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

RACHEL LEE
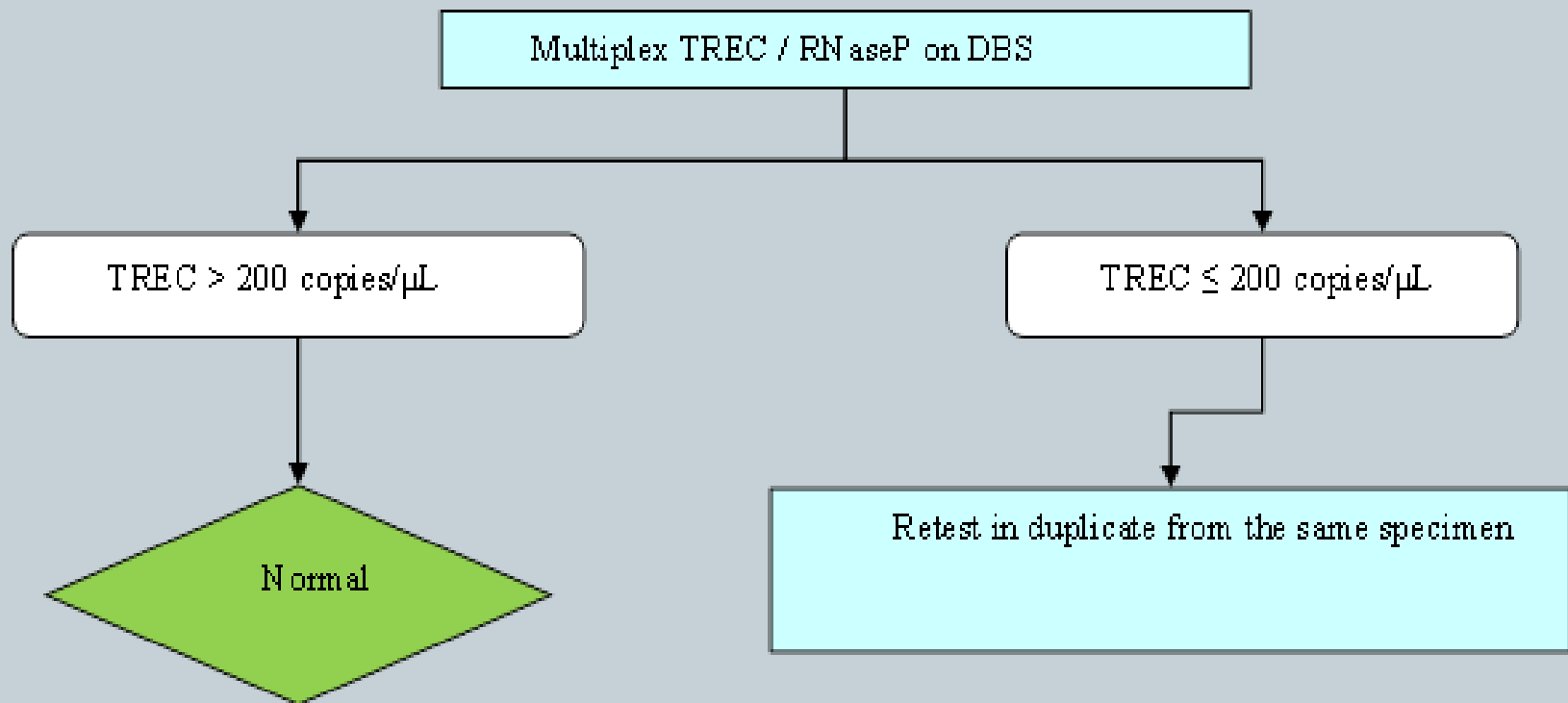
M. Nolen, C. Corrigan, D. Luna, D. Freedenberg, MD, PhD, S.M. Tanksley, PhD
Texas Department of State Health Services, Austin, TX

