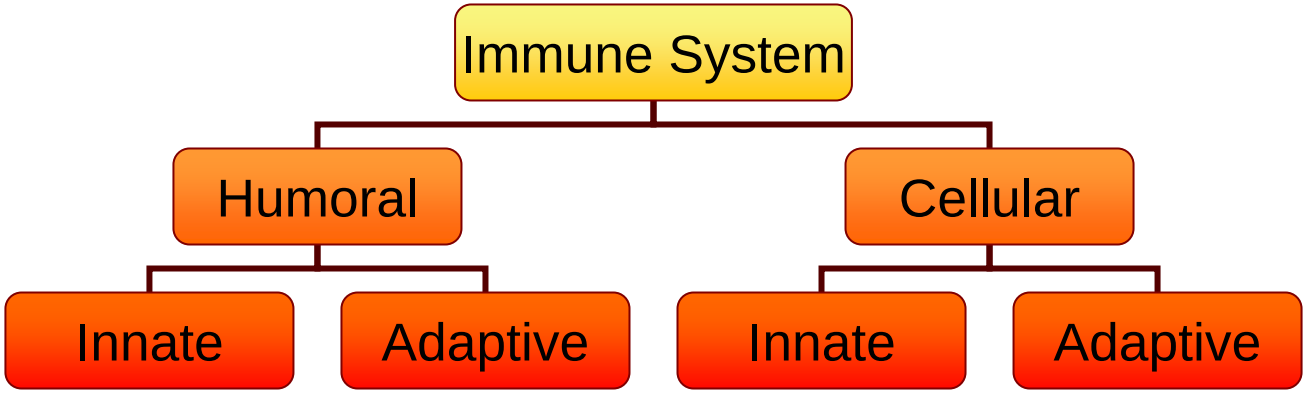
Basic Divisions



Humoral Immune System

- ✓ Liquid immunity
  - Antibodies
  - Complement
  - Acute Phase Reactants

Cellular Immune System

- ✓ Made up of living cells
- ✓ Includes lymphocytes, macrophages, granulocytes, dendritic cells
- ✓ Synthesize some components of humoral immune system

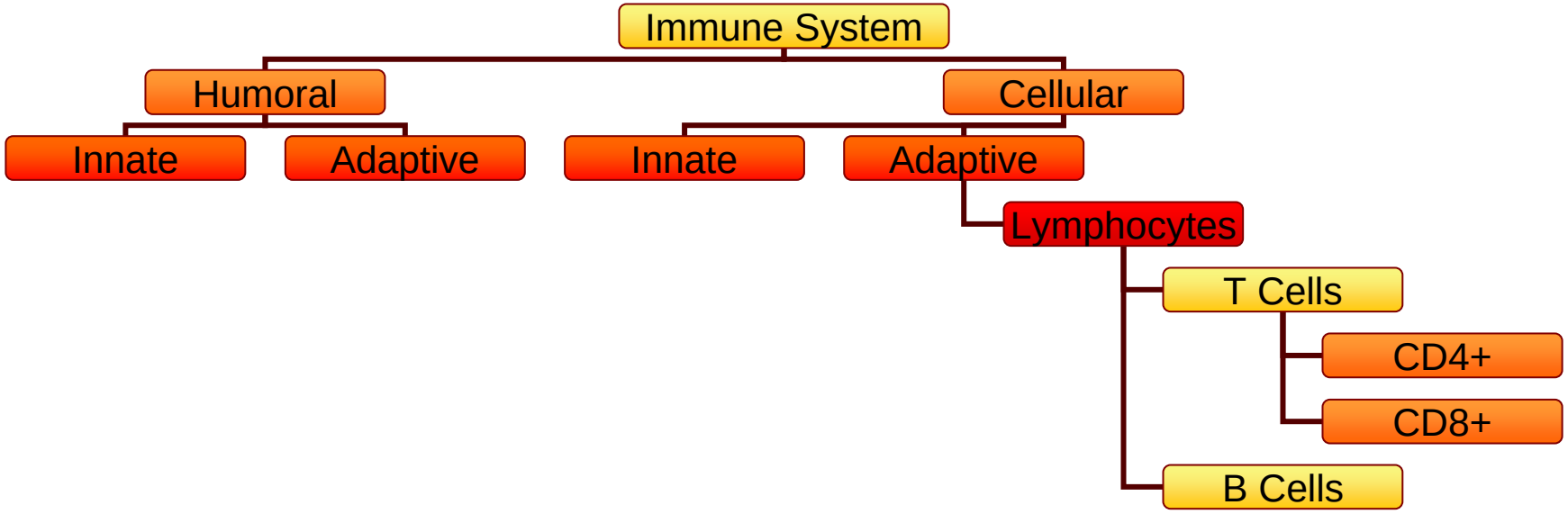
Innate Immunity

- ✓ Pre-existing immune response
  - Requires no previous exposure to antigen
- ✓ May not recognize everything it needs to…

Adaptive Immunity

- ✓ Developed in response to new antigens (“learned” immunity)
- ✓ Enables response to foreign proteins that have not been encountered before

Basic Divisions



Lymphocytes

- ✓ T cells
  - $CD4^+$
  - $CD8^+$
- ✓ B cells

B cells

- ✓ Synthesize antibody
- ✓ Develop specific receptors to foreign antigens that have not been encountered before
- ✓ Self vs. Non-self
- ✓ Reside in spleen and lymph nodes. When activated, create germinal centers that are clusters of B cells producing a specific antibody
- ✓ First IgM, then switch to IgG or other antibodies

T cells

- ✓ CD4+
  - Quarterbacks
  - Recognize antigen and provide instructions (cytokines) to other immune system effector cells
  - B cell help
  - CD4 is receptor for HIV
- ✓ CD8+
  - Hit Men
  - Cytotoxic T cells
  - Kill cells that are infected or cancerous (antibody can’t see organisms inside cells)

T cells' education

- ✓ Learn to be a T cell in the thymus
- ✓ Those that do poorly (attack self or don't recognize receptors) are killed off
- ✓ Also have unique receptors similar to B cells
  - But with one important difference

Major Histocompatability Complex

- ✓ Ship's flag
- ✓ Every cell samples antigens it produces and displays them on their membranes (MHC I)
- ✓ T cells bind to antigen in the context of MHC
- ✓ Some cells (macrophages, dendritic cells) also display antigens that they find around them (MHC II)
- ✓ CD4+ T cells recognize these and then know to send instructions to respond to these antigens (B cell help)

SCID as the absence of adaptive immunity

- ✓ No T-cell function
- ✓ No B-cell function
- ✓ Otherwise healthy appearance
- ✓ Results from defects in T-cell development or defective T-cell function

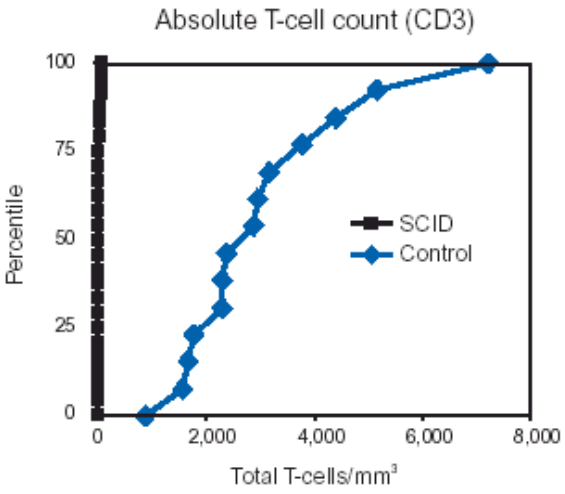
Typical SCID presentation

- ✓ Healthy at birth
- ✓ Within first few months
  - Pneumonia
  - Viral infections
  - Opportunistic infections
  - Diarrhea
  - Failure to thrive

Pathophysiology: A lack of functional T cells prevents adaptive response

- ✓ Lack of development
- ✓ Present but non-functional (rarely)
- ✓ B cells lack T-cell help and so fail to produce antibody
- ✓ Innate immune responses are intact

A Variety of Diseases Comprise SCID, But Most Are Identified By T-Cell Lymphopenia



Buckley (2004), Lindegren (2004)

