# Early Detection of Severe Immune Defects by Newborn Screening

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# Severe Combined Immunodeficiency, SCID

- Absent T cells; no specific antibody production by B cells.
- Recurrent infections and weight loss from age 2-4 months.
- Serious bacterial, viral, & fungal infections; opportunistic pathogens that do not cause disease in healthy infants.
- Early death unless a working immune system can be established.

# Many SCID Genes, Distinct T, B, NK profiles

| | | | |
|---|---|---|---|
| • *IL2RG* (common γ chain, X-linked) | T- | B+ | NK- |
| • *JAK3* (γ chain associated Janus kinase) | T- | B+ | NK- |
| • *IL7R* (IL-7 receptor α chain) | T- | B+ | NK+ |
| • *CD45* (membrane tyrosine kinase) | T- | B+ | NK+ |
| • *TCRD/E/Z* ( TCR CD3 δ,ε,ξ chains) | T- | B+ | NK+ |
| • *RAG1/RAG2* | T- | B- | NK+ |
| • *DCLRE1C* (Artemis) | T- | B- | NK+ |
| • *LIG4* (DNA ligase IV) | T- | B- | NK+ |
| • *PRKDC* (DNA PKcs) | T- | B- | NK+ |
| • *ADA* (adenosine deaminase) | T- | B- | NK- |
| • *AK2* (reticular dysgenesis, deafness) | T- | B+/- | NK+ |
| • *TTC7A* (multiple bowel atresias) | T- | B+/- | NK+ |
| • *RMRP* (cartilage hair hypoplasia) | T- | B+/- | NK+ |
| • *FOXN1* (nude mouse) | T- | B+ | NK+ |
| • *CORO1A* (Coronin 1A) | T- | B+/- | NK+ |

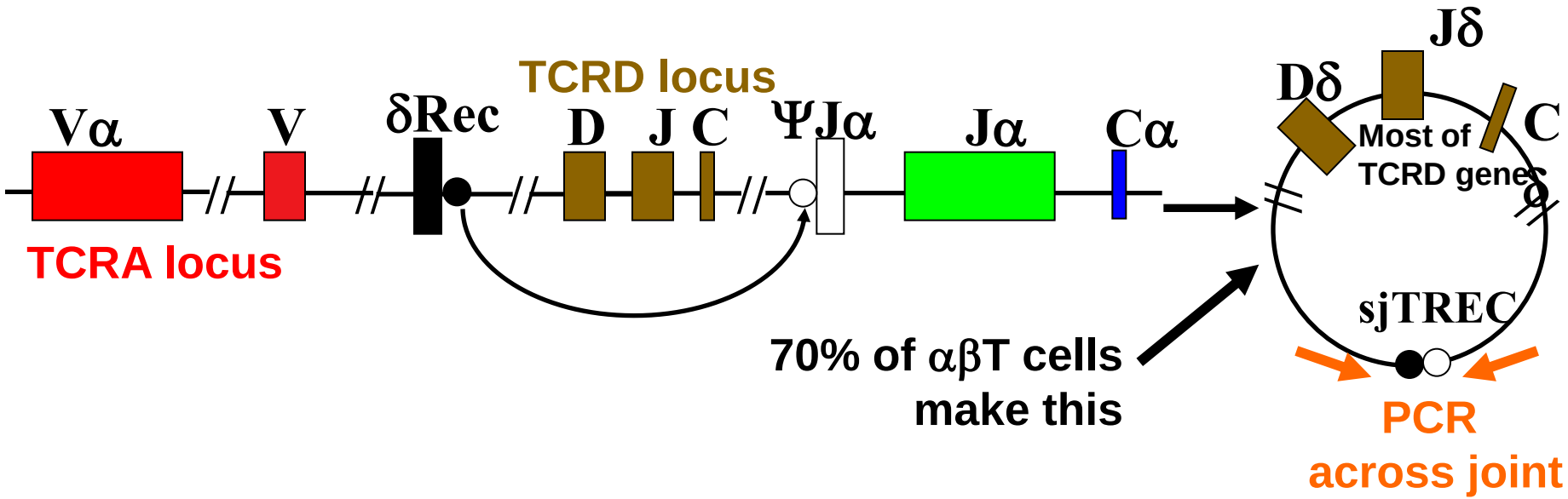
# What Are the Barriers to Early Primary Immunodeficiency Diagnosis?

- Immune defects are rare.
- Infections are common, not just in immune deficiency.
- Family history often missed, subtle or absent.
- Maternal IgG protects infants for their first months of life.
- Both gene defect and environmental exposure contribute, so presentation is variable.

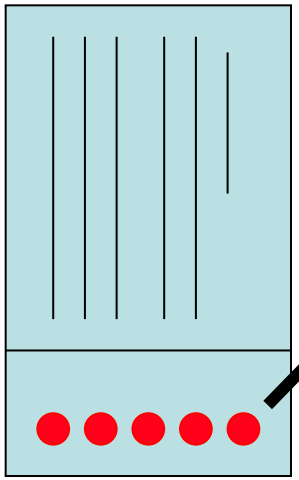
# Thymus produces T cells with a diverse repertoire

- Antigen specificity arises by DNA recombination of T cell receptor genes.
- Excised DNA segments form circles (TRECs) as a byproduct.
- TRECs are stable and are detected by PCR.
- Newborns have the most TRECs; TRECs are diluted out as T cells undergo many divisions in the periphery.

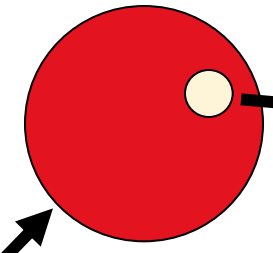
# Generation of T Cell Receptor Excision Circles, TRECs