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

TREC ≤ 200 copies/µL

Normal

Retest in duplicate from the same specimen

RETEST SCREEN ALGORITHM
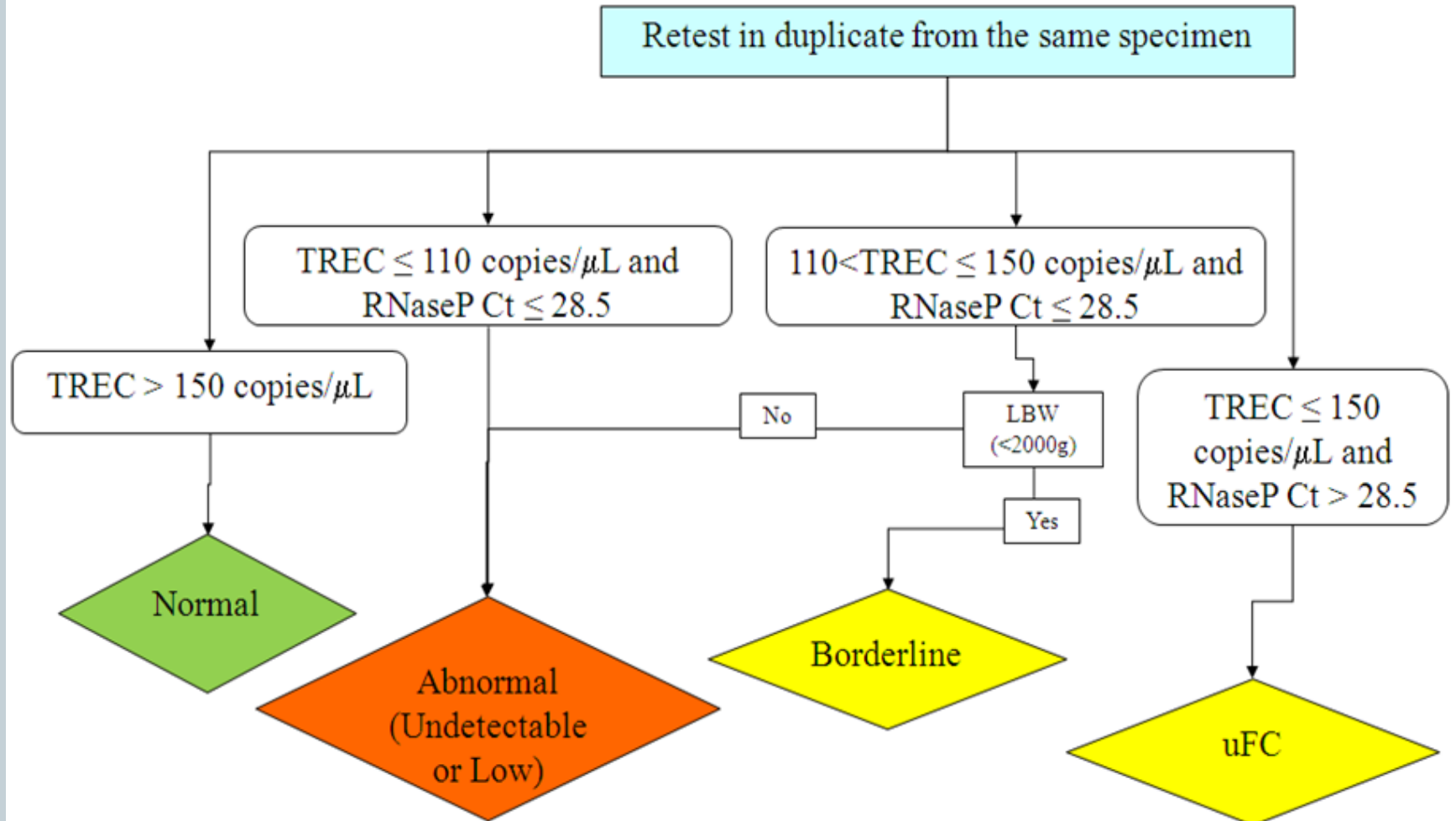
SCID RESULT NOTES

- 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

# of Specimens Screened	1,952,400
# of 1st Screen Specimens (~ # of Newborns)	1,010,063
# of Abnormal/Borderline Specimens	4,954 (0.25%)
# of Newborns Referred	681 (0.07%)
# of Diagnosed SCID Cases	25 (1:40,403)
# of Secondary Diagnosed Cases **	322 (1:3,137)

**Does not include Preterm alone

SENSITIVITY, SPECIFICITY, FALSE POSITIVE RATE, FALSE NEGATIVE RATE, AND POSITIVE PREDICTIVE VALUE—*SCID DIAGNOSED*

	Screen Positive	Screen Negative	Total
SCID Diagnosed	25	0	25
Cleared	656	1,009,382	1,010,038
Total	681	1,009,382	1,010,063

