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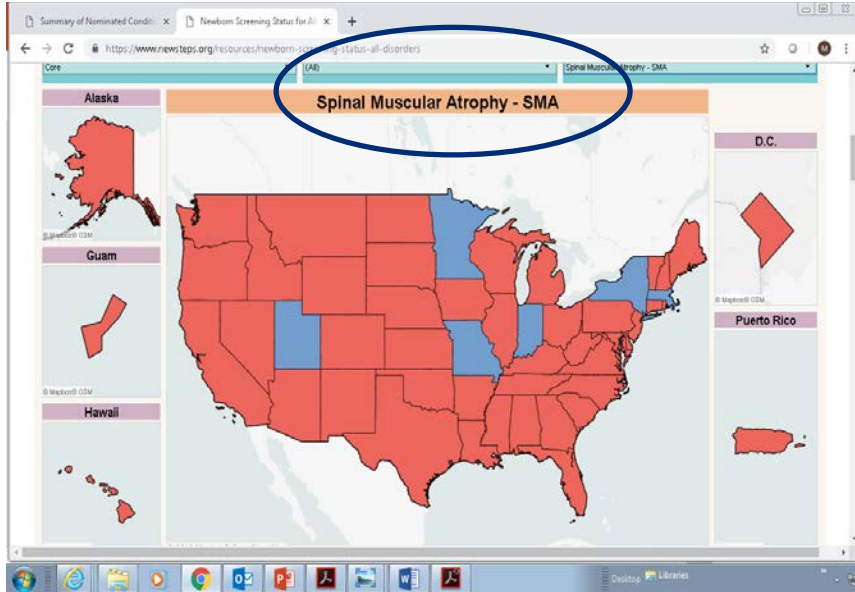
Challenges in Revalidation of the TREC qPCR Assay as a Multiplex SCID-SMA Assay

April 9, 2019

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

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Wadsworth Center, NYS Department of Health

Timeline and Screening Status



1st nomination 06/08
1st review 10/08
Nothing further 11/08

2nd nomination 02/17
2nd 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 (2nd round)



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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

Sample Type	Number specimens
NBS population	3,305
SCID controls	37
Affected, SCID	5
Affected, Other	32
SMA controls	36
Normal, 2 copies SMN1	15
Affected, 0 copies SMN1	2
Carriers, 1 copy SMN1	13
Patient samples (Biogen)	6
Total	3,378



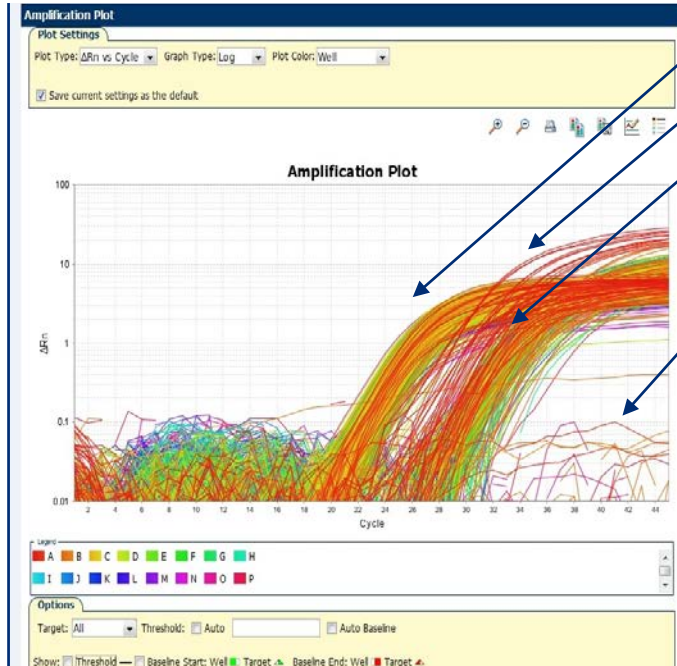
Just Add Probes and Primers....