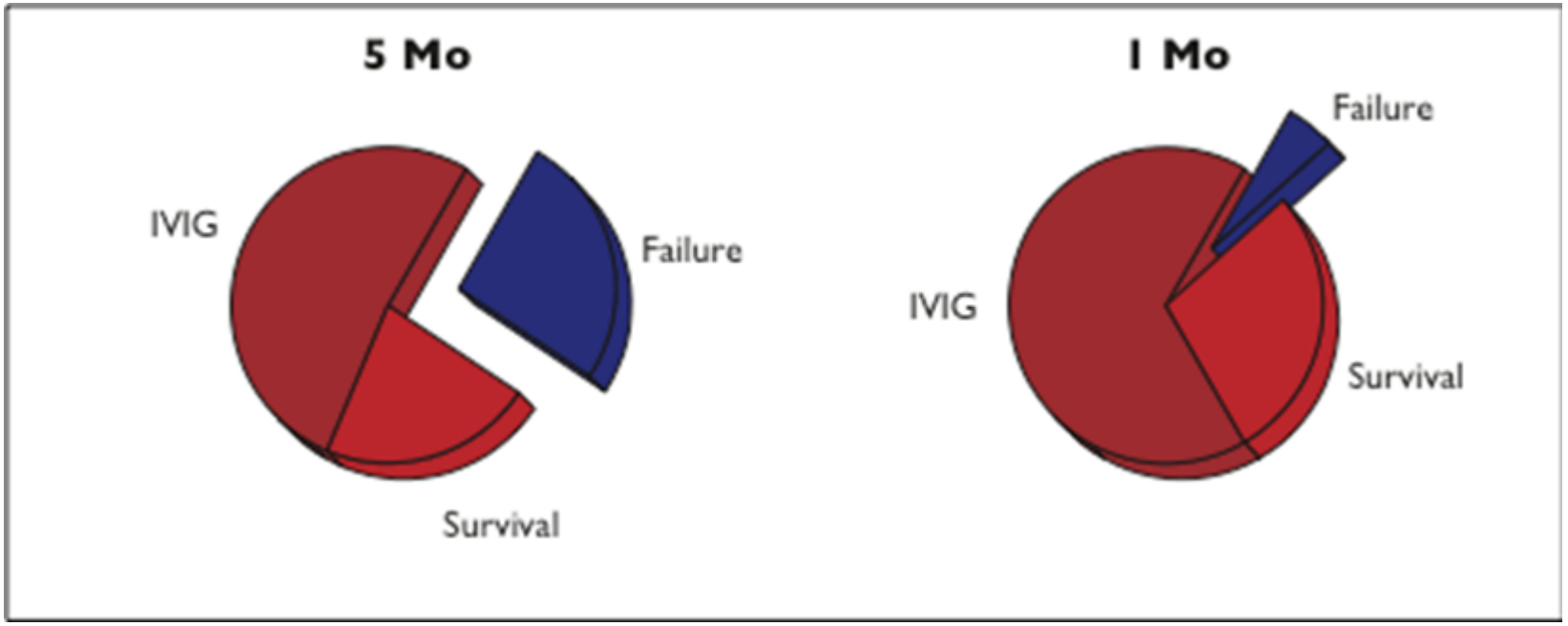
Early therapy for SCID

- ✓ No vaccination
- ✓ All transfusions irradiated, CMV negative
- ✓ IVIG for antibody replacement
- ✓ Bactrim prophylaxis for pneumocystis
- ✓ Transplant evaluation

Treatment of SCID requires stem cell transplant (usually)

- ✓ Matched related donor
- ✓ Matched unrelated donor
- ✓ Haploidentical T-cell depleted donor
- ✓ Method differs from oncologic
  - Minimally or non-ablative

Early Transplant Improves SCID Outcomes



Myers, *et al*. (2002)

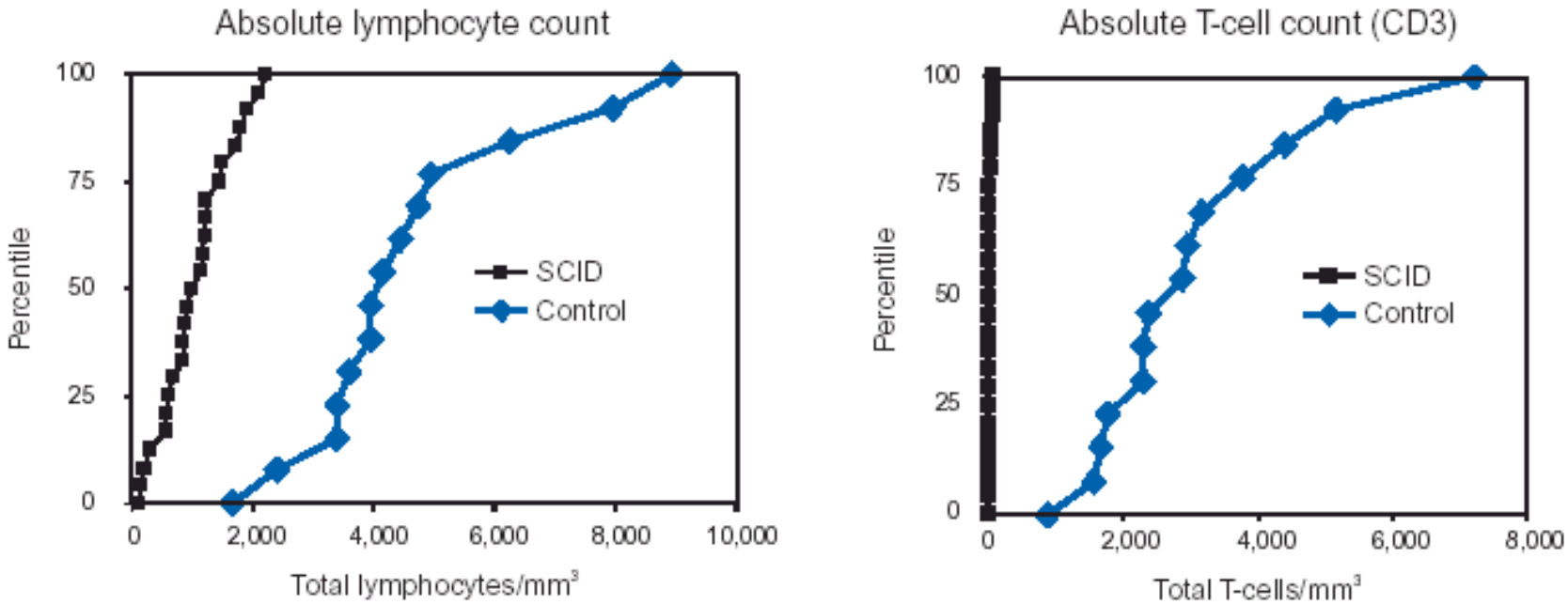
Long term treatment outcomes are excellent

- ✓ Decades long survival
- ✓ Approximately 20-30% may require monthly IVIG
- ✓ Premature immune senescence (unclear significance)
- ✓ Some patients with persistent warts (?10%)

Newborn Screening for Lymphopenia

California SCID Screening Pilot

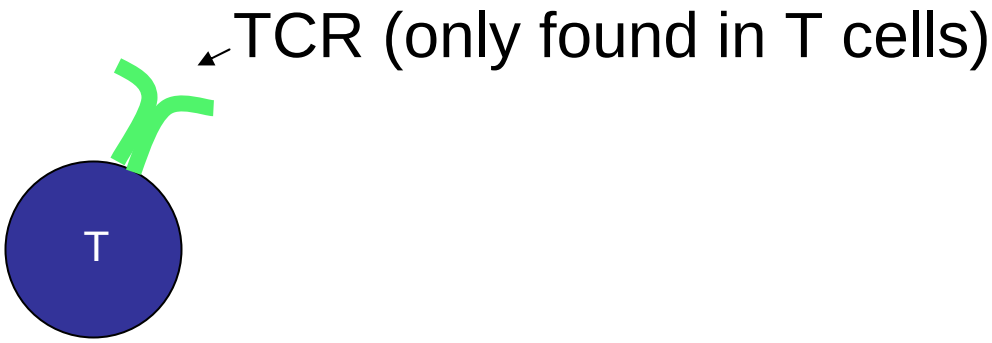
Figure 1. Absolute lymphocyte and T-cell counts in healthy and SCID infants (Lindegren *et al*., 2004).



Screening by CXR

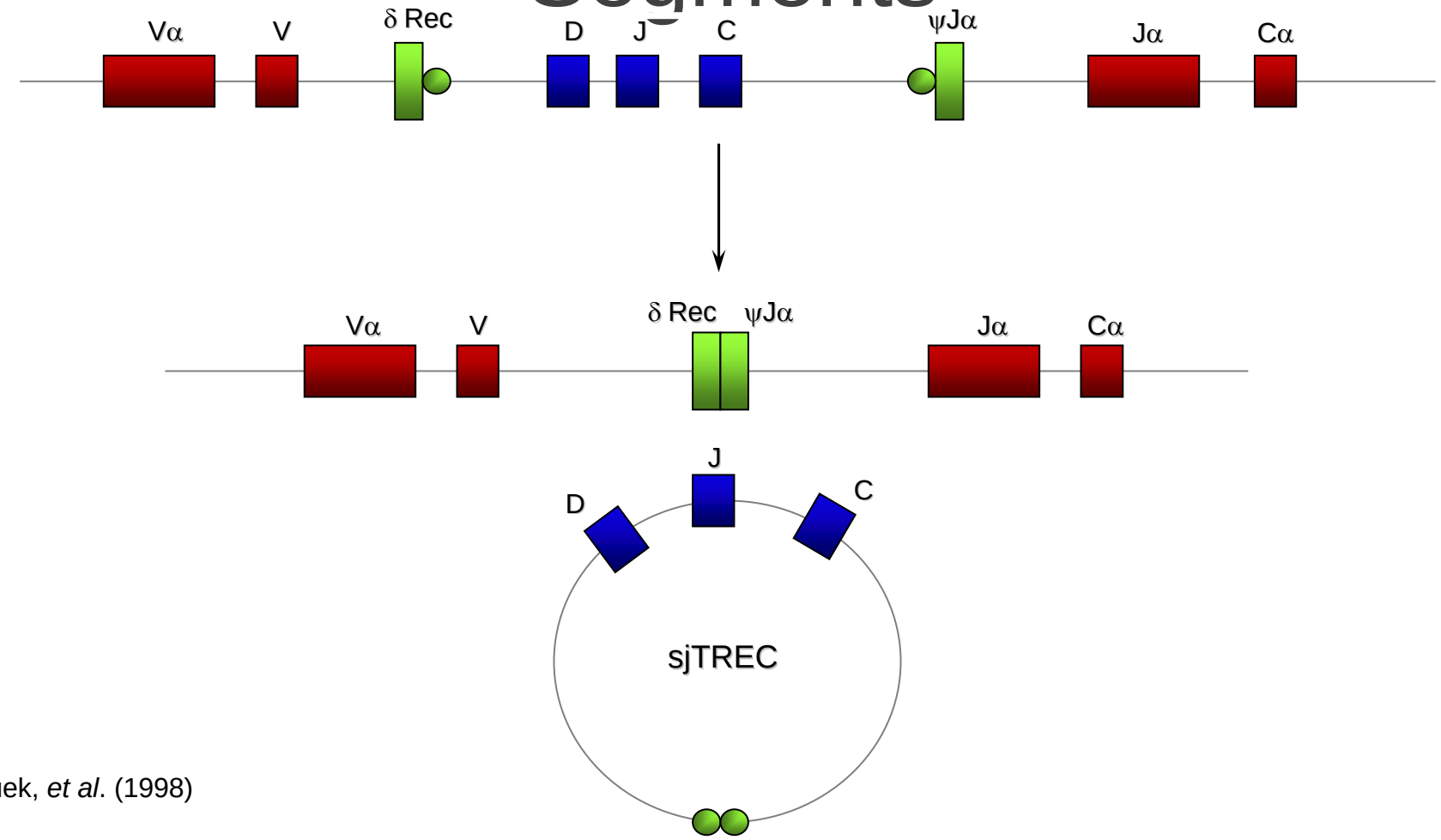
- ✓Chest X-ray shows no evidence of thymus
- ✓May show signs of pneumonia, including *Pneumocystis jirovecii*