# TREC Dried Blood Spot Assay



Guthrie Card



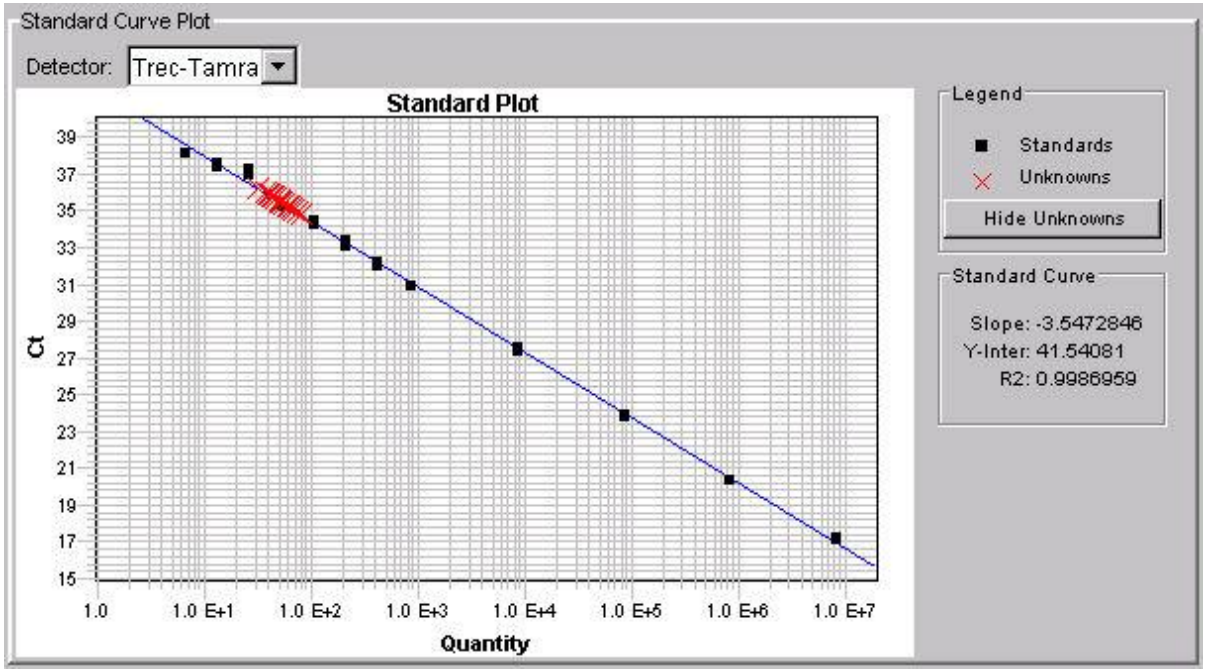
50 ul blood/drop

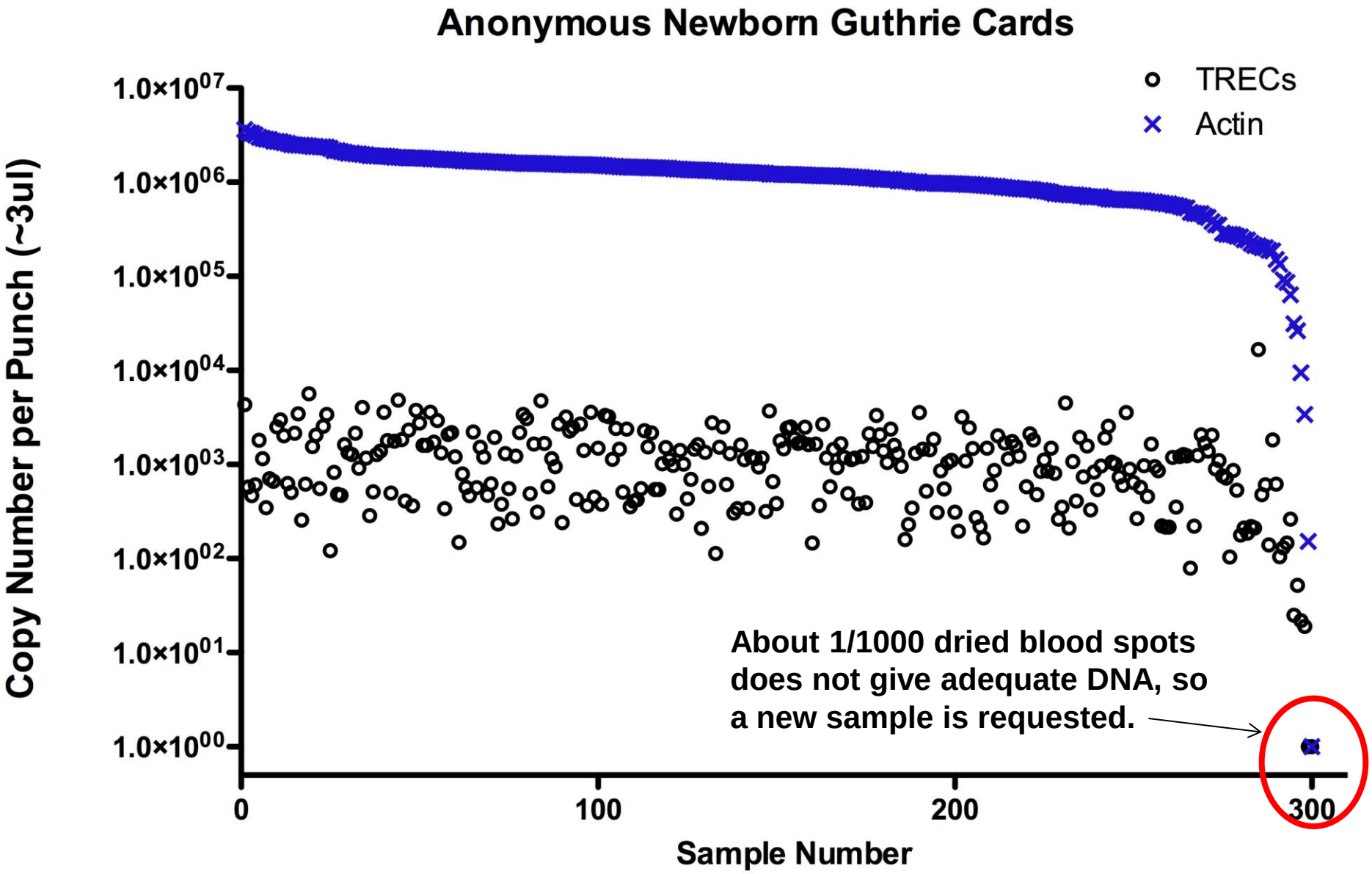
3 mm hole punched from blood spot

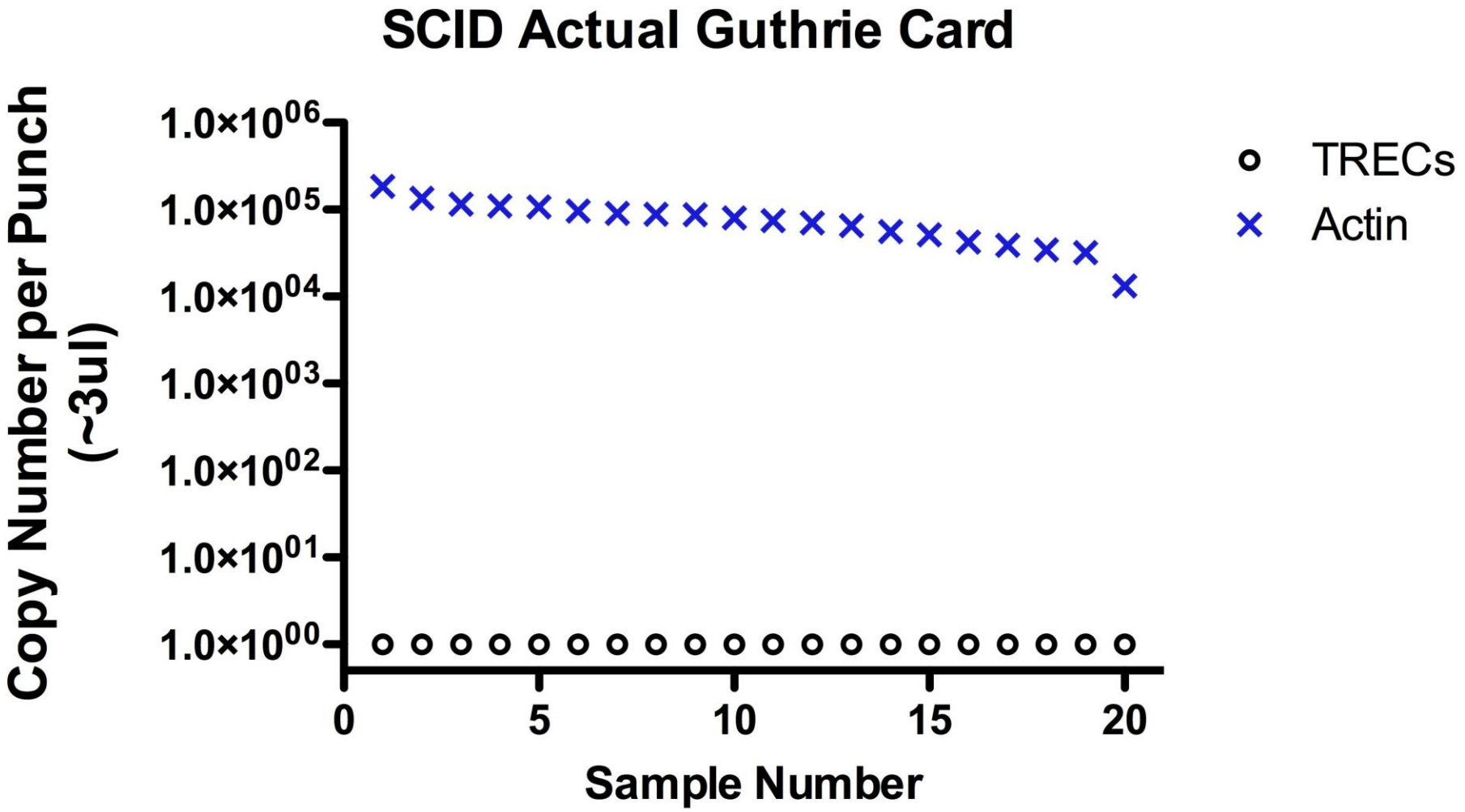
~3 ul blood

Extract DNA

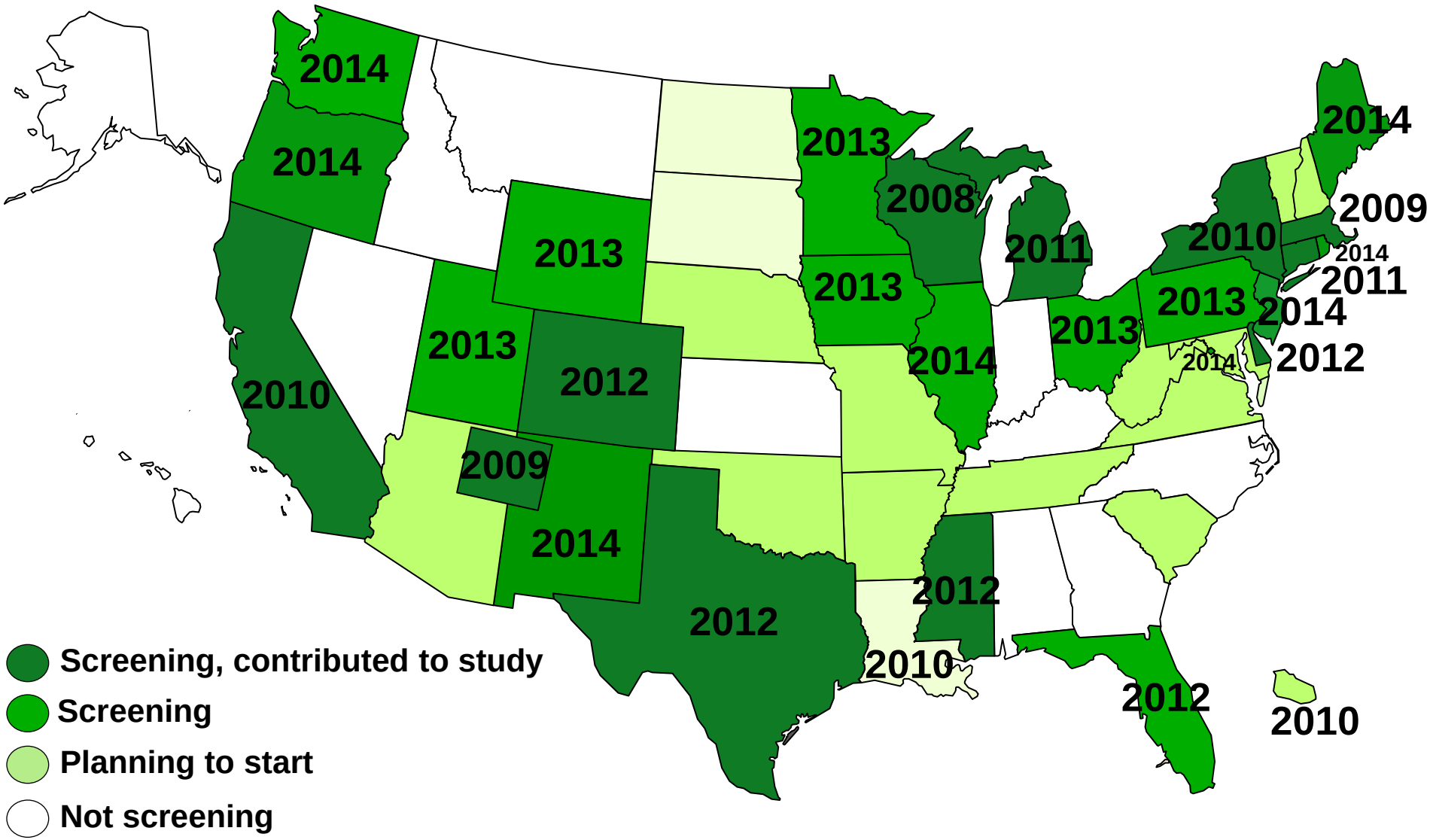
Measure TRECs by PCR




Anonymous Newborn Guthrie Cards
TRECs
Actin
Copy Number per Punch (~3ul)
1.0×10^07
1.0×10^06
1.0×10^05
1.0×10^04
1.0×10^03
1.0×10^02
1.0×10^01
1.0×10^00
0
100
200
300
Sample Number
About 1/1000 dried blood spots does not give adequate DNA, so a new sample is requested.