Sensitivity = 100%

Specificity = 99.9%

False Positive Rate = 0.065%

False Negative Rate = 0%

Positive Predictive Value = 3.7%

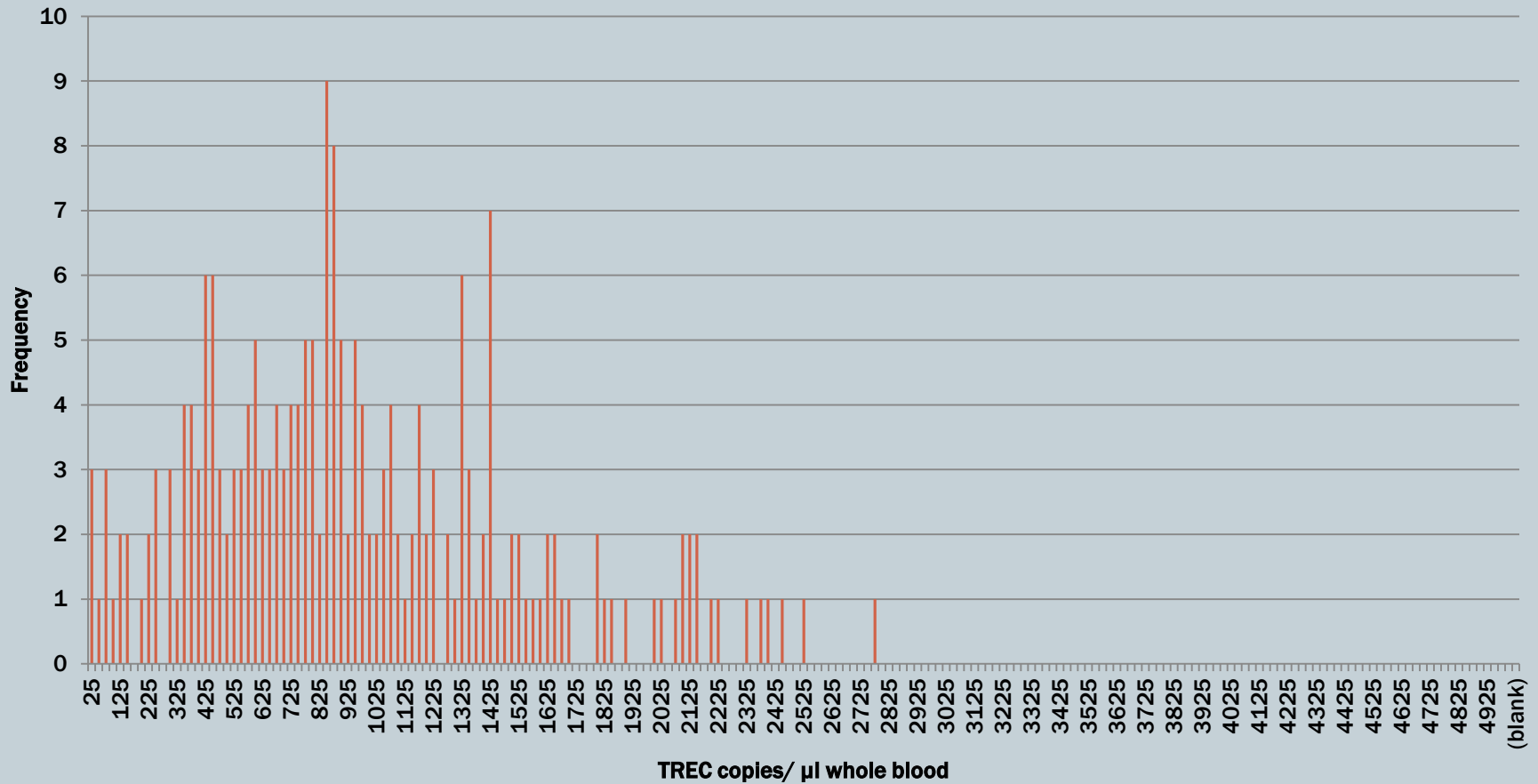# POSITIVE