T-cell Receptor Excision Circles (TRECS)



- ✓ Creation of T-cell receptor produces a unique fragment of DNA that does not occur in any other cell (TREC)
- ✓ Counting these fragments can provide a substitute for T-cell counts

Generation of Signal Joint TRECs During Removal of δ Gene Segments



Douek, *et al*. (1998)

Newborns have high TREC and lymphocyte counts relative to adults

- ✓ Infants born with large numbers of lymphocytes (> twice adult values)
  - – Frequently leads to misdiagnosis of lymphopenia in infants
- ✓ Number rises in first month, then contracts over first few years of life

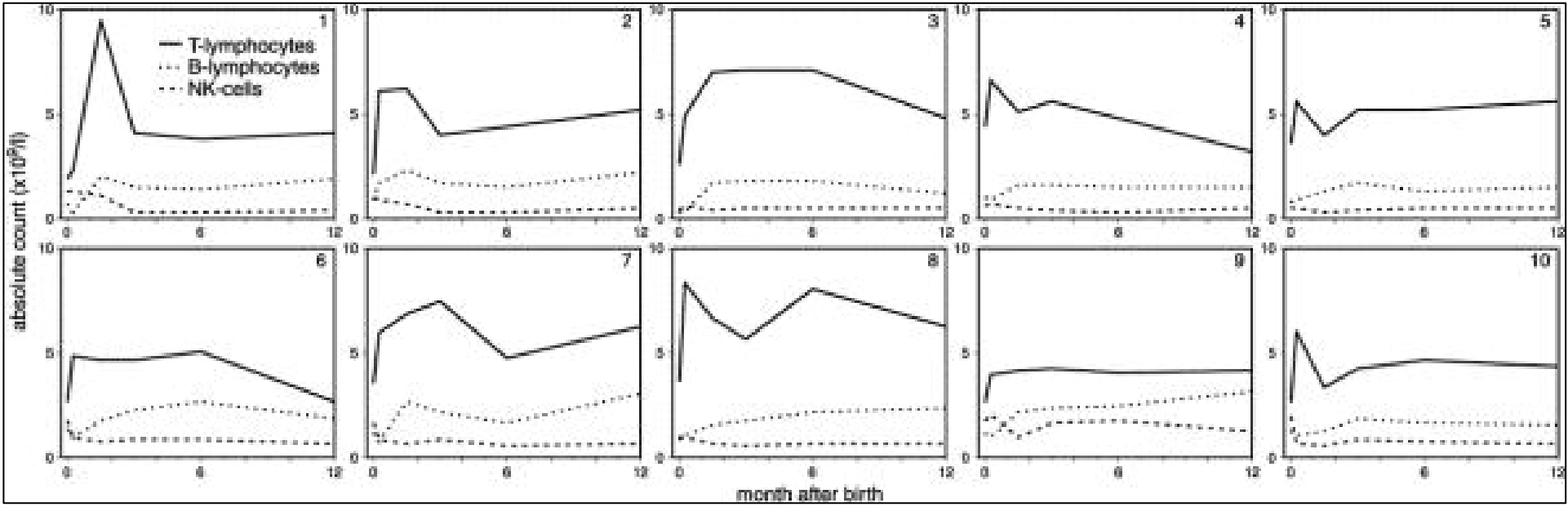

T-lymphocytes
B-lymphocytes
NK-cells
absolute count (x10⁹/l)
month after birth
1
2
3
4
5
6
7
8
9
10
0
5
10
0
6
12

Table 2. *Absolute counts of lymphocytes, CD3⁺ T lymphocytes, and CD3⁺ T lymphocyte subpopulations in 11 infants during follow-up from birth to 1 y of age and in nine adults*

