Wisconsin Newborn Screening Laboratory

Universal Newborn Screening for Severe Combined Immunodeficiency

APHL/CDC Newborn Screening Molecular Workshop
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Learning Objectives

- Understand SCID from a clinical perspective
- Understand the rationale for newborn screening for SCID
- Understand the screening test for SCID


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TIMELINE
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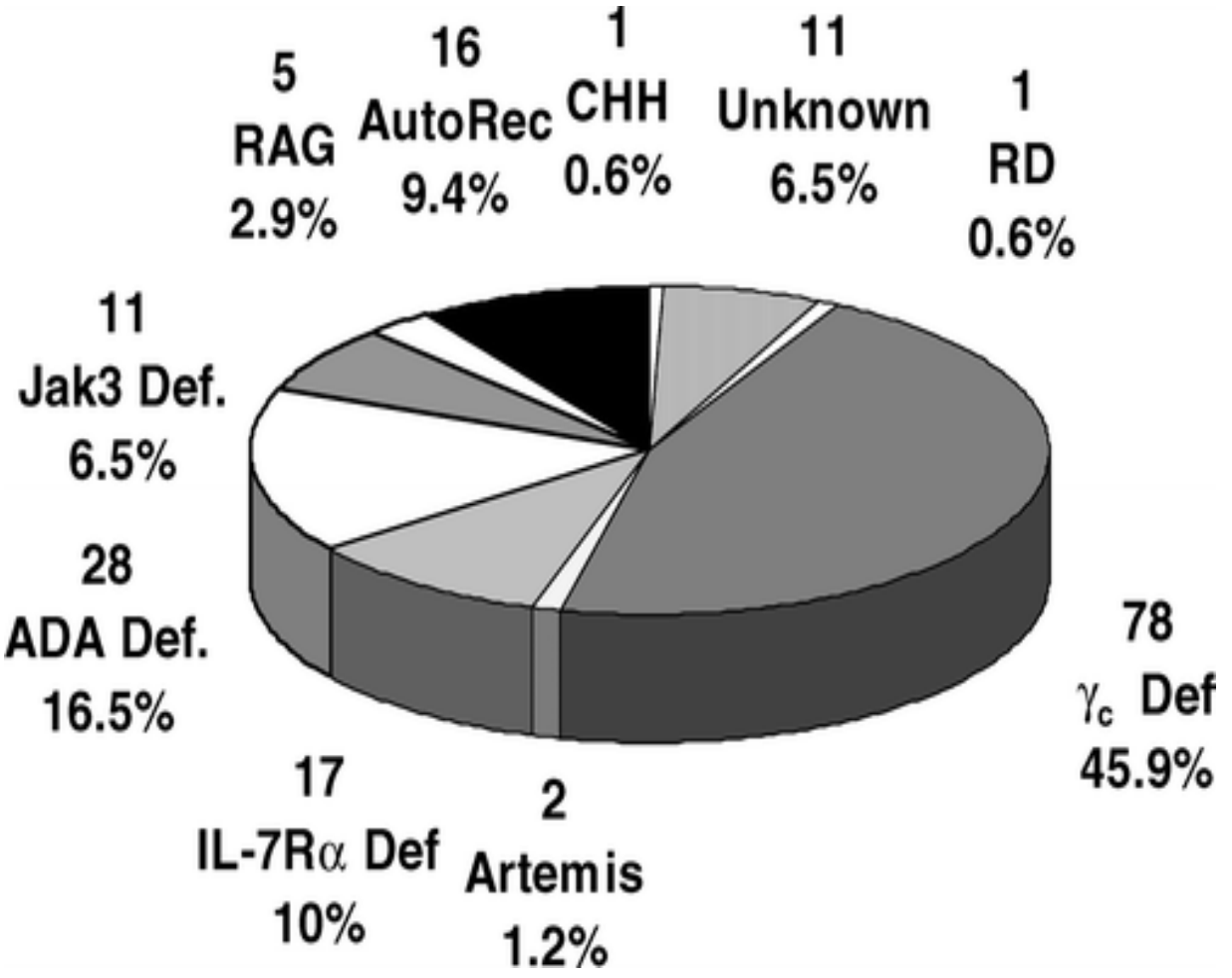
AMERICAN EXPERIENCE
THE BOY IN THE BUBBLE

Severe Combined Immunodeficiency (SCID)

- **Infections in first year of life**
  - recurrent, etiology bacterial, viral and fungal
  - persistent despite routine treatment
  - severe--including sepsis, meningitis
  - opportunistic pathogens, such as PCP (pneumonia)
- **Failure to thrive, chronic diarrhea**
- **T cells decreased or absent**
  - poor proliferation *in vitro* to mitogens
- **B cells absent or non-functional**
  - low Ig's after maternal IgG wanes; no specific antibody responses
- **Fatal without immune reconstitution**

SCID Genetic Analysis

- X-linked SCID is most common form (males)
- Specific gene defect can be found in 80% of cases (15 genes known)
- Clinical applications:
  - Carrier and prenatal dx
  - Predict response to BMT
  - Gene therapy