PREDICTIVE VALUE—*SCID & SECONDARY CONDITIONS, REFERRED*

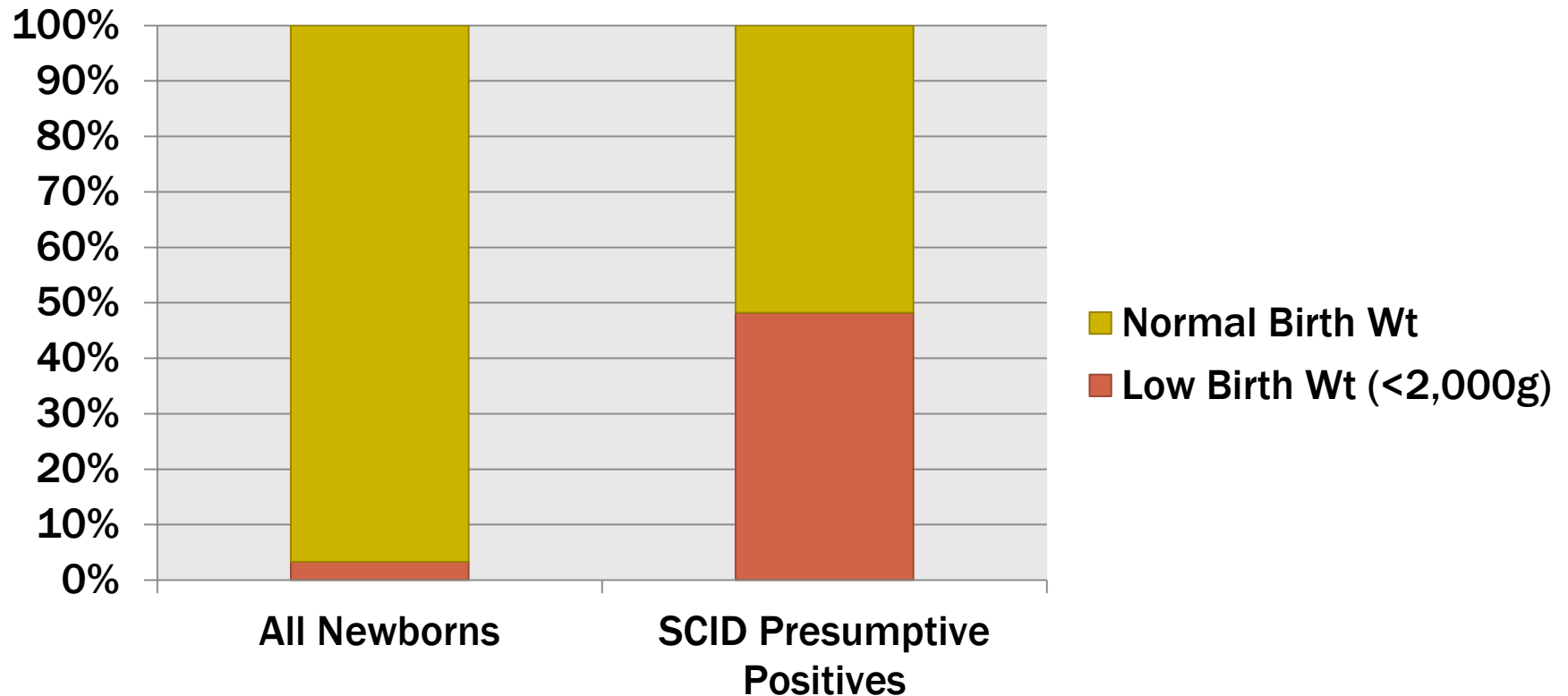|	Screen Positive
SCID and Secondary Conditions	316
Cleared	365
Total	681