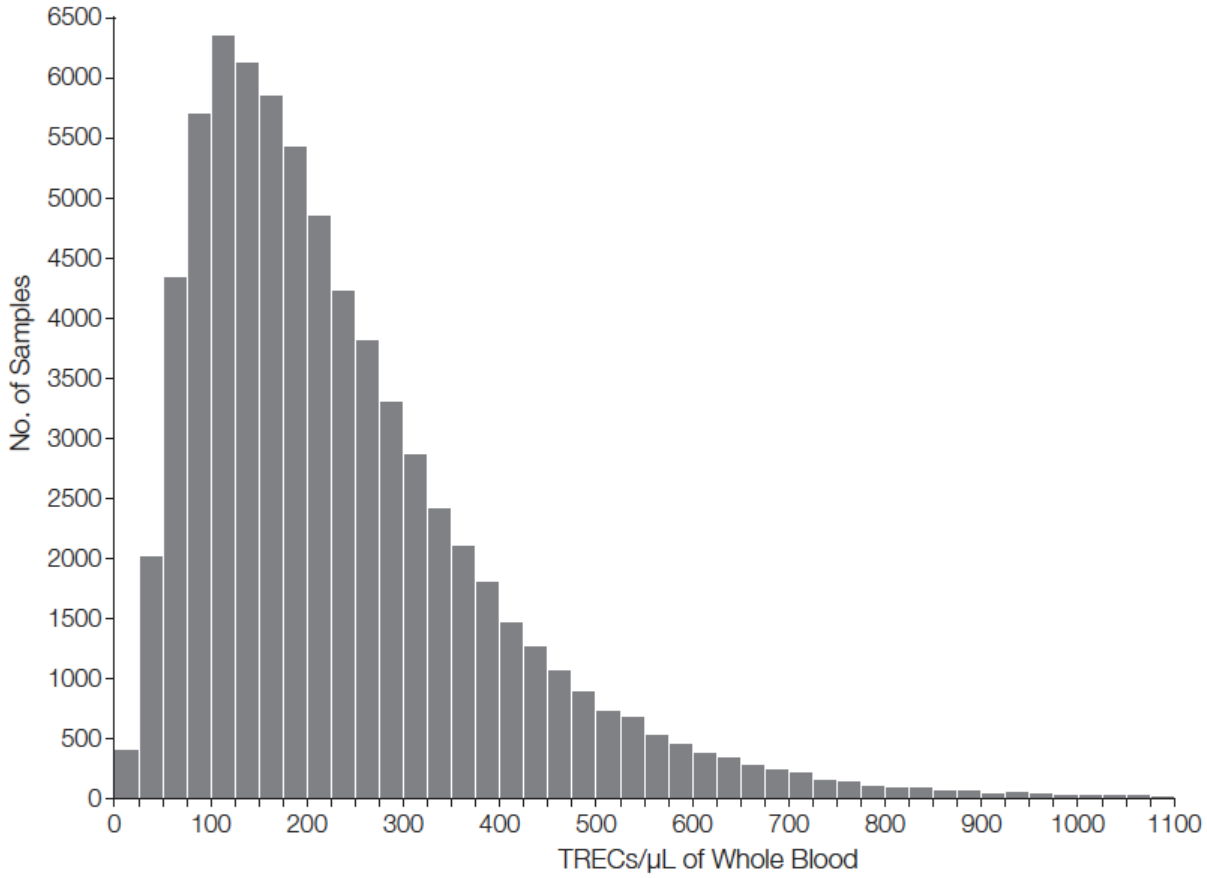
Cell type	Neonatal cord blood	1 wk	6 wk	3 mo	6 mo	1 y	p value*	Adults
Total lymphocytes	4.7 (3.5–6.2)†	6.9 (4.0–9.6)	7.2 (4.1–12.6)	7.1 (5.8–9.6)	6.8 (5.6–9.9)	7.1 (4.1–8.9)	<0.001	2.1 (1.1–2.4)
$CD3^+$ T lymphocytes	2.6 (1.9–4.4)	5.6 (2.4–8.0)	5.1 (3.0–9.5)	4.5 (3.9–7.1)	4.5 (3.7–7.7)	4.4 (2.3–5.9)	<0.001	1.5 (0.7–1.8)
T cell subpopulations								
$TcR\alpha\beta^+/CD3^+$	2.5 (1.8–4.3)	5.5 (2.2–7.7)	4.9 (2.9–8.5)	4.2 (3.5–7.0)	4.2 (3.3–7.5)	4.0 (2.0–5.8)	<0.001	1.4 (0.6–1.7)
$TcR\gamma\delta^+/CD3^+$	0.1 (0.0–0.1)	0.1 (0.0–0.2)	0.1 (0.1–0.8)	0.2 (0.1–0.3)	0.2 (0.1–0.3)	0.2 (0.1–0.3)	<0.001	0.1 (0.0–0.2)
$CD4^+/CD3^+$	2.0 (1.4–3.5)	4.1 (2.0–6.1)	3.3 (2.2–5.4)	3.4 (2.6–6.1)	3.5 (2.4–5.5)	3.2 (1.5–5.0)	<0.001	1.0 (0.4–1.1)
$CD8^+/CD3^+$	0.6 (0.5–1.0)	1.3 (0.4–2.4)	1.2 (0.7–5.8)	1.0 (0.8–1.4)	1.0 (0.5–2.2)	1.0 (0.6–1.5)	<0.001	0.5 (0.3–0.8)
$CD4^+/CD8^+/CD3^+$	0.1 (0.0–0.1)	0.1 (0.0–0.1)	0.1 (0.0–0.4)	0.1 (0.0–0.2)	0.0 (0.0–0.2)	0.0 (0.0–0.2)	NS	0.0 (0.0–0.1)
$CD1a^+/CD3^+$	0.1 (0.0–0.3)	0.0 (0.0–0.3)	0.0 (0.0–0.5)	0.0 (0.0–0.2)	0.0 (0.0–0.0)	0.0 (0.0–0.1)	NS	0.0 (0.0–0.1)
$CD45RA^+/CD3^+$	1.7 (1.0–3.9)	4.3 (1.9–5.5)	3.6 (2.1–5.2)	3.8 (2.7–5.8)	3.7 (2.8–6.7)	3.8 (1.8–5.1)	<0.001	0.9 (0.3–1.1)
$CD45RO^+/CD3^+$	0.6 (0.3–1.8)	1.2 (0.7–2.4)	0.9 (0.4–6.1)	1.1 (0.6–1.9)	0.8 (0.6–2.6)	0.8 (0.5–2.1)	<0.001	0.8 (0.5–1.0)
$CD38^+/CD3^+$	2.5 (1.8–4.3)	5.4 (2.2–7.7)	4.8 (2.9–9.3)	4.1 (3.6–7.0)	4.2 (3.3–7.4)	4.0 (2.0–5.6)	<0.001	0.7 (0.3–0.9)
$CD4^+/CD45RA^+/CD3^+$	1.2 (0.6–3.1)	2.4 (0.1–4.0)	2.3 (1.3–4.2)	2.8 (1.9–4.8)	2.9 (1.9–4.9)	2.7 (1.1–4.3)	<0.001	0.4 (0.1–0.8)
$CD8^+/CD45RA^+/CD3^+$	0.6 (0.4–0.8)	1.1 (0.4–1.7)	1.0 (0.6–2.5)	0.9 (0.7–1.2)	0.9 (0.5–1.9)	1.0 (0.6–1.3)	<0.001	0.3 (0.1–0.6)
$CD4^+/CD45RO^+/CD3^+$	0.4 (0.2–1.2)	1.0 (0.1–1.9)	0.7 (0.3–2.2)	0.7 (0.4–1.6)	0.6 (0.5–2.3)	0.6 (0.4–1.5)	<0.001	0.6 (0.3–0.7)
$CD8^+/CD45RO^+/CD3^+$	0.1 (0.1–0.5)	0.2 (0.1–0.7)	0.1 (0.1–4.8)	0.2 (0.1–0.5)	0.1 (0.1–0.7)	0.1 (0.1–0.5)	0.002	0.2 (0.2–0.4)
$CD38^-/CD45RA^+/CD3^+$	0.1 (0.0–0.1)	0.1 (0.0–0.2)	0.1 (0.0–0.2)	0.1 (0.1–0.2)	0.1 (0.1–0.2)	0.2 (0.1–0.3)	<0.001	0.4 (0.2–0.7)
$CD38^+/CD45RA^+/CD3^+$	1.8 (1.1–3.7)	4.2 (1.9–5.4)	3.5 (2.0–5.1)	3.6 (2.4–5.8)	3.5 (2.7–6.4)	3.6 (1.6–4.9)	<0.001	0.5 (0.2–0.7)
$CD38^-/CD45RO^+/CD3^+$	0.1 (0.0–0.2)	0.1 (0.1–0.2)	0.2 (0.1–0.3)	0.1 (0.1–0.3)	0.2 (0.1–0.4)	0.2 (0.1–0.3)	<0.001	0.6 (0.4–0.8)
$CD38^+/CD45RO^+/CD3^+$	0.5 (0.3–1.6)	1.1 (0.6–2.2)	0.8 (0.4–6.0)	0.8 (0.5–1.5)	0.7 (0.5–2.0)	0.6 (0.3–2.1)	<0.001	0.3 (0.1–0.4)
$CD38^-/CD4^+/CD3^+$	0.1 (0.0–0.1)	0.1 (0.1–0.2)	0.2 (0.1–0.2)	0.2 (0.1–0.2)	0.2 (0.1–0.2)	0.2 (0.1–0.2)	<0.001	0.1 (0.0–0.5)
$CD38^+/CD4^+/CD3^+$	1.9 (1.3–3.5)	4.0 (1.9–6.0)	3.1 (2.2–5.3)	3.2 (2.4–6.0)	3.4 (2.3–5.4)	3.0 (1.3–4.8)	0.002	0.5 (0.2–0.8)
$CD38^-/CD8^+/CD3^+$	0.1 (0.0–0.1)	0.1 (0.0–0.2)	0.1 (0.0–1.2)	0.1 (0.0–0.1)	0.1 (0.0–0.2)	0.2 (0.0–0.3)	0.001	0.1 (0.0–0.8)
$CD38^+/CD8^+/CD3^+$	0.6 (0.5–0.9)	1.3 (0.4–2.2)	1.0 (0.5–5.4)	1.0 (0.7–1.3)	0.9 (0.5–2.1)	0.8 (0.5–1.3)	NS	0.1 (0.0–0.1)
$CD25^+/CD3^+$	0.2 (0.2–0.4)	0.7 (0.5–0.8)	0.5 (0.3–0.8)	0.4 (0.3–0.7)	0.4 (0.3–0.5)	0.3 (0.2–0.5)	<0.001‡	0.4 (0.2–0.5)
$HLA\text{-}DR^+/CD3^+$	0.2 (0.0–0.9)	0.2 (0.1–1.6)	0.2 (0.1–6.6)	0.1 (0.1–1.3)	0.1 (0.1–0.2)	0.1 (0.1–0.3)	NS	0.4 (0.0–0.8)
$CD57^+/CD3^+$	0.0 (0.0–0.0)	0.0 (0.0–0.1)	0.0 (0.0–0.8)	0.0 (0.0–0.5)	0.0 (0.0–0.3)	0.1 (0.0–0.6)	NS	0.1 (0.0–0.2)

† Median absolute count followed by minimal and maximal absolute count in parentheses $\times 10^9$/L.

‡ also 1 wk > 6 wk–1 y, 6 wk > 3 mo–1 y, 3 mo > 6 mo–1 y, all $p < 0.001$.

* Neonatal cord blood < 1 wk, 6 wk, 1 mo, 3 mo, 6 mo, and 1 y.

