Challenges in Revalidation of the TREC qPCR Assay as a Multiplex SCID-SMA Assay

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The Association of Public Health Laboratories
Newborn Screening and Genetic Testing Symposium

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Timeline and Screening Status

1\textsuperscript{st} nomination 06/08
1\textsuperscript{st} review 10/08
Nothing further 11/08

2\textsuperscript{nd} nomination 02/17
2\textsuperscript{nd} review 04/17
Evidence review 05/17
Discussion and vote 02/18
HHS approval 07/18

As of December 2018 all states are screening for SCID
Approved by HHS in 2010 (2\textsuperscript{nd} round)
Population-wide SMA Screening in NYS

- Regulatory amendment
- Specialty Care Center meeting *(certified)*
  - Genetics, neuromuscular specialists (n = 9)
- No carrier reporting
- Multiplex with severe combined immunodeficiency (SCID) qPCR assay; singlicate
- Retests done as “SMA only” in triplicate
- qPCR for SMN2 copy number; ddPCR validated
Validation Specimens

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Number specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBS population</td>
<td>3,305</td>
</tr>
<tr>
<td>SCID controls</td>
<td>37</td>
</tr>
<tr>
<td>Affected, SCID</td>
<td>5</td>
</tr>
<tr>
<td>Affected, Other</td>
<td>32</td>
</tr>
<tr>
<td>SMA controls</td>
<td>36</td>
</tr>
<tr>
<td>Normal, 2 copies SMN1</td>
<td>15</td>
</tr>
<tr>
<td>Affected, 0 copies SMN1</td>
<td>2</td>
</tr>
<tr>
<td>Carriers, 1 copy SMN1</td>
<td>13</td>
</tr>
<tr>
<td>Patient samples (Biogen)</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>3,378</td>
</tr>
</tbody>
</table>
Just Add Probes and Primers.... Impacted SCID Assay

- SCID-only reaction is 10 µL (8 µL mastermix + 2 µL DNA)
- Multiplex reaction is 8 µL (6 µL mastermix + 2 µL DNA); helps reduce cost
- SCID only reaction uses TaqMan Environmental Master Mix 2.0
- Multiplex reaction uses PerfeCTa Multiplex qPCR ToughMix
- Reduced SMN1 primer concentration by half
- Changed plasmid, RPPH1 formulation and standard
Multiplex Screening -- Results

SMN1 / RPPH1 amplification
Standard Curve
TREC amplification

NTC, blanks, failures, positive samples

Controls per 384 well plate:
3 SMA positive; 3 SCID positive;
2 reagent only; 4 blank punch;
1 normal TREC / normal SMA (triplicate)
Multiplex – Lower Ct Values

SCID only

Multiplex
Mean TREC Values Increase

Old TREC mean: 998 + 555
Multiplex TREC mean: 1665 + 1066
Validation Results

- All real SCID cases were detected
- All syndromic/T-cell impairment, 2ndary T-cell lymphopenia; and idiopathic T-cell lymphopenia flagged with TREC < 300
- Cases referred by older assay and not by multiplex resolved as “No Disease”
- Higher rate of referrals (0 v. 9)*
- Higher rate of borderlines (2 v. 26)**
- Higher rate of preterm borderline (3 v. 12)**
- High sensitivity with known cases (n=37)

* no infants were referred on old assay during the validation
** no infants were determined to have disease
Screening – Multiplex SCID

Multiplex TREC and e7del using qPCR

TREC > 300

SCREEN NEGATIVE
No Further Action Required

TREC ≤ 300

TREC “old” 2nd tier triplicate

SCREEN POSITIVE
Referral for Evaluation & Diagnostic Testing

Probes
5’-VIC (SMN1)
5’-ABY (RPPH1)
5’-FAM (TREC)
3’ quencher – MGB-NFQ (SMN1 + TREC)
3’ quencher – QSY (RPPH1)
Purple Haze standard

^ refer regardless of GA
<37 weeks: repeat when reached
≥37 weeks; repeat immediately

0 TRECS^ 1-124 avg TRECS 125-200 avg TRECS > 200 avg TRECS

Borderline Repeat Screen negative
Changes with Universal SMA Screening

Carriers
• Not reported

Late onset SMA
• SMN2 copy number
• When to treat?
• How will detection impact the incidence of SMA?