Positive Predictive Value = 46.4%

# ALL BIRTH WEIGHT
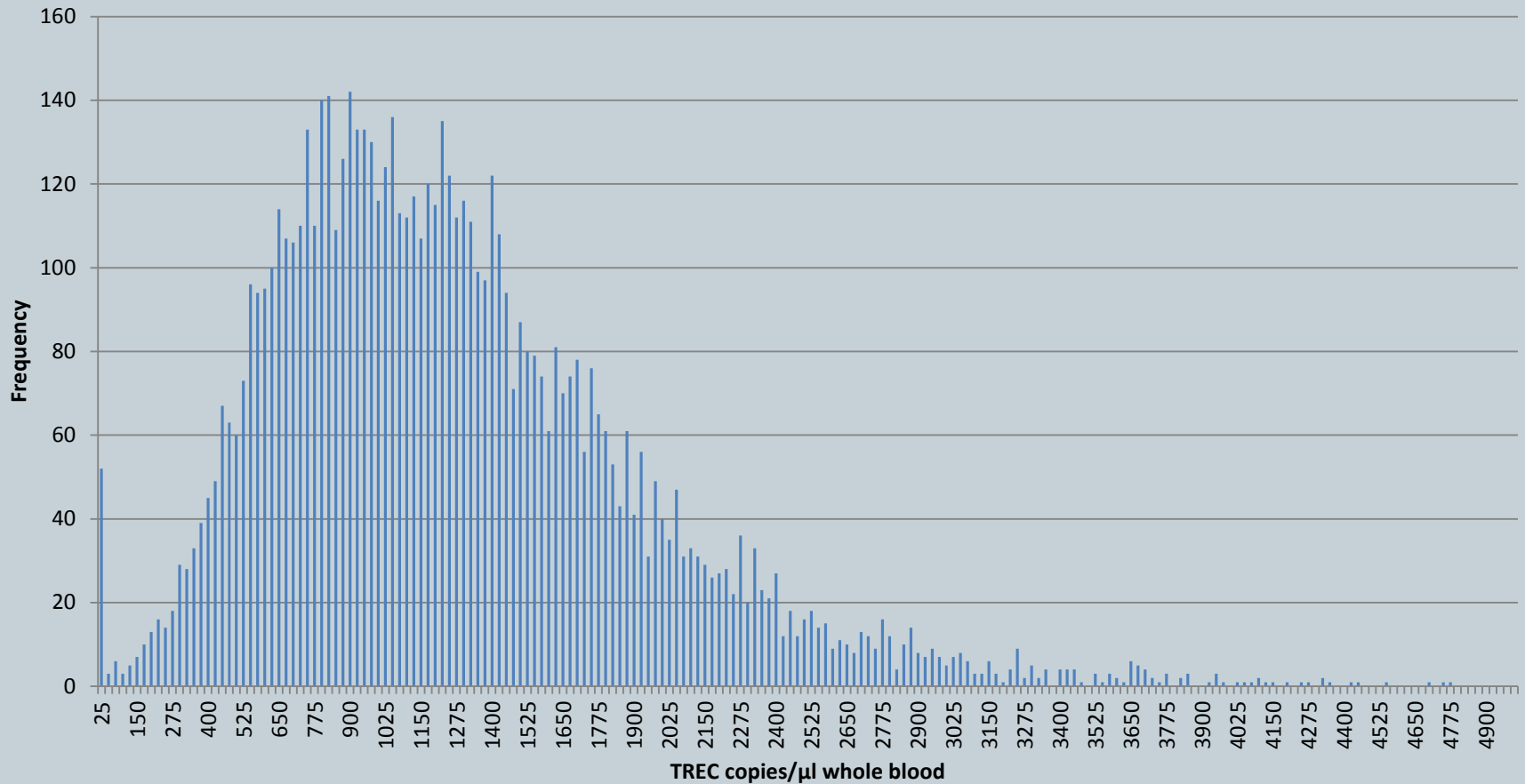
LOW BIRTH WEIGHT ONLY

BIRTH WEIGHT

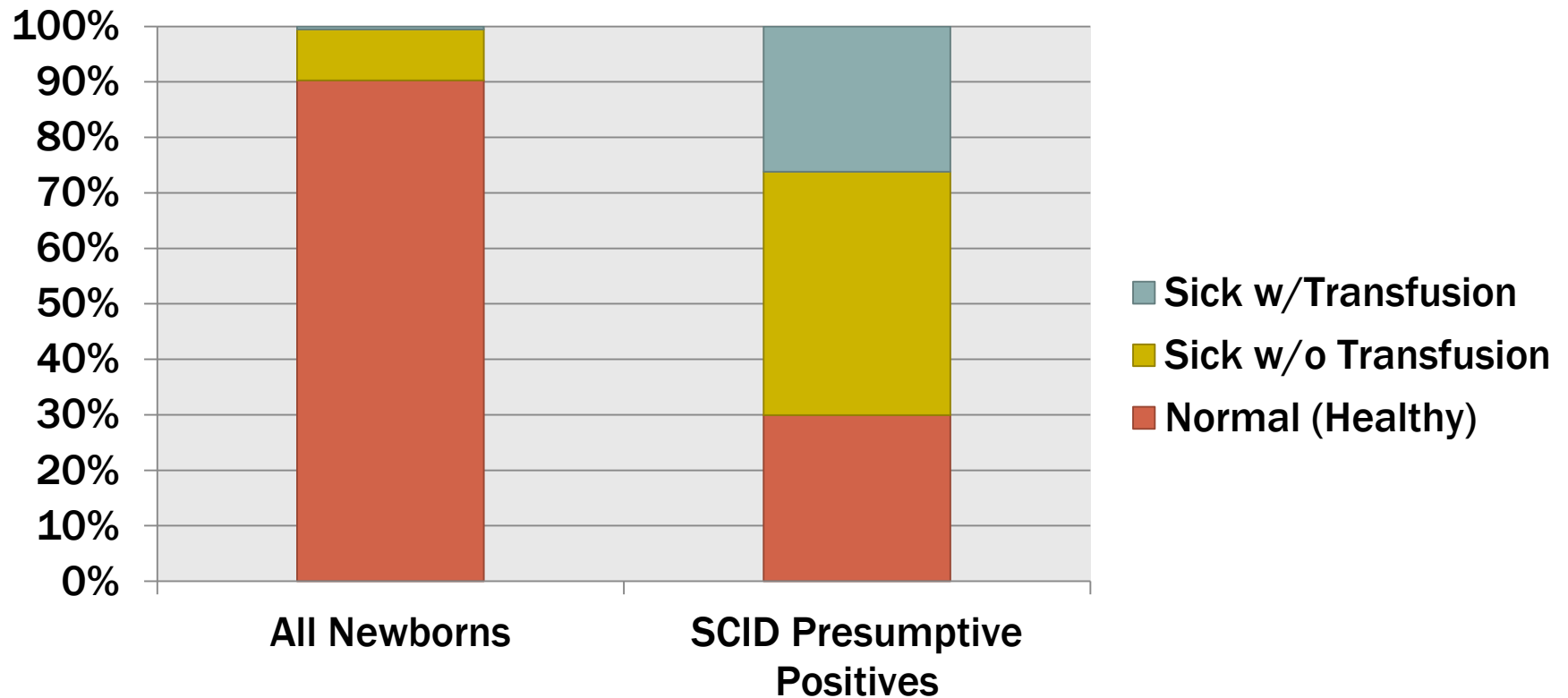**Presumptive Positive Rate: Normal birth weight 0.14%**
Low birth weight 4%