Figure 1. Distribution of TRECs in NBS Cards From 71 000 Infants in 2008

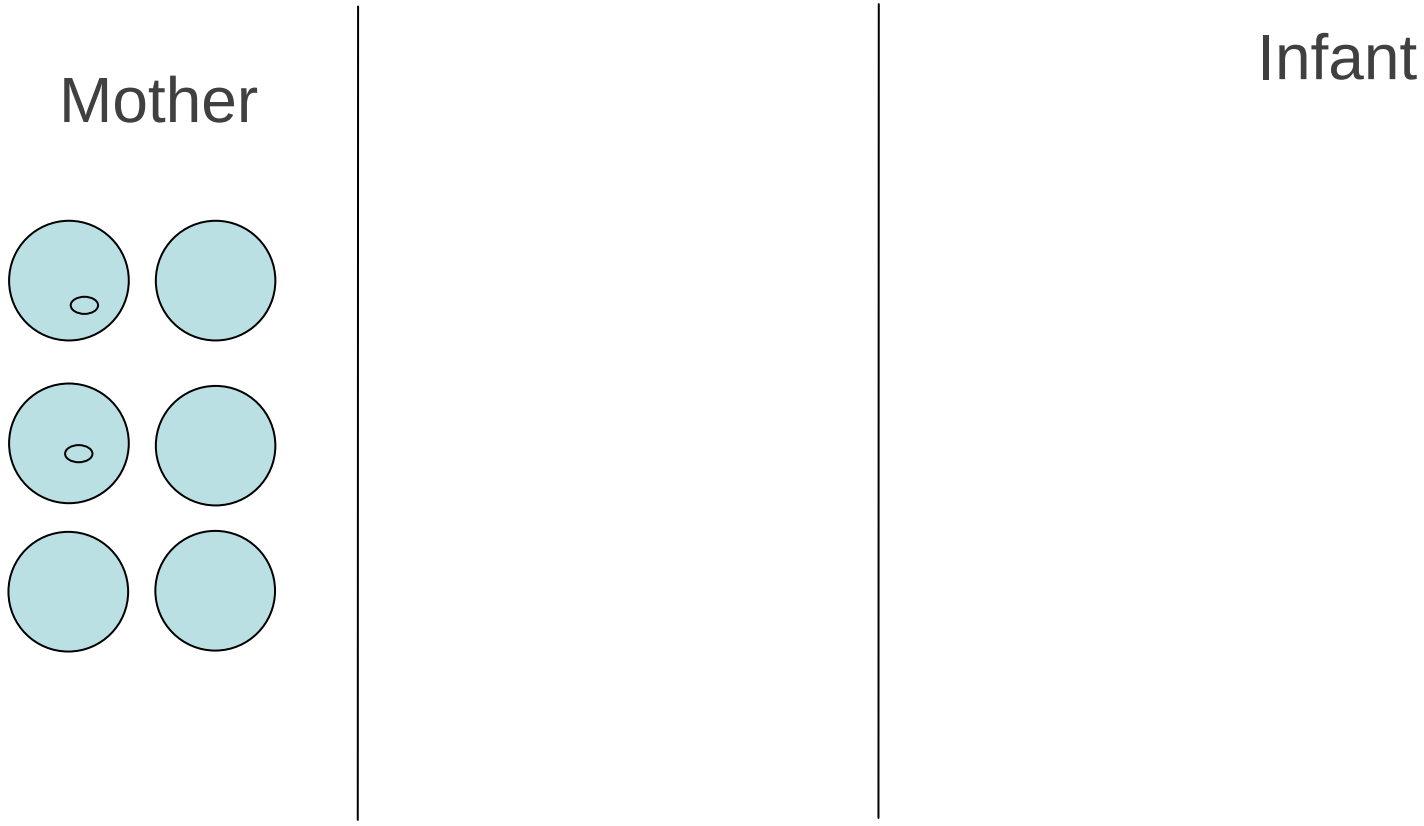


TRECs indicate T-cell receptor excision circles; NBS, newborn blood screening. The mean number of TRECs was 225 TRECs/μL of whole blood and the median was 186 TRECs/μL of whole blood. A total of 115 samples have TRECs of more than 1050/μL, ranging from 1051/μL to 5184/μL.

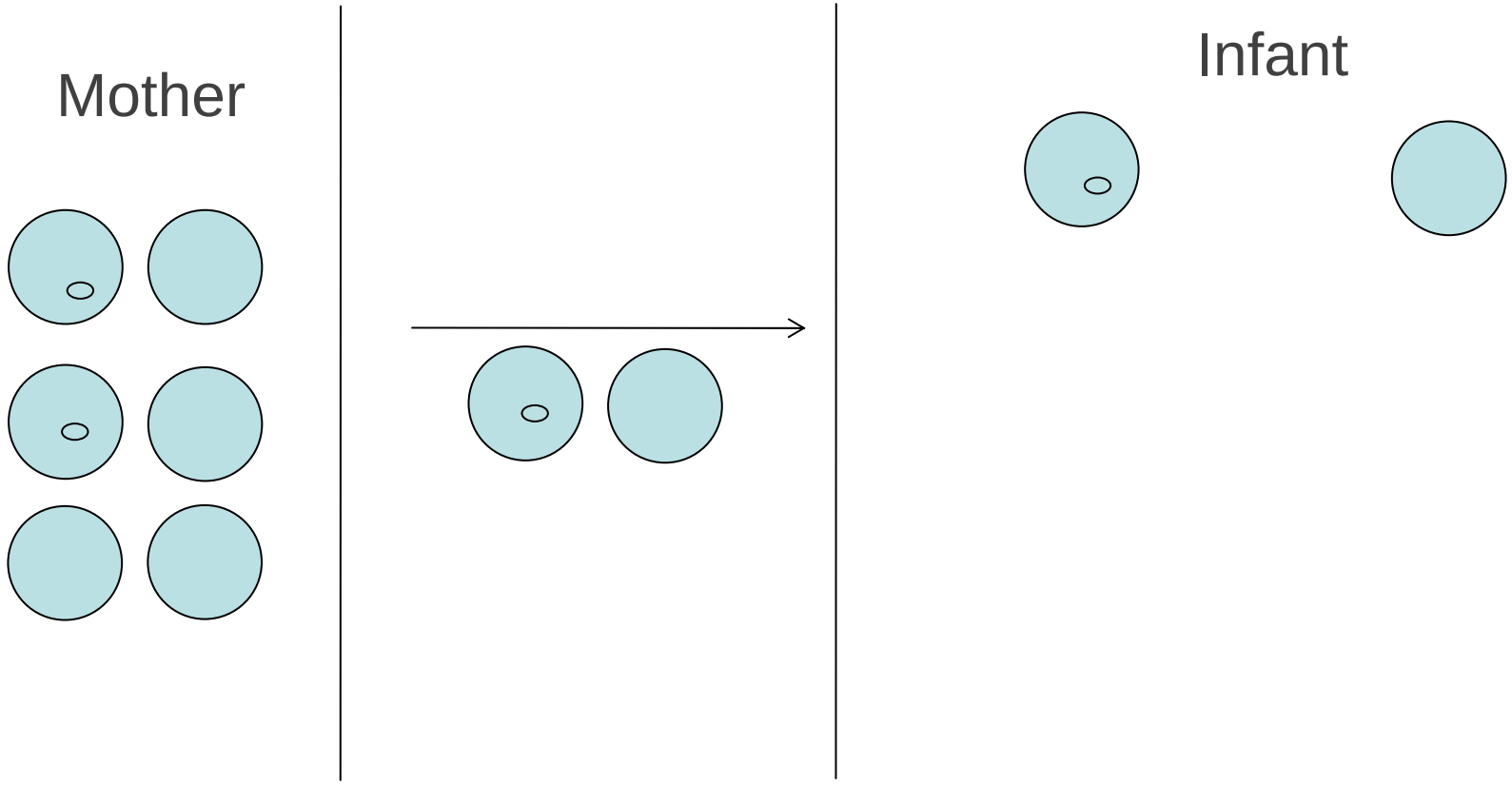
SCID infants can rarely have normal numbers of T cells

- ✓ Maternal engraftment
- ✓ Peripheral expansion
  - – Leaky SCID
  - – Omenn syndrome
- ✓ Inappropriate transfusion
- ✓ TREC numbers remain low