Impacted SCID Assay

- **SCID-only reaction is 10 μ L (8 μ L mastermix + 2 μ L DNA)**
- **Multiplex reaction is 8 μ L (6 μ L mastermix + 2 μ L 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

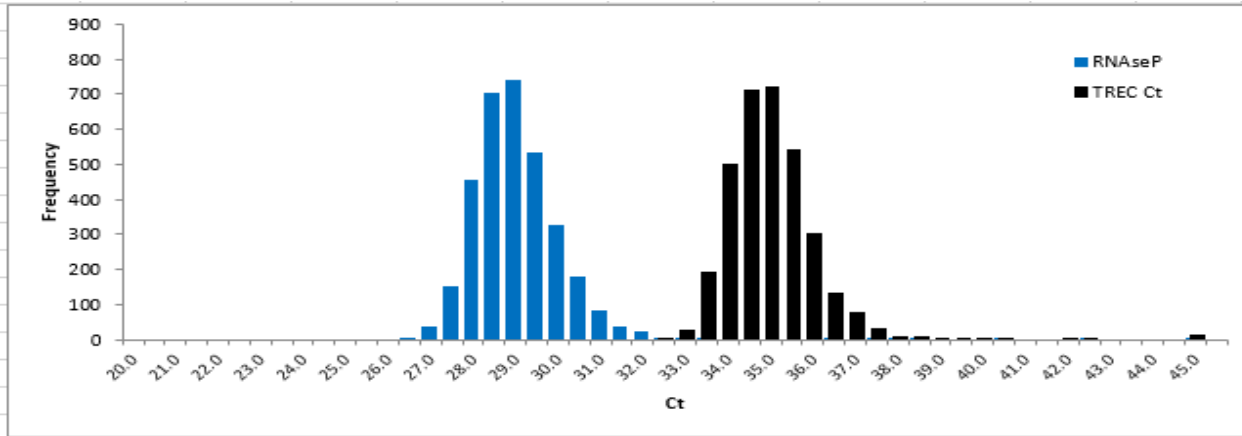
Controls per 384 well plate:
3 SMA positive; 3 SCID positive;
2 reagent only; 4 blank punch;
1 normal TREC / normal SMA (triplicate)



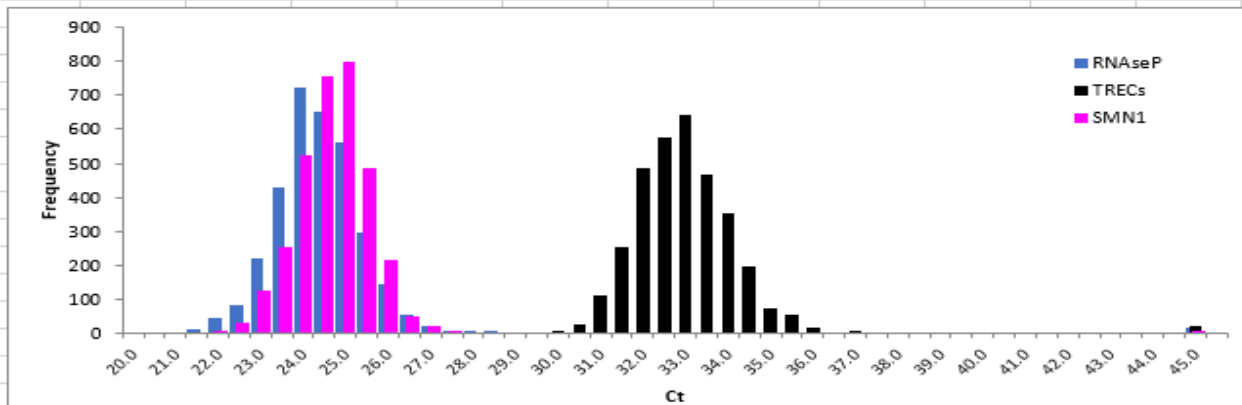
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Multiplex – Lower Ct Values