Non-deletion mutations
• Will not be detected; report language important
• 2 – 5%

Treatment
• Long-term effects? Renal toxicity?
• Availability, cost and compliance?
• Insurance coverage

Currently incidence rate is lower than expected
What About Spinal Muscular Atrophy?

- **Old assay:**
  - mean \( RPPH1 \) Ct = 28.7 ± 0.6 (27.7 - 30.4)
  - mean \( SMN1 \) Ct = 28.8 ± 1.0* (26.9 – 30.7)

- **New assay:**
  - mean \( RPPH1 \) Ct = 24.3 ± 1.0 (22.9 – 26.6)
  - mean \( SMN1 \) Ct = 24.8 ± 1.3* (23.0 – 29.0)

- Better amplification of \( SMN1 \); similar to TRECs

- All specimens from known cases returned consistent results using the new multiplex assay

*includes \( SMN1 \) 1, 2 or greater than 2 copies
Screening – Multiplex SMA

Multiplex TREC and e7del using qPCR

< 30 Ct SMN1

SCREEN NEGATIVE
No Further Action Required

≥ 30 Ct SMN1

SCREEN POSITIVE
Referral for Evaluation & Diagnostic Testing

Retest SMN1 and SMN2 dosage qPCR/ddPCR in triplicate

Universal screening
Ct cutoff rather than ΔΔCt; only ‘presence’

Probes
5’-VIC (SMN1)
5’-ABY (RPPH1)
5’-FAM (TREC)

3’ quencher – MGB-NFQ (SMN1 + TREC)
3’ quencher – QSY (RPPH1)

Purple Haze standard

• Looking into eliminating RPPH1
• Using <30 as Ct cutoff
• Looking at another master mix
Retest Rates!

SCID/Multiplex Retest* and Abnormal Rates

*Only includes first-tier TREC or RP fails (old 99941, 99942; MP: 99963, 99972, 99974); RETEST, RF_RL, AP_RL, TEC_RL, AT_RL, SMA probe only fail excluded. Entire plate/run fail not merged into UIMs, so excluded.

Day 274
RPPH1 Fails

Oct 1, 2017 – Sep 30, 2018*
8 (0 - 31)
0.7% (0 - 4.1%)

Oct 1, 2018 – Dec 4, 2018*
8 (1 - 52)
0.7% (0.1 - 5.9%)

Dec 5, 2018 – Feb 28, 2019*
6 (1 - 30)
0.6% (0.1 - 2.1%)

*Median number or percentage specimens with abnormal RPPH1 per day (range)
Low TREC\(s\) and/or SMN1

\[
\text{Oct 1, 2017 – Sep 30, 2018*} \\
\quad 19 (1 - 66) \\
\quad 2.0\% (0.4 - 5.5\%)
\]

\[
\text{Oct 1, 2018 – Dec 4, 2018*} \\
\quad 83 (4 - 166) \\
\quad 7.7\% (1.8 - 18.4\%)
\]

\[
\text{Dec 5, 2018 – Feb 28, 2019*} \\
\quad 56 (14 - 111) \\
\quad 5.8\% (2.2 - 10.0\%)
\]

\*Median number or percentage specimens with low TREC\(s\) that resolved on retest per day (range)
“SMN1 Only” Fails

Oct 1, 2018 – Dec 4, 2018*
2 (0 - 63)
0.1% (0 – 9.0%)

Dec 5, 2018 – Feb 28, 2019*
0 (0 - 47)
0% (0 - 3.6%)

*Median number or percentage specimens with abnormal SMN1 per day (range)
Diagnoses

Month and Year

Number Infants w/ Diagnosis

Number Infants Referred

SCID
Other Immunodeficiency
No SCID (False Positives)
Infants Referred


0  5  10  15  20  25

0  5  10  15  20  25
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- Denise Kay, PhD
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