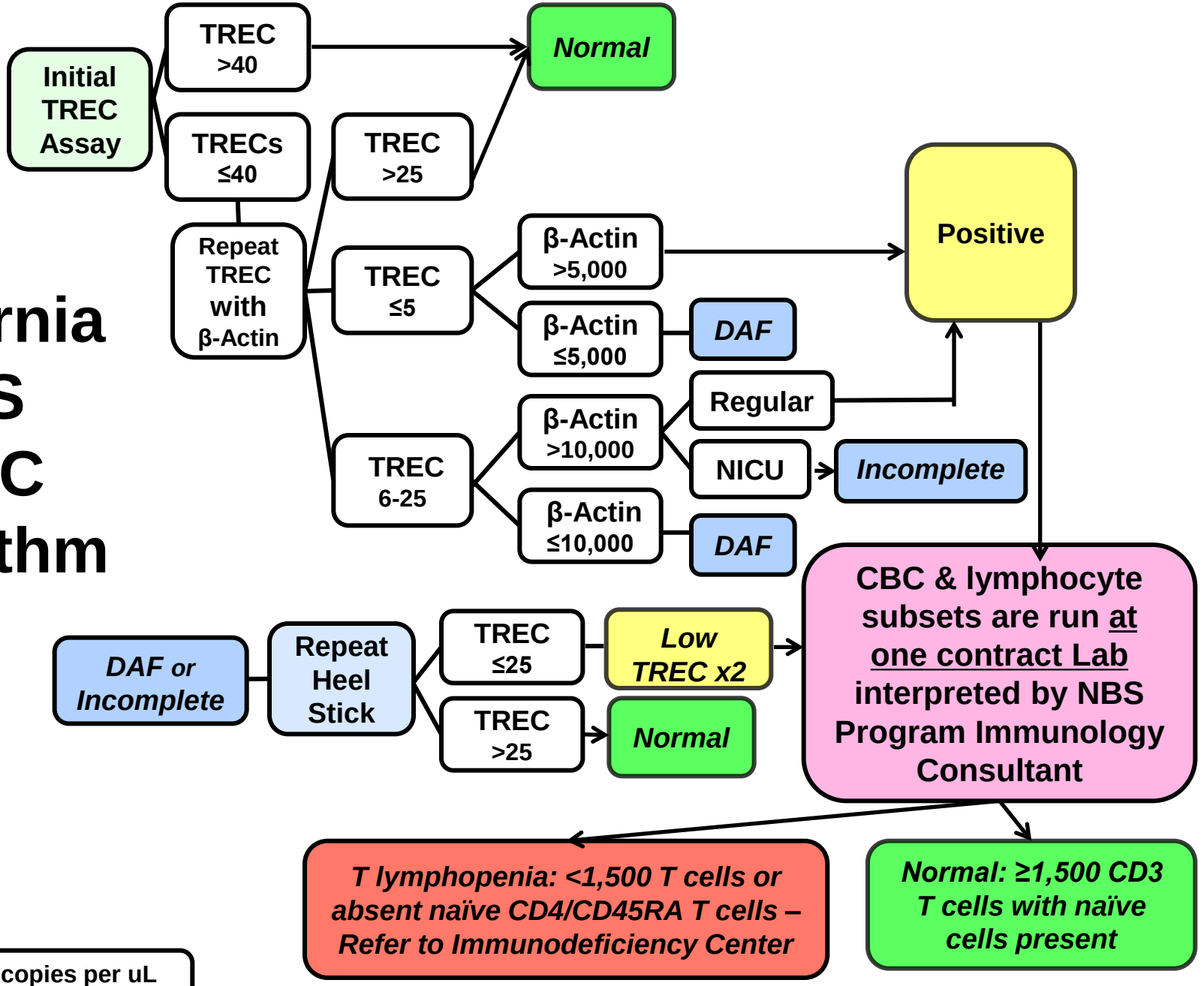

SCID Actual Guthrie Card
Copy Number per Punch (~3ul)
1.0×10^06
1.0×10^05
1.0×10^04
1.0×10^03
1.0×10^02
1.0×10^01
1.0×10^00
0
5
10
15
20
Sample Number
TRECs
Actin

# SCID Newborn Screening, October 2014




Amy Brower, NBSTRN; Emily Hovermale, IDF

# California NBS TREC Algorithm




TREC and Actin copies per uL of blood

# Severe Combined Immunodeficiency, SCID

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- Early death unless a working immune system can be established.

# SCID with Newborn Screening

- Absent T cells; no specific antibody production by B cells.
- ~~Recurrent infections and weight loss from age 2-4 months.~~
- ~~Serious bacterial, viral, & fungal infections; opportunistic pathogens that do not cause disease in healthy infants.~~
- ~~Early death unless a~~ working immune system can be established.
- Incidence 1/58,000 births.
- Typical SCID: <300/uL autologous T cells; <10% of normal proliferation to mitogens such as PHA; no production of specific antibodies.