SCID only



Multiplex



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Mean TREC Values Increase



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*

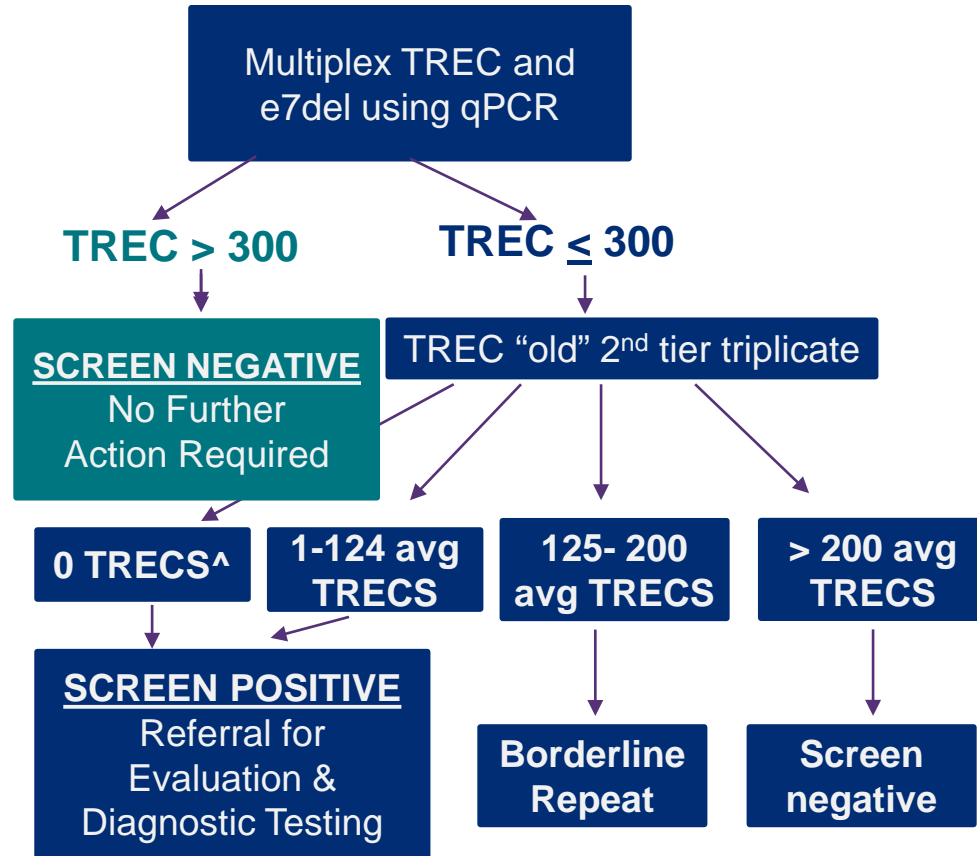
** *no infants were determined to have disease*



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Screening – Multiplex SCID



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
≥37weeks; repeat immediately



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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



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What About Spinal Muscular Atrophy?

- Old assay: mean *RPPH1* Ct = 28.7 ± 0.6 (27.7- 30.4)
 mean *SMN1* Ct = $28.8 \pm 1.0^*$ (26.9 – 30.7)
- New assay: mean *RPPH1* Ct = 24.3 ± 1.0 (22.9 – 26.6)
 mean *SMN1* Ct = $24.8 \pm 1.3^*$ (23.0 – 29.0)
- Better amplification of *SMN1*; similar to TRI
- All specimens from known cases returned using the new multiplex assay



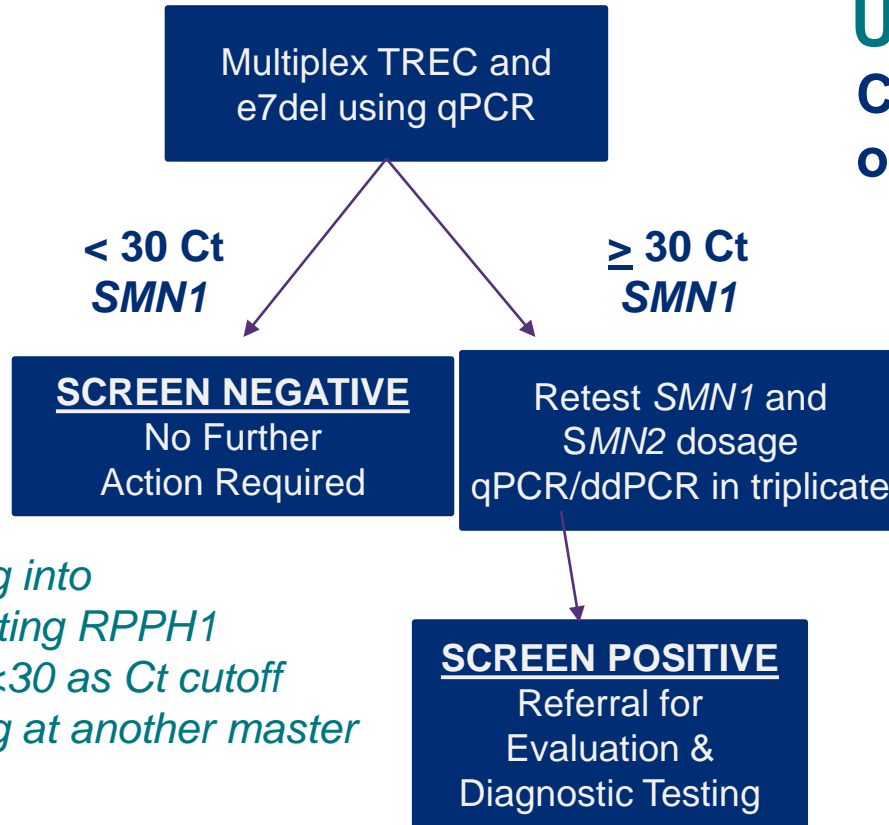
*includes *SMN1* 1, 2 or greater than 2 copies