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**

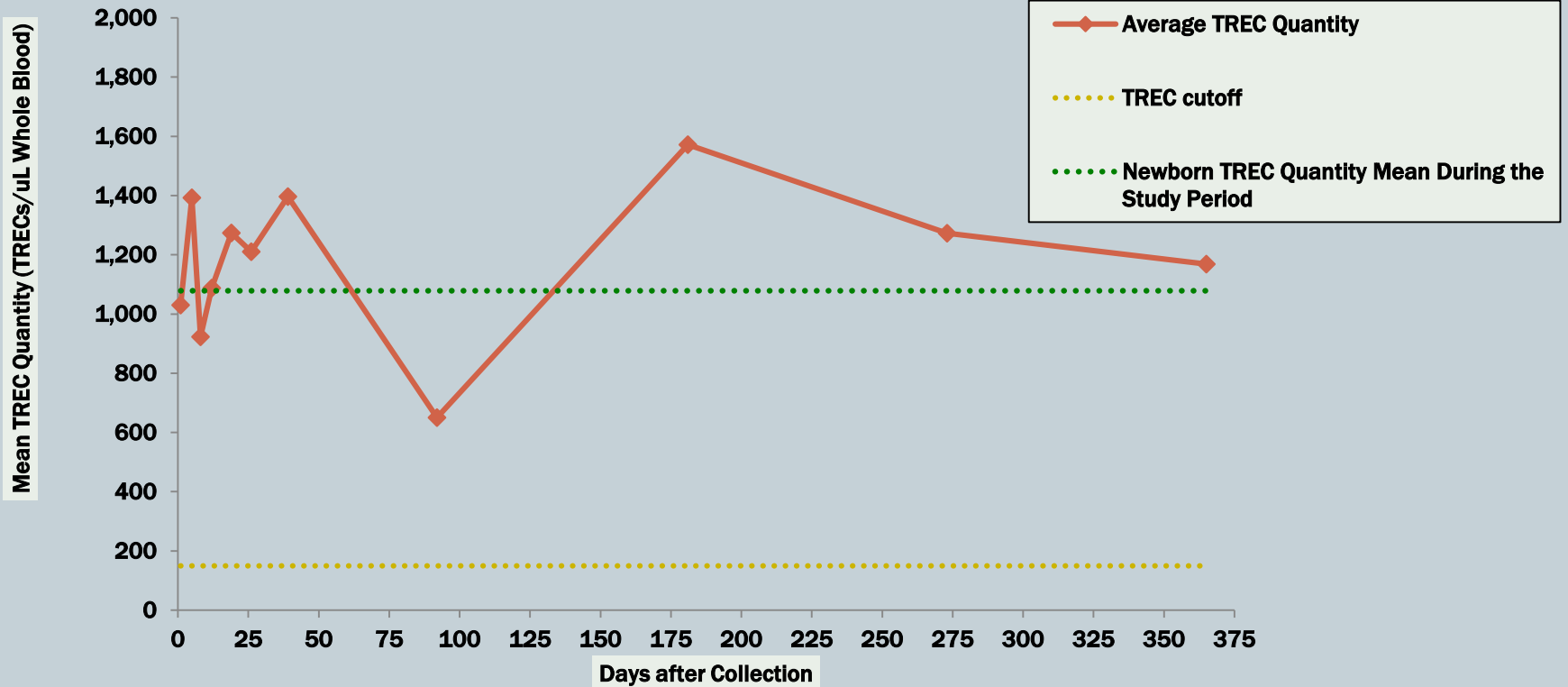- No SCID cases with a Borderline result

AGE AT SPECIMEN COLLECTION
