tr>
<td>+2SD</td>
<td>27.70</td>
<td>28.76</td>
<td>29.83</td>
<td>30.83</td>
<td>32.83</td>
<td>33.98</td>
<td>34.96</td>
<td>37.35</td>
</tr>
</tbody>
</table>