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Screening – Multiplex SMA



- Looking into eliminating RPPH1
- Using <30 as Ct cutoff
- Looking at another master mix

Universal screening
Ct cutoff rather than $\Delta\Delta Ct$;
only 'presence'

Probes

5'-VIC (SMN1)

5'-ABY (RPPH1)

5'-FAM (TREC)

3' quencher – MGB-NFQ
(SMN1 + TREC)

3' quencher – QSY
(RPPH1)

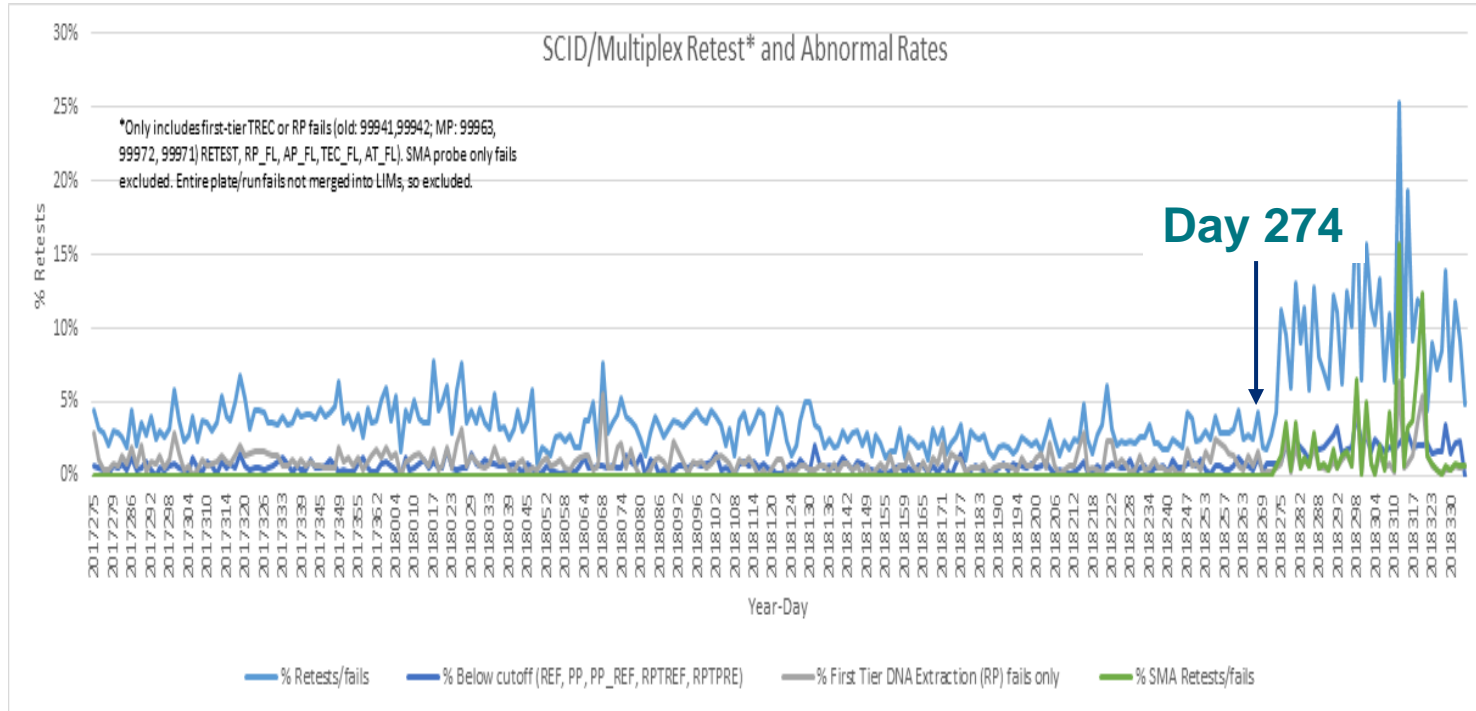
Purple Haze standard



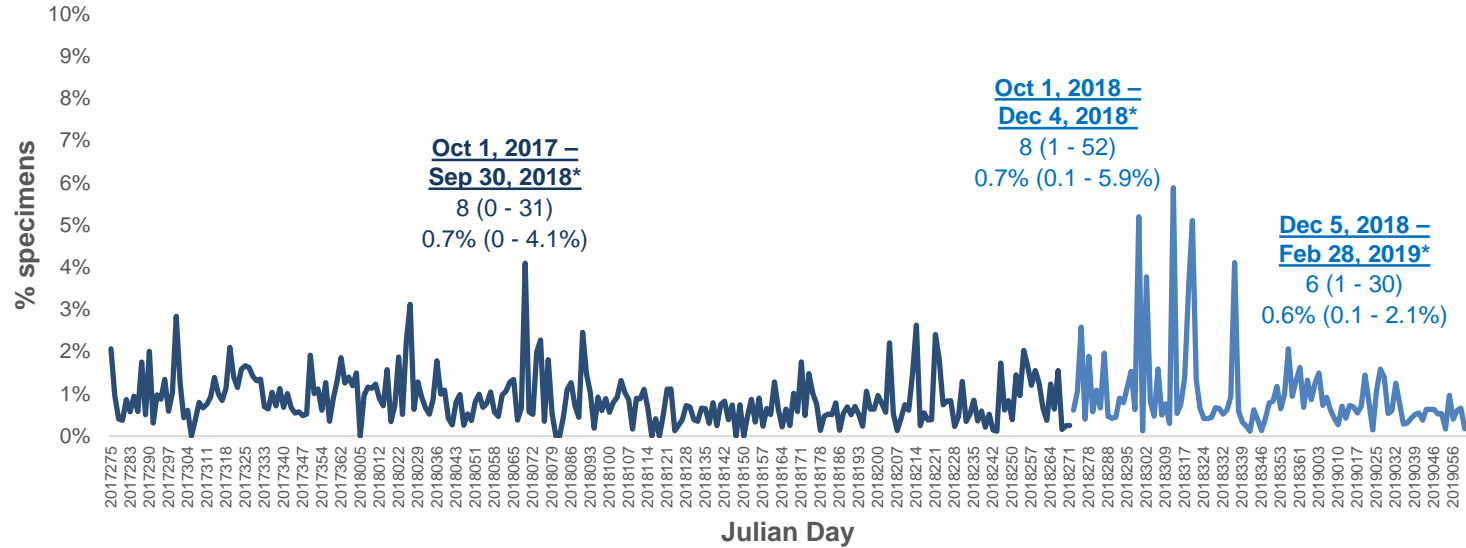
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Retest Rates!