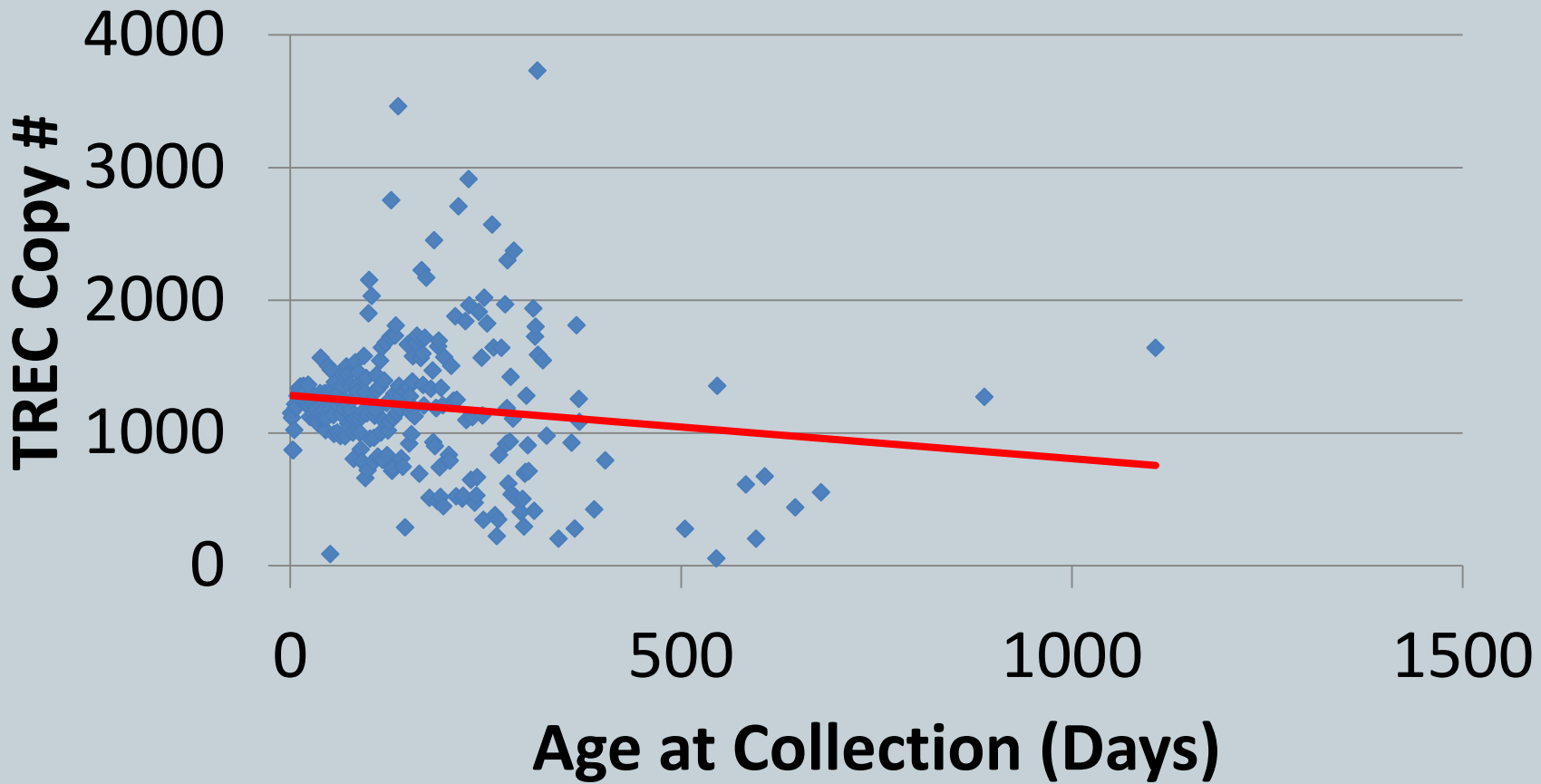
CLINICAL STATUS

Presumptive Positive Rate: Normal status 0.09%
Sick w/o transfusion 1.3%
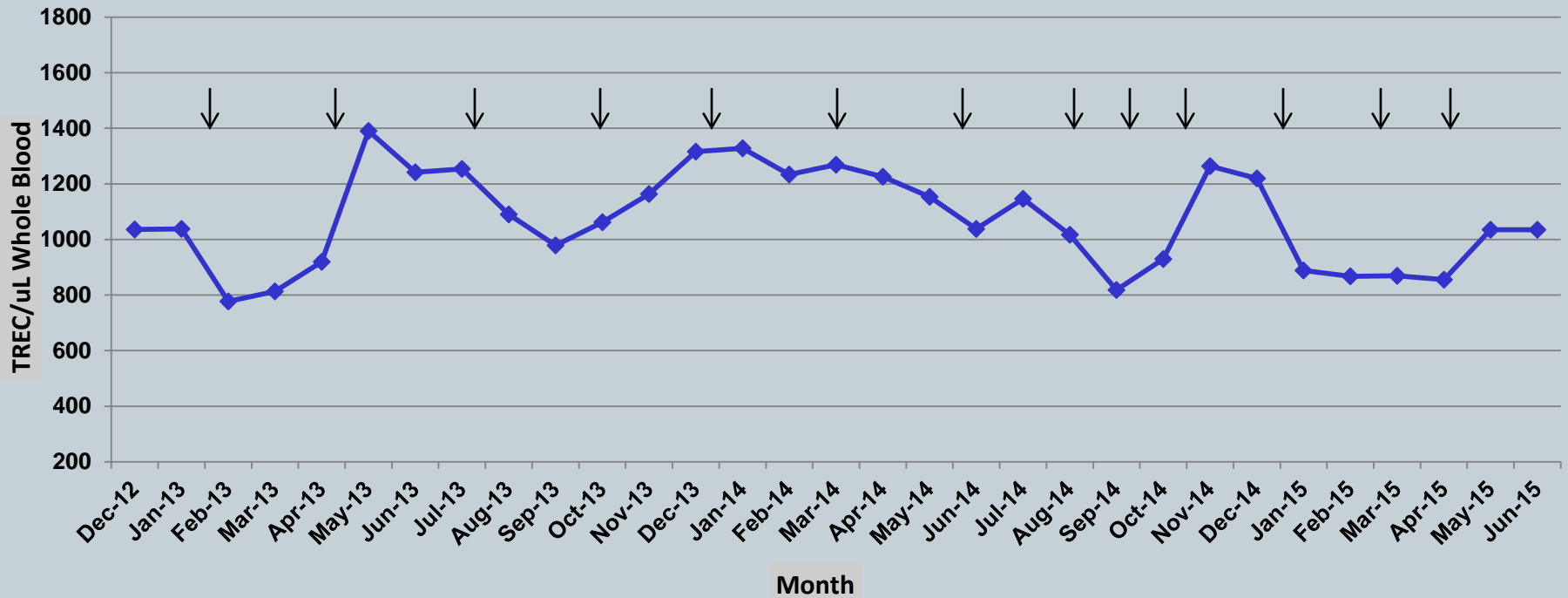**Sick w/ transfusion 13.3%**