# Sample Report by State Contract Flow Lab: (*IL7R* SCID)

| | | | |
|---|---|---|---|
| WBC | 3.0* | Thousand/uL | 5.0-19.5 |
| RBC | 2.83* | Million/uL | 3.10-5.30 |
| HGB | 9.7* | g/dL | 10.7-17.1 |
| HCT | 28.6* | % | 33.0-55.0 |
| MCV | 101.0 | fL | 88.0-123.0 |
| MCH | 34.3 | pg | 27.0-36.0 |
| MCHC | 34.0 | g/dL | 28.0-36.0 |
| PLT | 592* | Thousand/uL | 150-400 |
| MPV | 7.9 | fL | 7.5-11.6 |
| RDW | 14.9 | % | 11.5-16.0 |
| Absolute Neutrophils | 1680 | cells/uL | 1000-9000 |
| Absolute Band Neutrophils | 0* | cells/uL | 500-1700 |
| Absolute Metamyelocytes | 0 | cells/uL | 0 |
| Absolute Myelocytes | 0 | cells/uL | 0 |
| Absolute Promyelocytes | 0 | cells/uL | 0 |
| Absolute Lymphocytes | 630* | cells/uL | 2500-16500 |
| Absolute Monocytes | 660 | cells/uL | 200-1400 |
| Absolute Eosinophils | 30 | cells/uL | 15-800 |
| CD3 T-Cells, Absolute | <20* | cells/uL | 2550-5500 |
| CD3 T-Cells, Percent | <1* | % of lymphocytes | 53-84 |
| CD4 T-Helper, Absolute | <20* | cells/uL | 1600-4000 |
| CD4 T-Helper, Percent | <1* | % of lymphocytes | 35-64 |
| CD8 T-Cytotoxic, Absolute | <20* | cells/uL | 560-1700 |
| CD8 T-Cytotoxic, Percent | <1* | % of lymphocytes | 12-28 |
| CD19 B-Cells, Absolute | 328 | cells/uL | 300-2000 |
| CD19 B-Cells, Percent | 52* | % of lymphocytes | 6-32 |
| CD16/56 NK-Cell, Absolute | 132* | cells/uL | 170-1100 |
| CD16/56 NK-Cell, Percent | 21* | % of lymphocytes | 4-18 |
| CD3/CD4/CD45RA, Absolute | <20* | cells/uL | 1200-3700 |
| CD3/CD4/CD45RA, Percent | <1* | % of CD3 Cells | 64-95 |
| CD3/CD8/CD45RA, Absolute | <20* | cells/uL | 450-1500 |
| CD3/CD8/CD45RA, Percent | <1* | % of CD3 Cells | 56-88 |
| CD3/CD4/CD45RO, Absolute | <20* | cells/uL | 60-900 |
| CD3/CD4/CD45RO, Percent | <1* | % of CD3 Cells | 2-22 |
| CD3/CD8/CD45 RO, Absolute | <20* | cells/uL | 30-330 |
| CD3/CD8/CD45 RO, Percent | <1* | % of CD3 Cells | 1-9 |