RPPH1 Fails



— Triplex: RNaseP/DNA/assay fail, resolved on retest

— Duplex: RNaseP/DNA/assay fail, resolved on retest

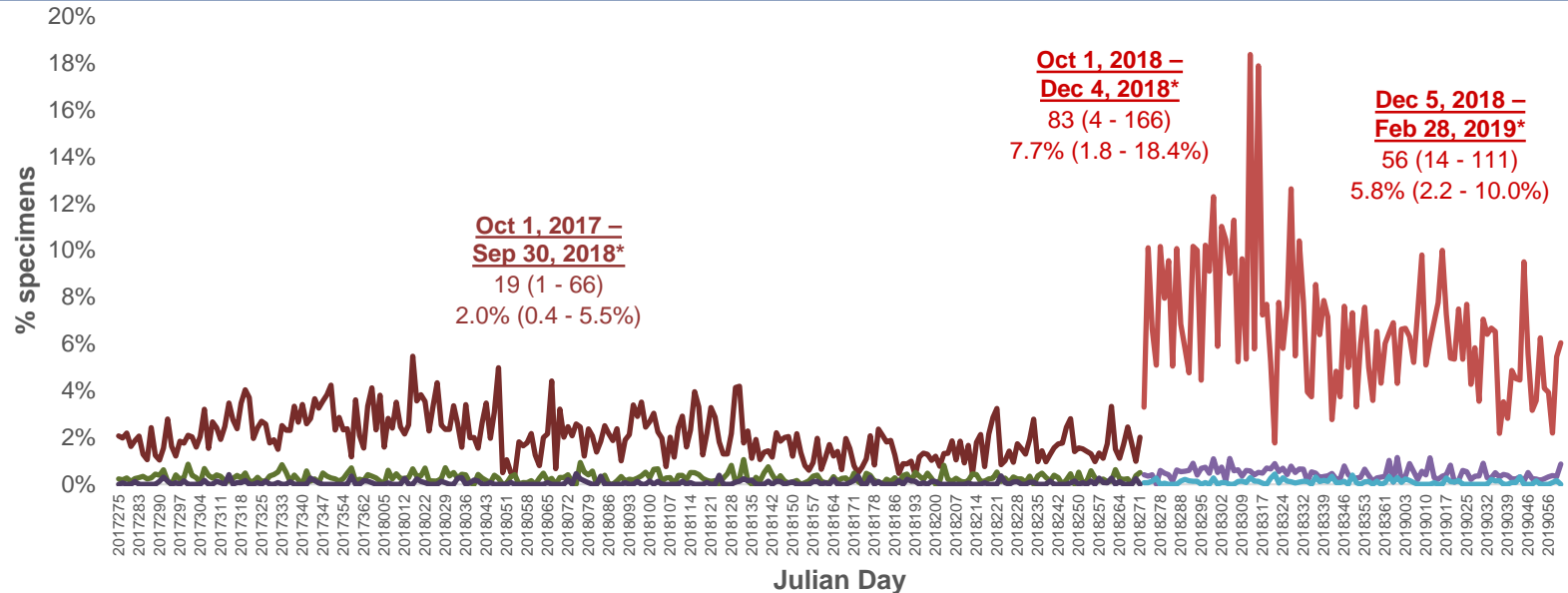
*Median number or percentage specimens with abnormal RPPH1 per day (range)



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Low TRECs and/or SMN1



— Triplex: TRECs abnormal, resolved on retest