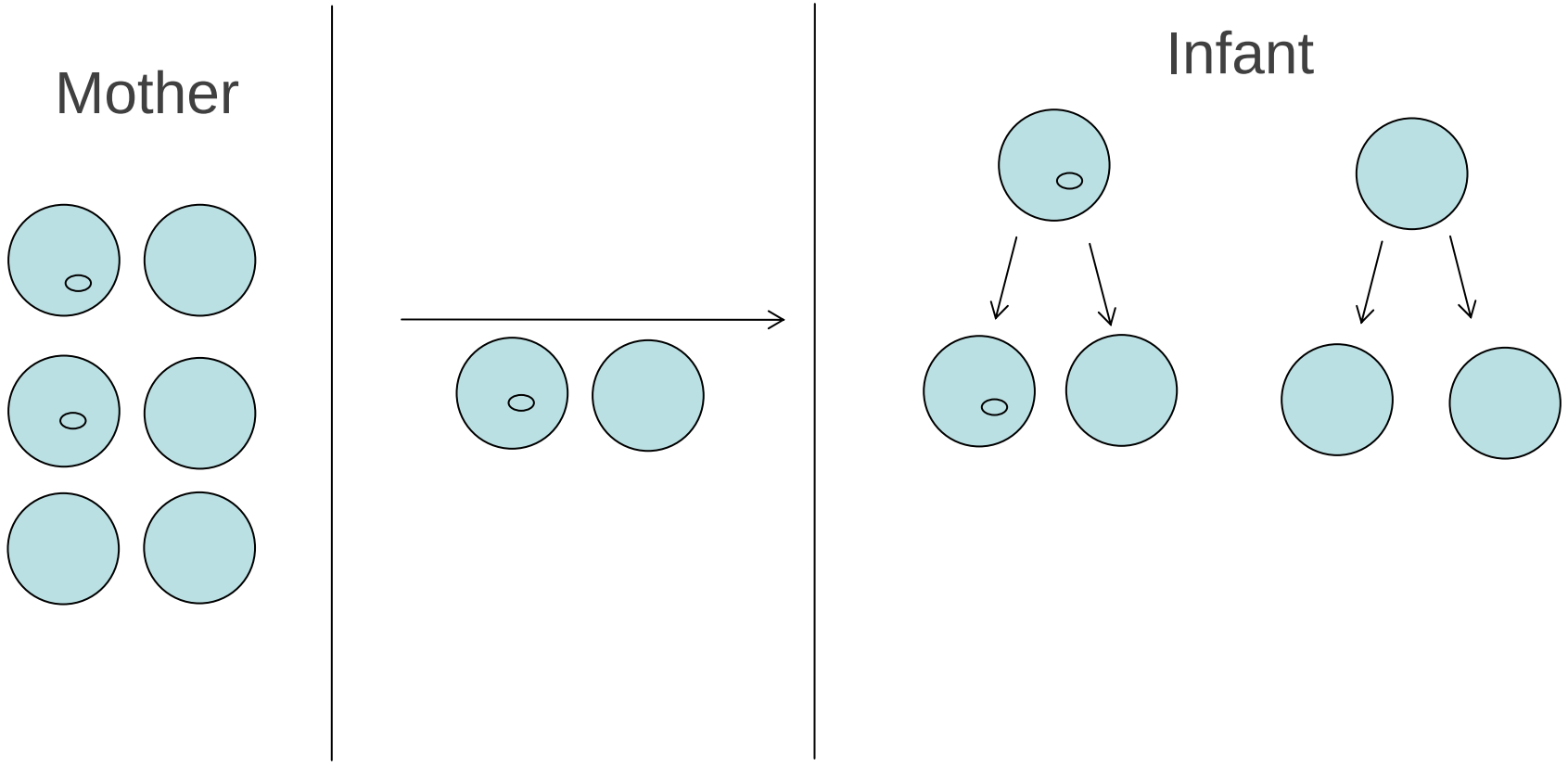
Peripheral expansion of T cells



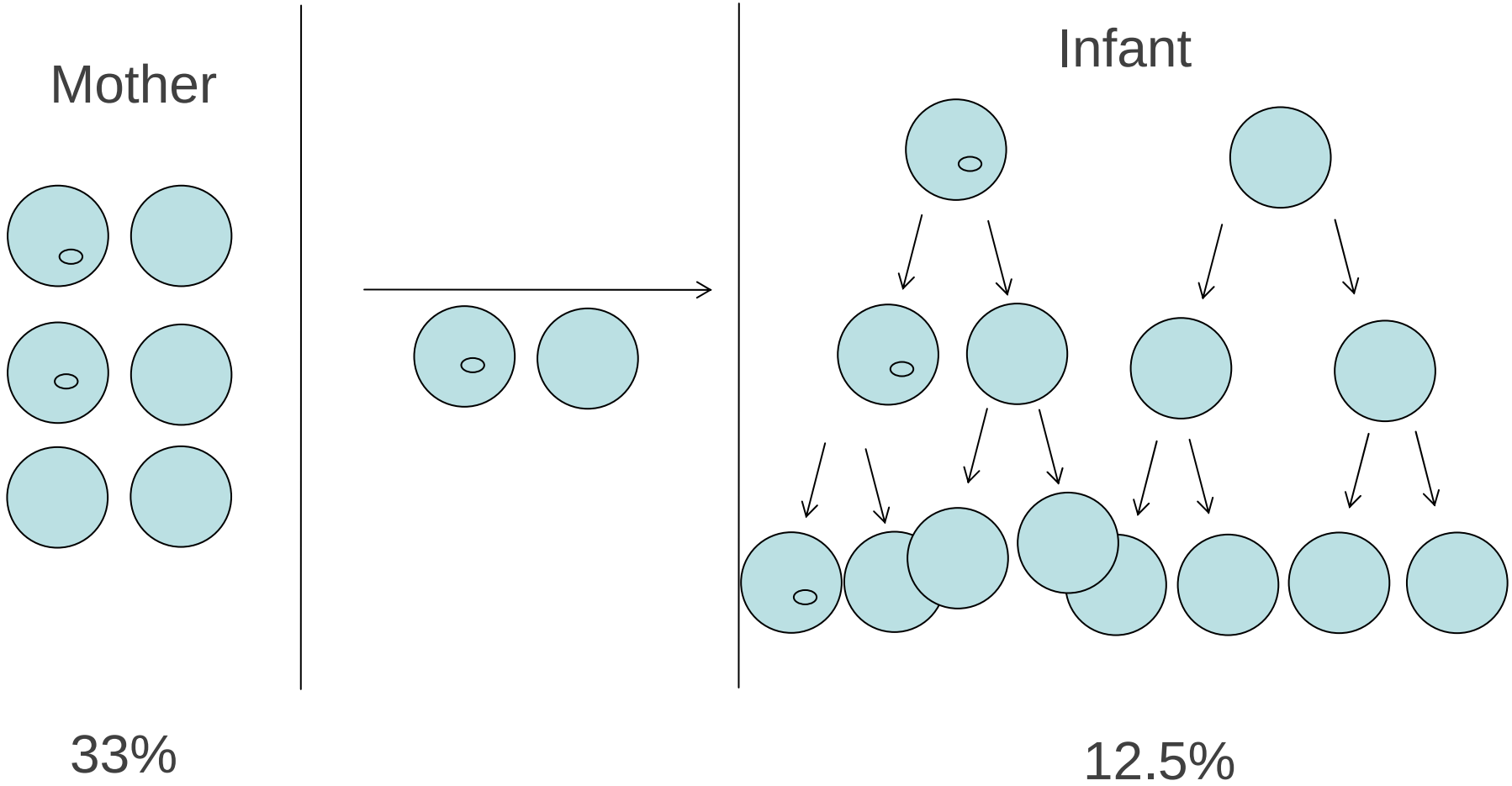
Peripheral expansion of T cells



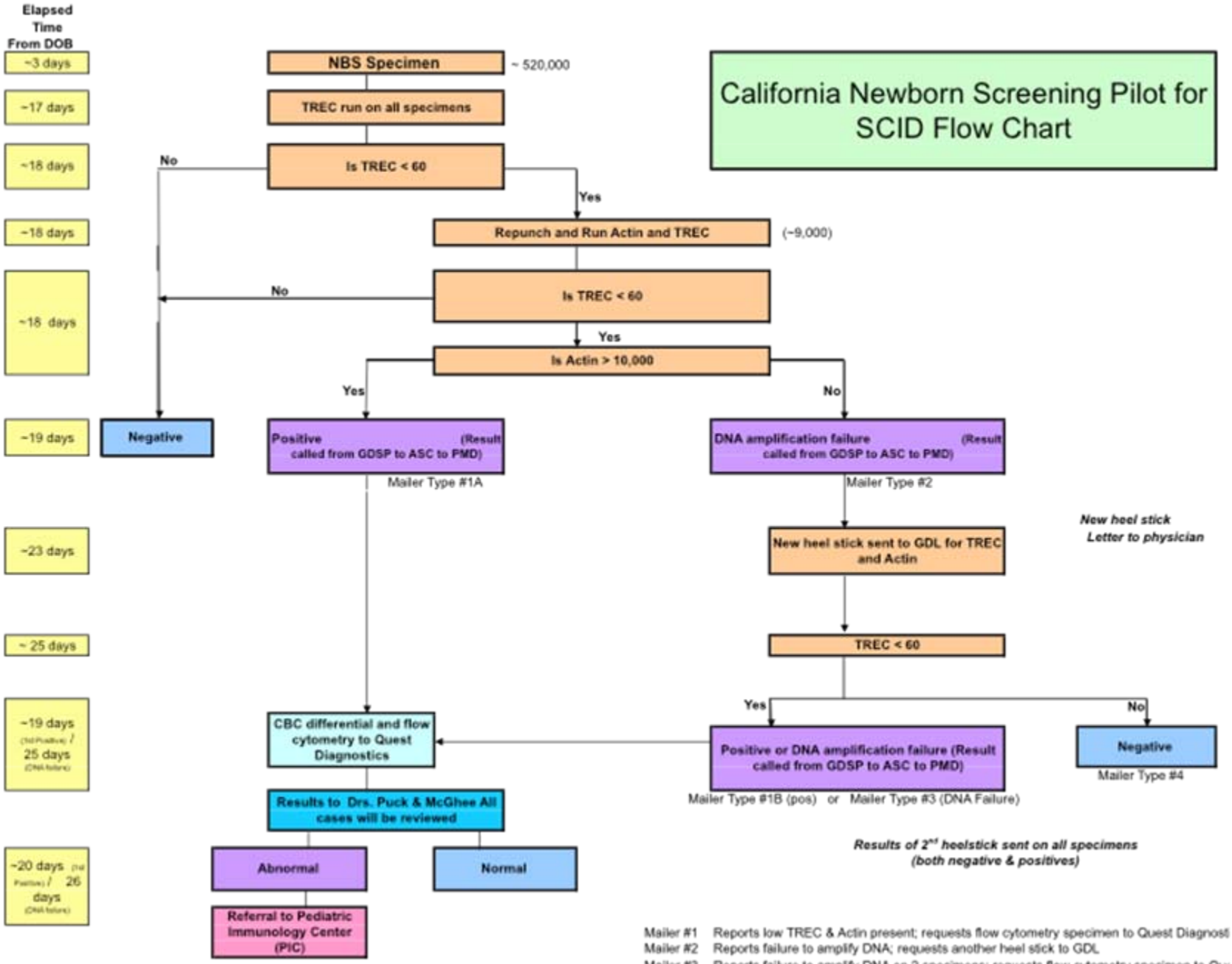
Peripheral expansion of T cells



Peripheral expansion of T cells

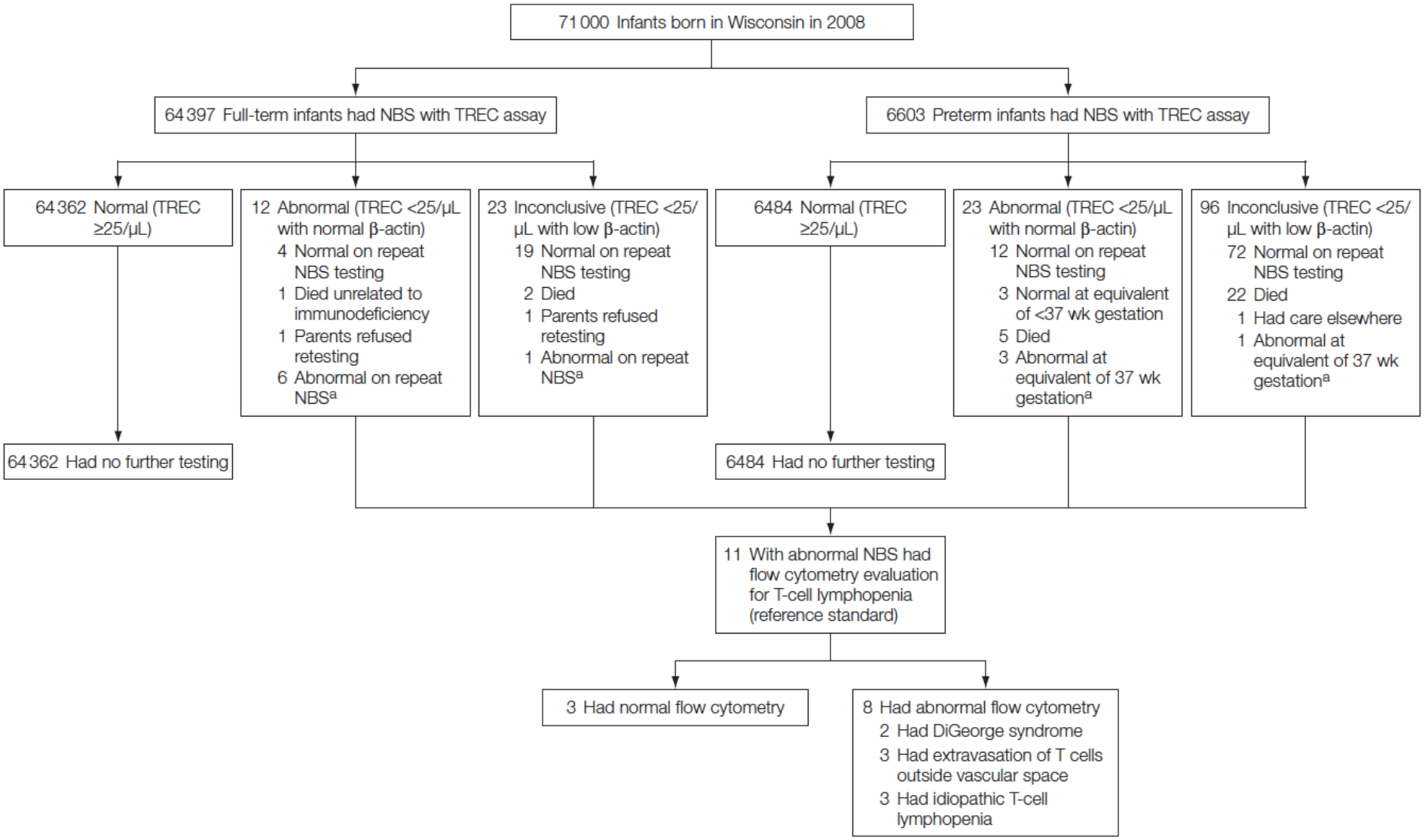


California Newborn Screening Pilot for SCID Flow Chart

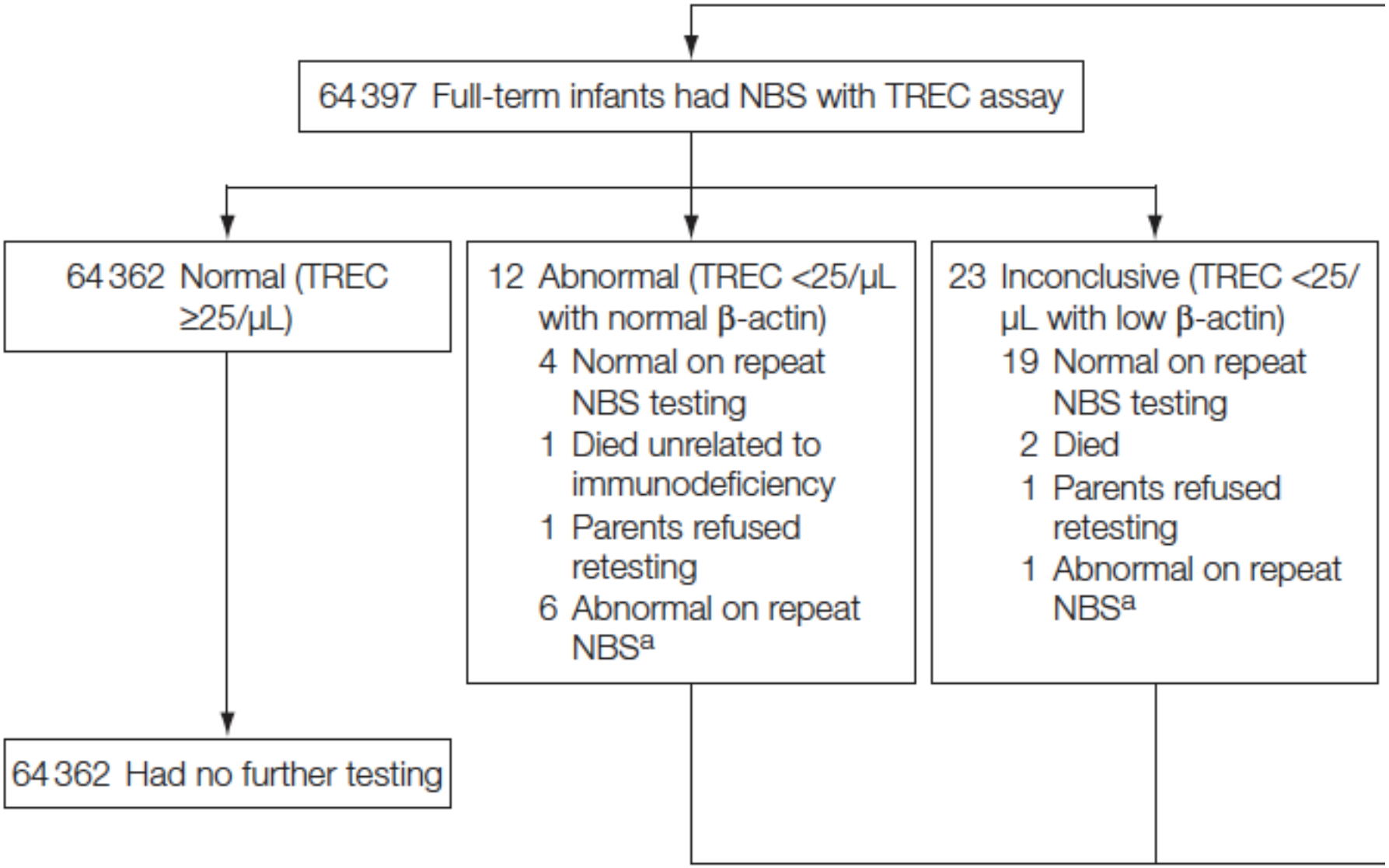