# 4 Years of California SCID Newborn Screening (8/2010-8/2014)

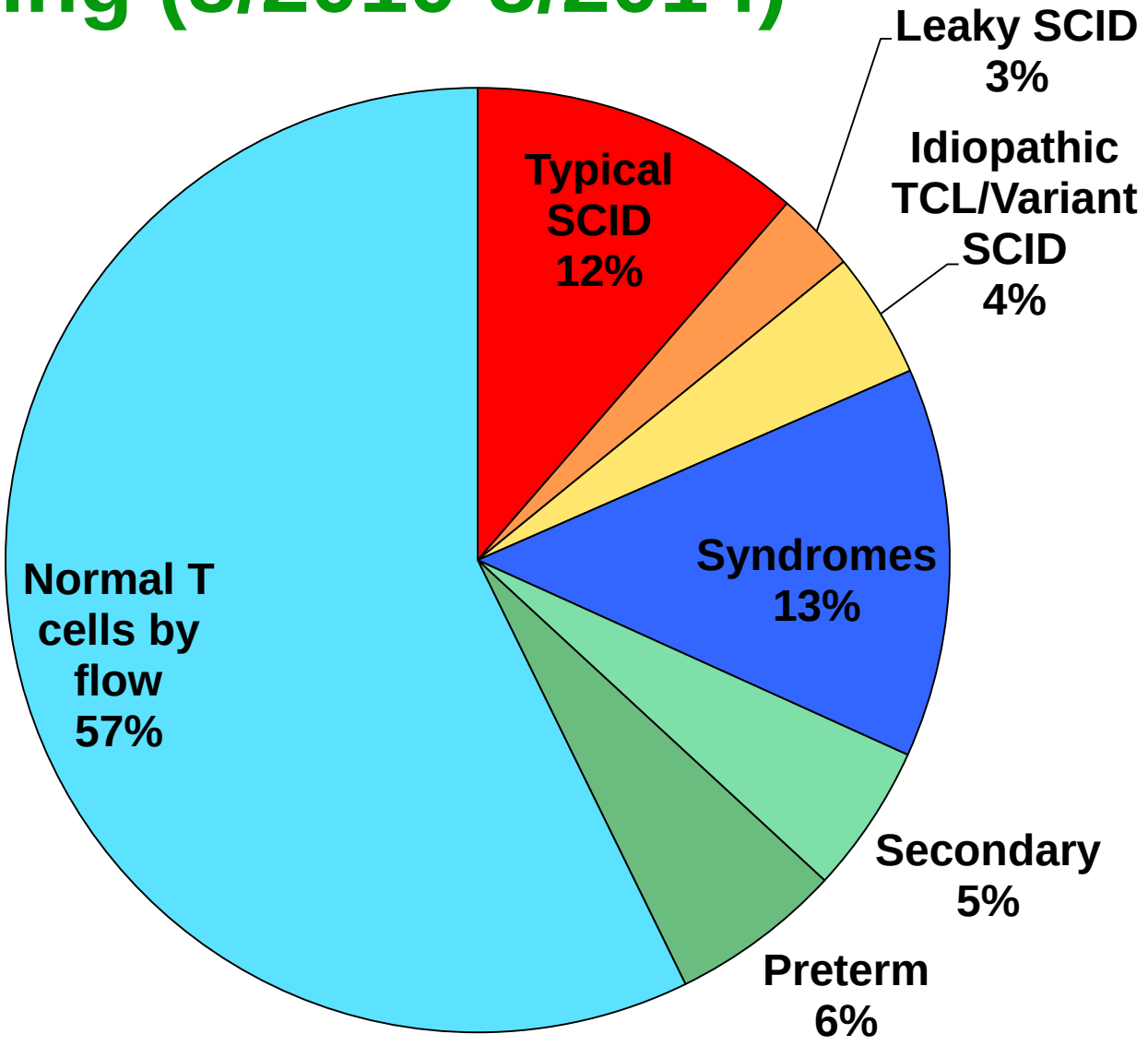
**1,980,133 infants screened**

**1.3 infants per 10,000 (255) required flow cytometry**

**109/255 had <1500 T cells/uL (43%)**

**<u>1/55,000 SCID (Typical and Leaky)</u>**

**1/180,000 Idiopathic TCL**



# First Summary of SCID Newborn Screening in the Navajo Nation 2009-14

**7,900 infants screened**

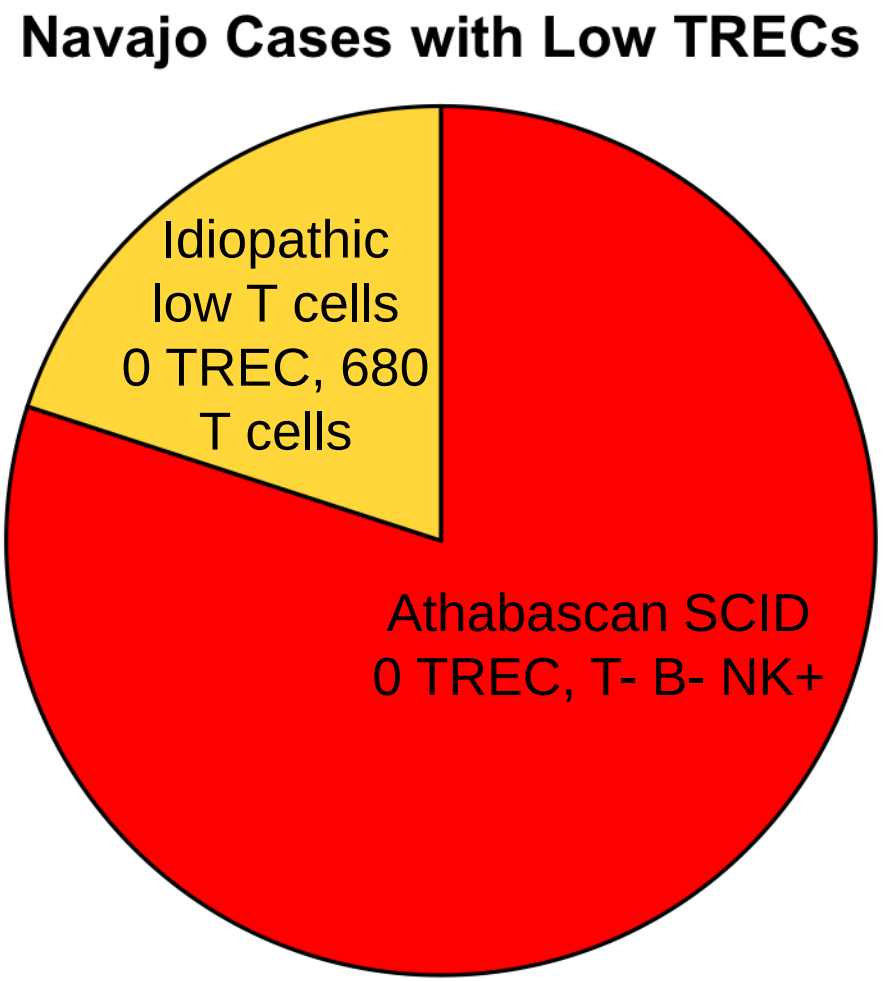
**5 low TRECs, needed flow cytometry (6/10,000 *vs. 1.3/10,000 in CA*)**

**5/5 had <1500 T cells/uL (100% *vs. 43% CA*)**

**4/7,900 SCID (1/1,975; All founder DCLRE1C *vs. previous est. 1/2,000*)**

**1/7,900 Idiopathic low T**

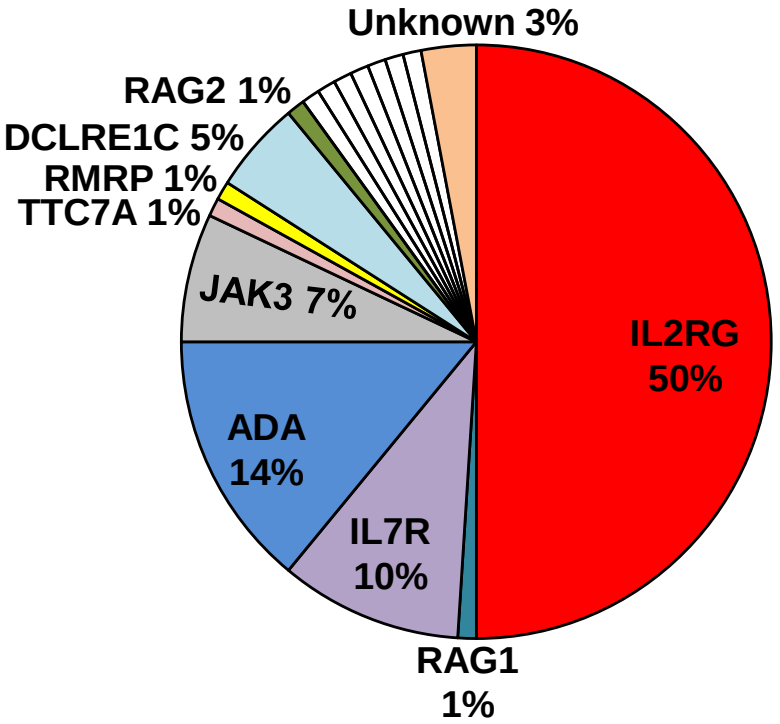
***Positive predictive value for TCL 100%, SCID 80%***