— Triplex: TRECs borderline, repeat requested

— Triplex: TRECs abnormal, referral

— Duplex: TRECs abnormal, resolved on retest

— Duplex: TRECs borderline, repeat requested

— Duplex: TRECs abnormal, referral

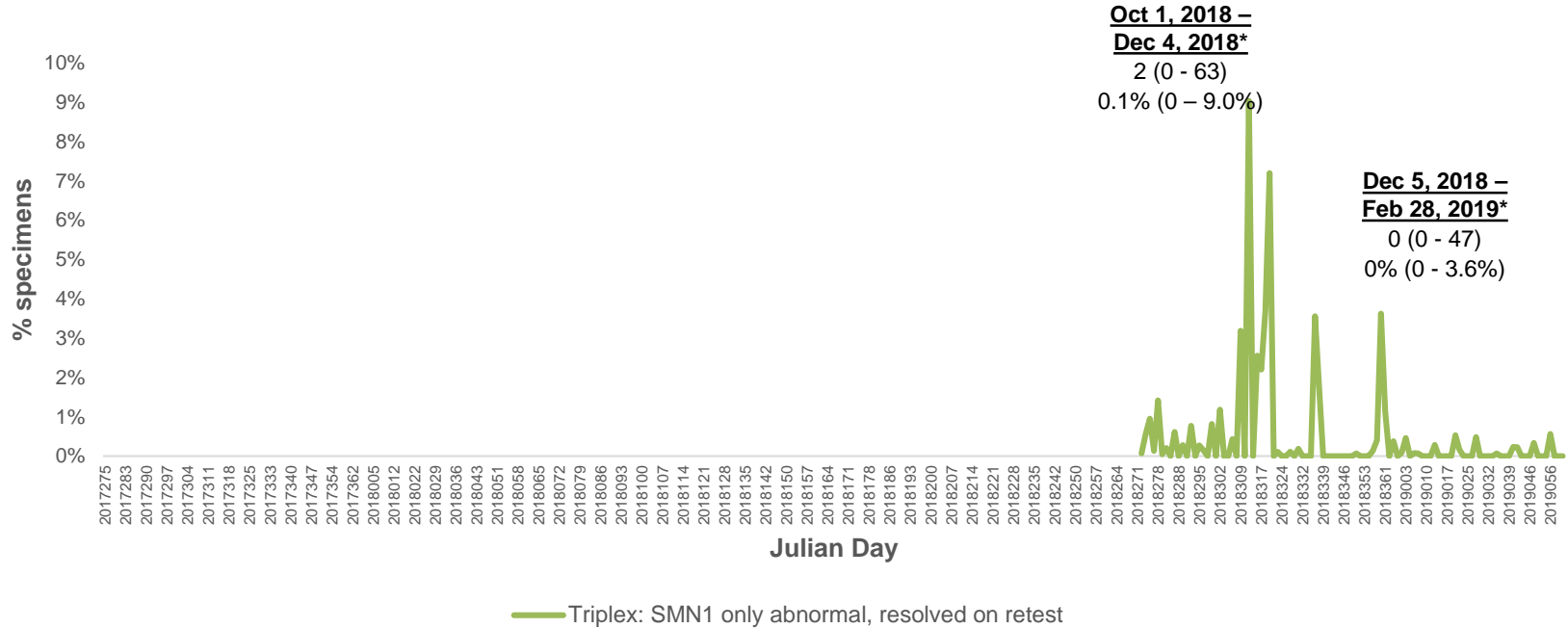
*Median number or percentage specimens with low TRECs that resolved on retest per day (range)