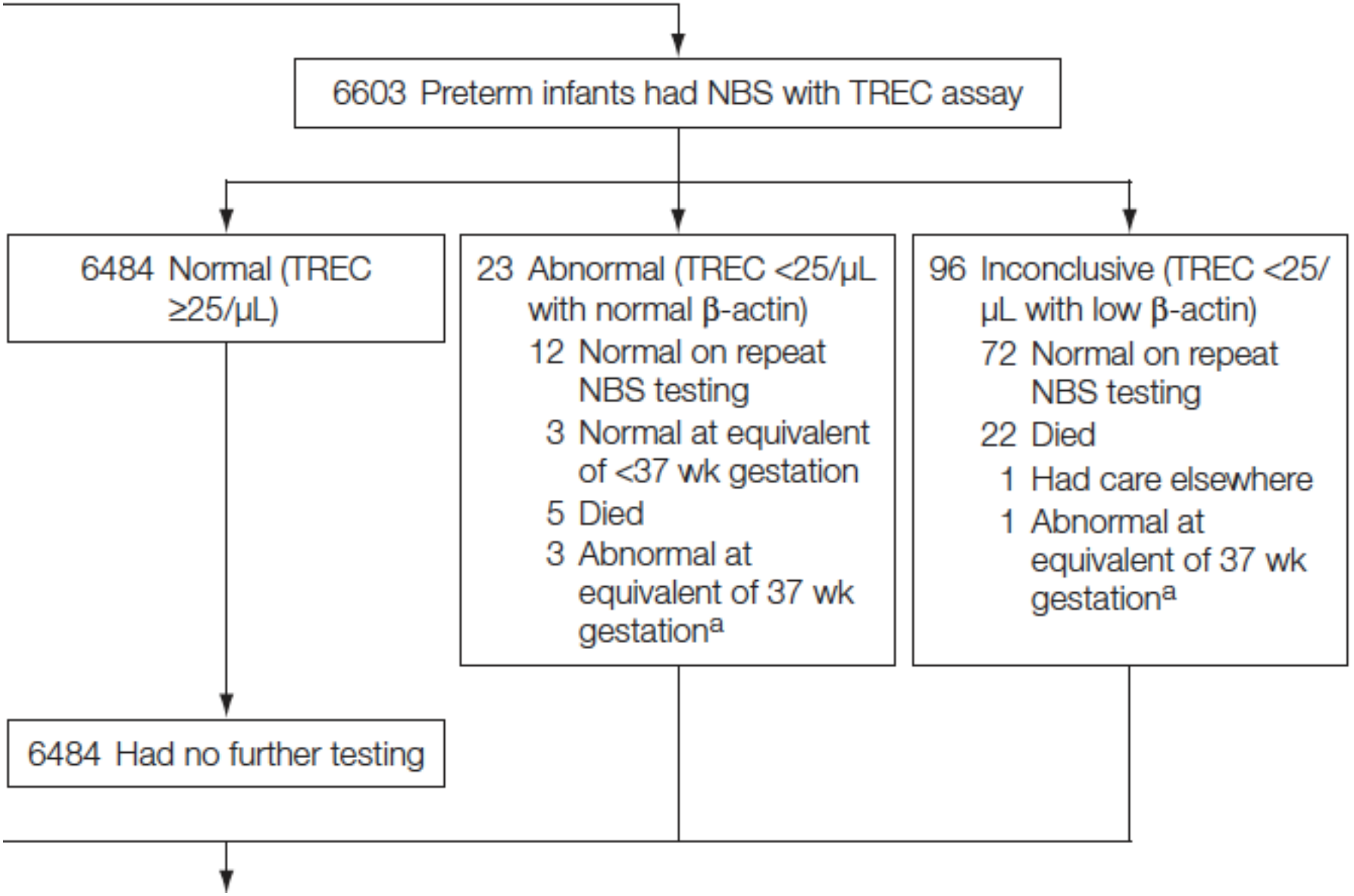
Mailer #1 Reports low TREC & Actin present; requests flow cytometry specimen to Quest Diagnost
Mailer #2 Reports failure to amplify DNA; requests another heel stick to GDL
Mailer #3 Reports failure to amplify DNA on 2 specimens; requests flow cytometry specimen to Qu
Mailer #4 Reports normal TREC numbers on 2nd heelstick