# Genotypes of Typical and Leaky SCID

## Reports from Transplant Centers, no Screening

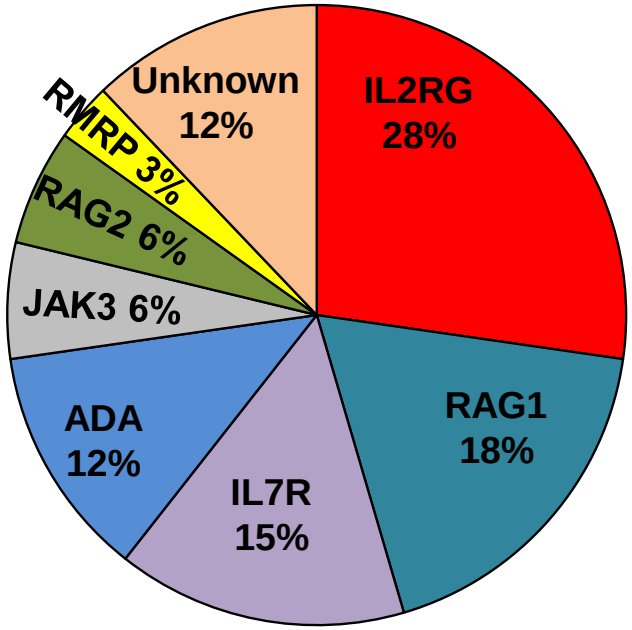
*Duke University, European centers (estimates)*




**Overall Survival ~74% or lower**

## California, with TREC Screening

*4 years, ~2 million infants*




**Overall Survival 95%**

# Non-SCID Conditions Detected (Secondary Targets of TREC Screening)

## Multisystem syndromes with variable T cell deficiency

**57% DiGeorge/chromosome 22q11.2 deletion**

**15% Trisomy 21**

**3% Ataxia telangiectasia**

**2% CHARGE syndrome**

**Many others…**

# DiGeorge Velo-Cardio-Facial Syndrome

Chromosome 22q11.2

Most common interstitial chromosome deletion (1/4,000 births)

90% de novo, 10% inherited as a dominant trait

Variable expressivity:

- Congenital heart defects
- Hypoparathyroidism (neonatal hypocalcemic seizures)
- Craniofacial anomalies (poor swallowing, speech)
- Behavioral and learning disorders
- >60% have low T cells in infancy, rarely no T cells

# Non-SCID Conditions Detected (Secondary Targets of TREC Screening)

**Multisystem syndromes with variable T cell deficiency**

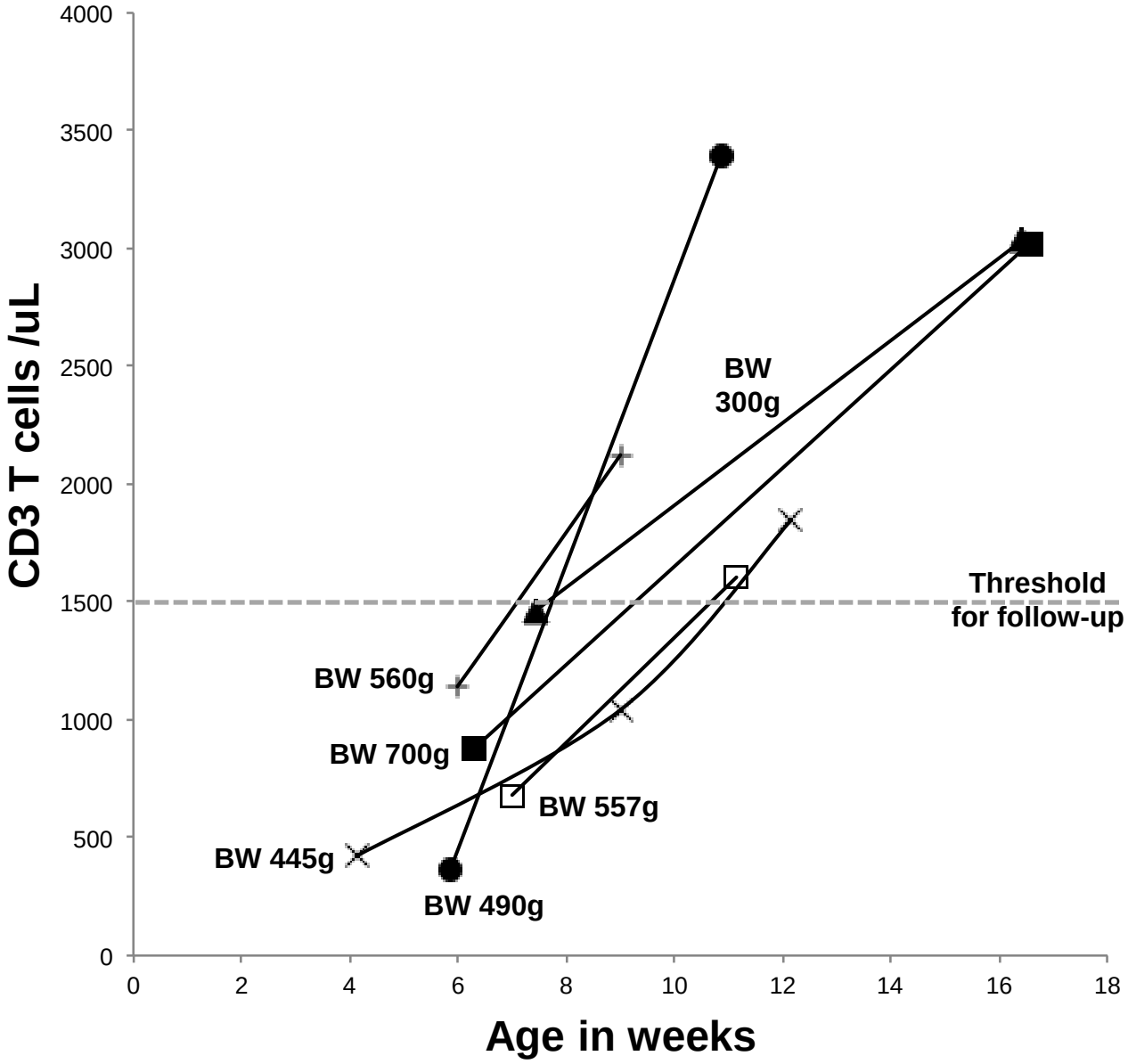
- 57% DiGeorge/chromosome 22q11.2 deletion
- 15% Trisomy 21
- 3% Ataxia telangiectasia
- 2% CHARGE syndrome