AGE OF SPECIMENS
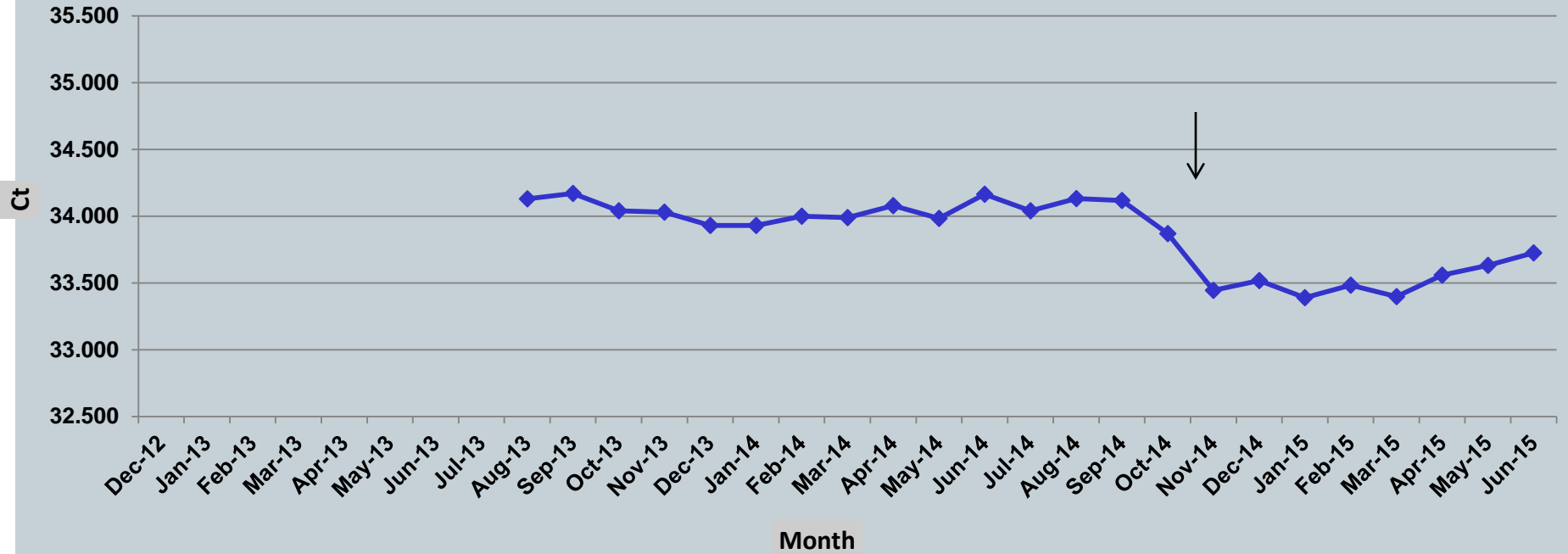

Average TREC Quantity

CALIBRATOR LOT

Median TREC Quantity

Average Median TREC Quantity: 1073 ± 173

REAGENT LOT

Median TREC Ct

Average Median TREC (copies/µL): 33.859 ± 0.273

1ST SCREEN VS. 2ND SCREEN

- 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

Screen	Age of Collection	SCID Screening Results
First	1 day	Normal
Second	7 days	Unsatisfactory
Third	15 days	Abnormal
Fourth	36 days	Abnormal

CASE #2 – ZAP70

Screen	Age of Collection	SCID Screening Results
First*	~3 days	Normal
Second	159 days	Abnormal
Third	180 days	Abnormal

* Collected by birthing hospital and tested in newborn screening laboratory out of Texas

LESSON LEARNED

- 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.

LESSON LEARNED (CONT.)

- 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!