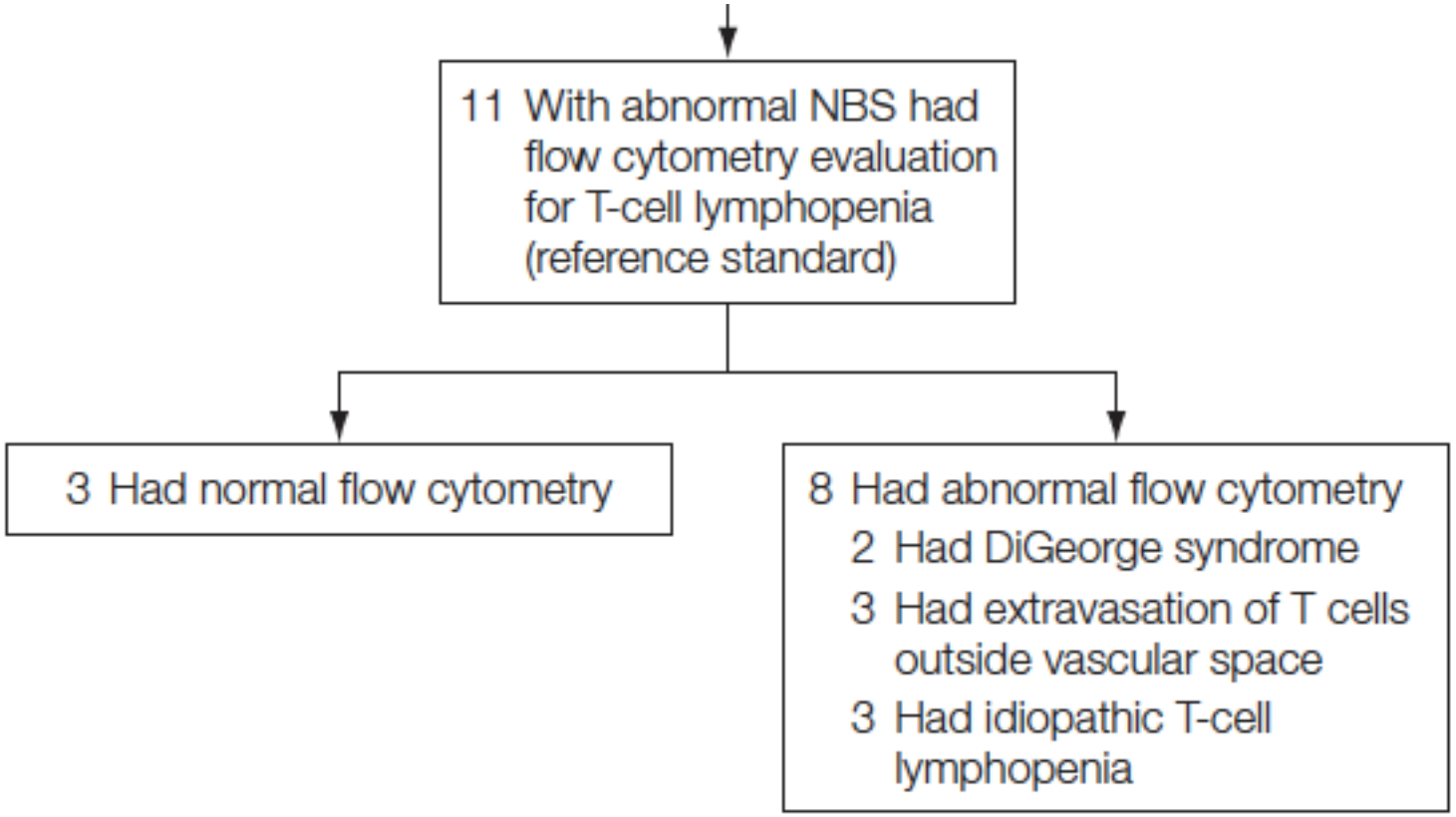
Figure 2. TREC Analysis for Newborn Screening for T-Cell Lymphopenia in Wisconsin



TREC indicates T-cell receptor excision circle; NBS, newborn blood screening. The numbers of TRECs were enumerated by real-time quantitative polymerase chain reaction from NBS cards, and a cutoff value of less than 25 TRECs/μL with a normal β-actin level constituted an abnormal test.
[a] Final NBS with abnormal TREC assay result, proceed to flow cytometry evaluation (reference standard).


64 397 Full-term infants had NBS with TREC assay
64 362 Normal (TREC ≥25/μL)
12 Abnormal (TREC <25/μL with normal β-actin)
4 Normal on repeat NBS testing
1 Died unrelated to immunodeficiency
1 Parents refused retesting
6 Abnormal on repeat NBS[a]
23 Inconclusive (TREC <25/μL with low β-actin)
19 Normal on repeat NBS testing
2 Died
1 Parents refused retesting
1 Abnormal on repeat NBS[a]
64 362 Had no further testing


6603 Preterm infants had NBS with TREC assay
6484 Normal (TREC ≥25/μL)
23 Abnormal (TREC <25/μL with normal β-actin)
12 Normal on repeat NBS testing
3 Normal at equivalent of <37 wk gestation
5 Died
3 Abnormal at equivalent of 37 wk gestation[a]
96 Inconclusive (TREC <25/μL with low β-actin)
72 Normal on repeat NBS testing
22 Died
1 Had care elsewhere
1 Abnormal at equivalent of 37 wk gestation[a]
6484 Had no further testing


11 With abnormal NBS had flow cytometry evaluation for T-cell lymphopenia (reference standard)
3 Had normal flow cytometry
8 Had abnormal flow cytometry
2 Had DiGeorge syndrome
3 Had extravasation of T cells outside vascular space
3 Had idiopathic T-cell lymphopenia

One additional year of screening and one new state

- ✓ Wisconsin
  - Now has detected one classic AR-SCID
  - Rac2 deficiency patient transplanted
- ✓ Massachusetts
  - Reportedly identified one classic SCID

Possible rates of non-amplification in preterm infants versus term infants

- ✓ California 550,000 live births/yr
  - Approx. 10% <37 wks (55,000)
  - At WI rates, 550 would need 2nd card
  - 180 would need flow

Possible reasons for DNA amplification failure

- ✓ PCR is sensitive to heparin (line draws or anticoagulated patients theoretically problematic)
- ✓ Little sample on collection paper
- ✓ Excessive degradation of DNA

Lymphocytes develop by third trimester in fetal development

- ✓ TRECs present down to 22 wks GA at least
  - Mean lower in premature infants
  - Cutoff for screening is much lower
- ✓ Flow should have roughly similar lymphocyte numbers with normal proportions for infants

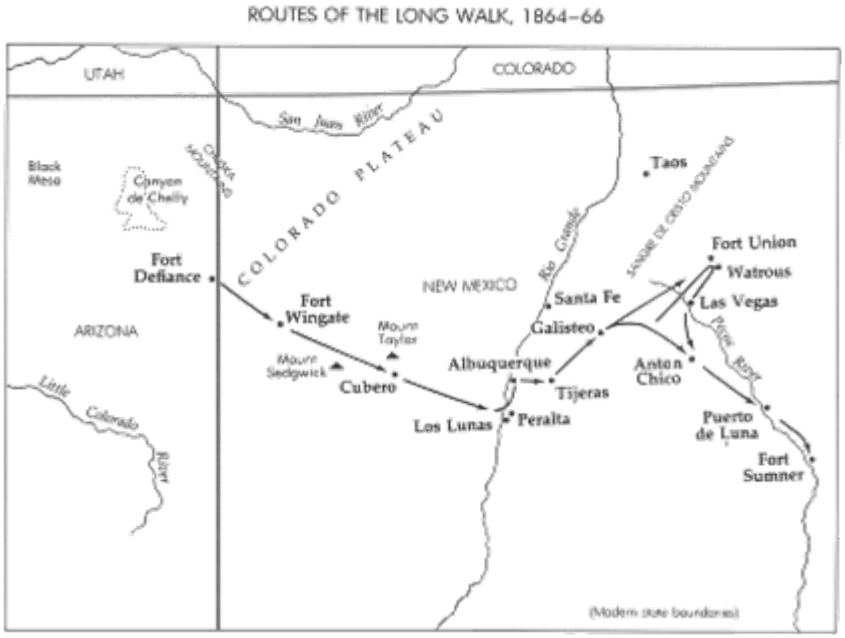
Preterm and NICU infants receive same protocol as term infants

- ✓ Is the most conservative course
- ✓ Will allow collection of data on preterm infants to determine most appropriate course
- ✓ Flow should be unaffected

Cost of pilot screening could be reduced by screening in high incidence populations

- ✓ Athabascan SCID (SCID-A)
  - Artemis deficiency (1:2000)
- ✓ Iran (1:27,000)
- ✓ Amish/Mennonite (?)

Athabascan SCID (Artemis deficiency) results from a DNA repair defect



- ✓ High incidence in Navajo (1:2000)
- ✓ Possibly due to founder effect from the Long Walk
- ✓ Diana Hu, IHS Tuba City

SCID incidence is also increased in the Amish

- ✓ Variety of forms
  - Mennonite
    - IL7Rα, CD3 deficiency, ZAP-70
  - Amish
    - ADA, RAG1

Discussion