**Secondary T lymphopenia**

- 25% Congenital cardiac anomalies
- 38% Other congenital anomalies
- 13% Vascular leakage, third spacing, hydrops
- 3% Neonatal leukemia

**Extreme preterm birth—T cells become normal over time**

# Preterm Low Birthweight Infants with Low TRECs and T Lymphopenia



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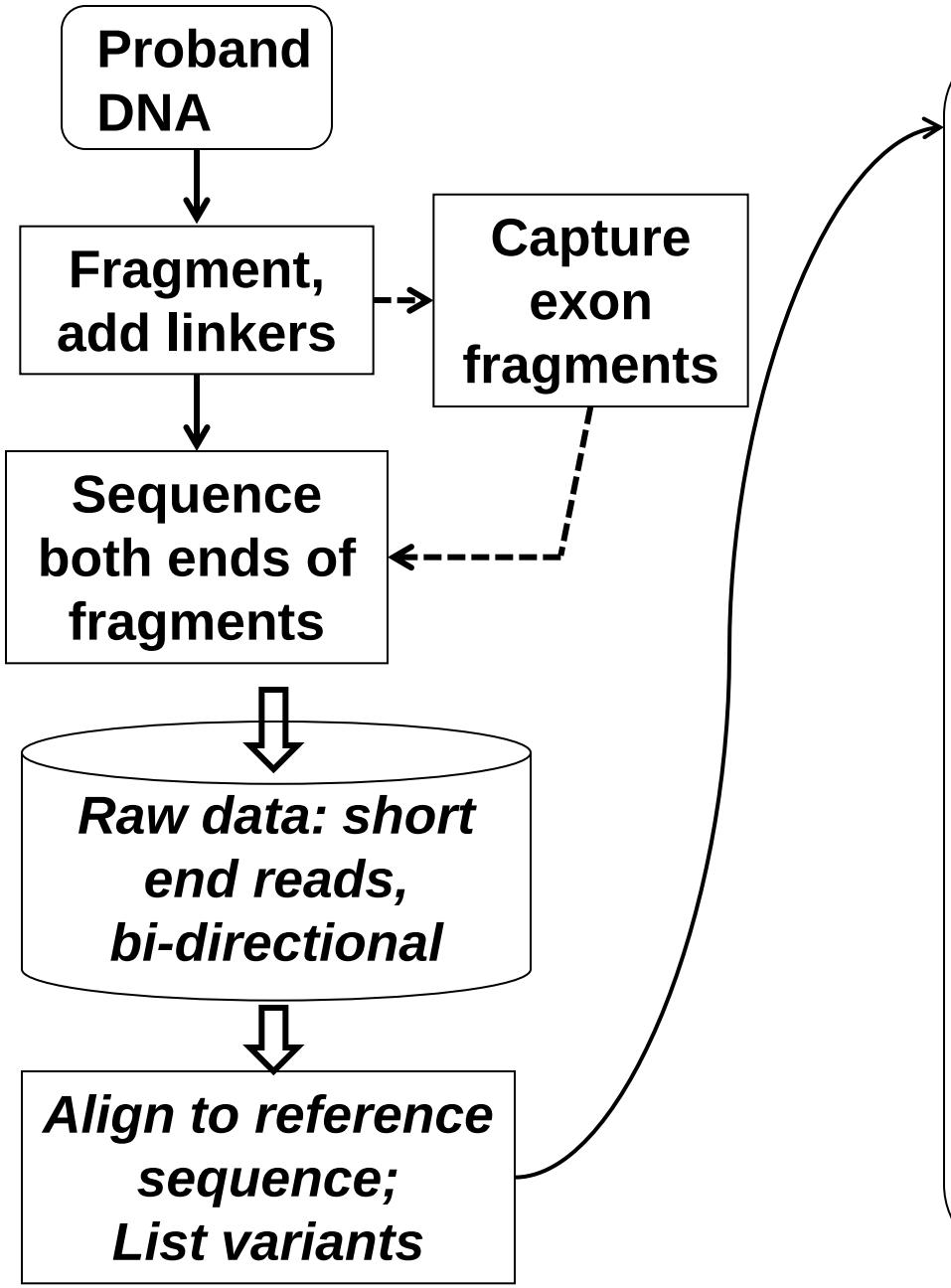
**Extreme preterm birth—T cells become normal over time**

**"Variant SCID" or Idiopathic T lymphopenia—few naïve T cells, no maternal engraftment, impaired T cell or antibody responses, no known gene defect**

# Variant SCID or Idiopathic T Lymphopenia

- **Persistent low but not absent T cells and TRECs, low naïve CD45RA T cells, no maternal engraftment.**
- **No known SCID gene mutation.**
- **Impaired T cell and/or antibody responses.**
- **When an etiology is found, case is moved to the appropriate category.**

# Search for Gene Diagnosis Using Deep Sequencing




***Filter: retain variants with***

**Good sequence quality**

**Predicted change in gene product**

**Absence in controls**

**Location in gene or pathway or tissue of interest,**

**Presence in relatives according to expected inheritance pattern**

**Same or other variants in same gene in unrelated individuals with same phenotype**

**Damage to protein function**

# What Does the TREC Test Miss?

- SCID infants who are not screened or not followed up
- Infants with a T cell defect *after* TCR recombination in thymus; T cells can develop, but function is affected
  - CD40L, MHC II, ZAP70 deficiency and others
- SCID sufficiently leaky to allow TRECs to be normal, or with temporary maternal compensating effects (ADA)
- Infants with a syndromes including variable T cell defects who have enough T cells for TRECs to be above cutoff (most DiGeorge)
- PIDs other than those causing low T cells, or that are not evident at birth
  - XLA, CVID, CGD, WAS

# Conclusions

1. SCID is a treatable serious, genetic immune deficiency affecting around 1/50,000 births.
2. Early diagnosis permits optimal treatment and best outcomes.
3. Population based newborn screening with TRECs identifies SCID, and also non-SCID conditions with low T cells, offering clinical benefit and opportunities to define the spectrum of disorders caused by a wide range of known and unknown gene defects.
4. A high index of suspicion is still needed for primary immune defects not picked up by TREC screening.

# Thanks to Many Collaborators

**California Dept. of Pub Health**
Bob Currier and colleagues
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