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“SMN1 Only” Fails



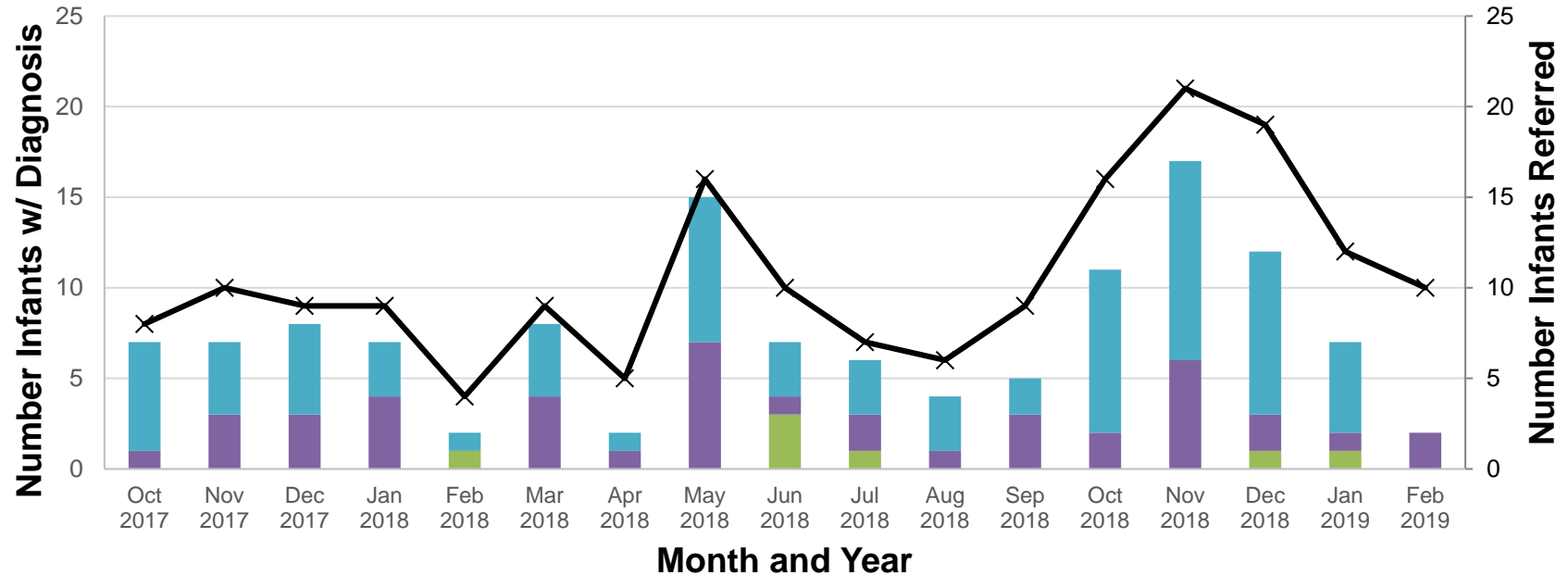
*Median number or percentage specimens with abnormal SMN1 per day (range)



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Diagnoses



■ SCID
 ■ Other Immunodeficiency
 ■ No SCID (False Positives)
 —x— Infants Referred



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