Buckley Ann. Rev Imm 2004

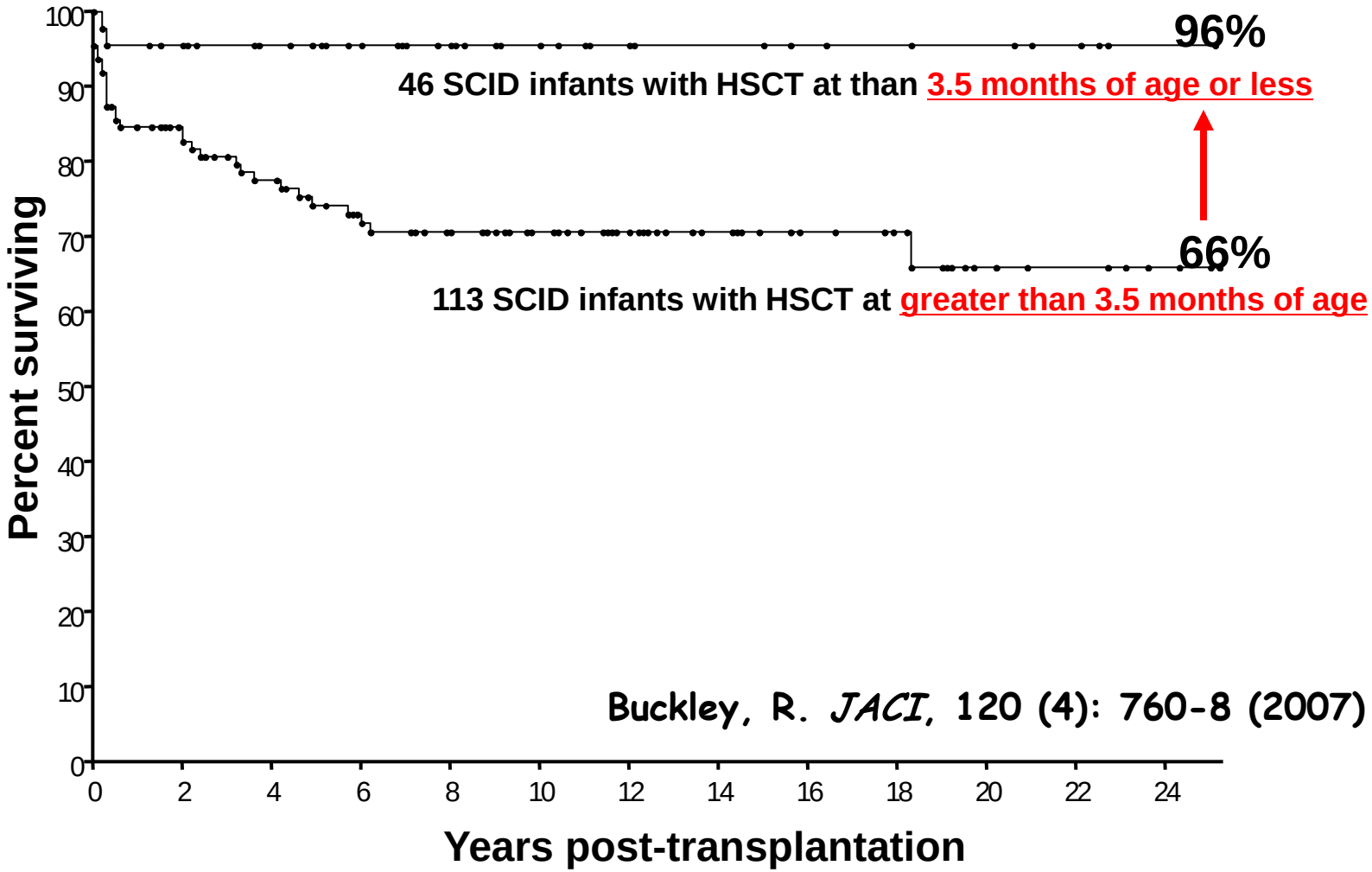
Available Curative Treatment Modalities for SCID

- Bone Marrow Transplantation
- Gene Therapy (X-linked and ADA SCID)

Does SCID fulfill NBS criteria?

- Prevalence of the disease (1:100,000 or greater)
  - SCID: 1:66,000 (conservative estimate)
- Can the disorder be detected by routine physical exam?
  - SCID: No, SCID baby appears normal at birth.
- Does the disorder have a short asymptomatic period after birth?
  - SCID: Yes, SCID baby can be protected by passive maternal immunity.
- Does the disease cause serious medical complications?
  - SCID: Yes, universally fatal within the first year of life
- Is there potential for successful treatment?
  - SCID: Yes, hematopoietic stem cell transplantation
- Is there a confirmatory test?
  - SCID: Yes, lymphocyte subpopulation analysis (flow cytometry)
- Does early intervention leads better outcome?
  - SCID: Yes!
- Is there a screening test?
  - SCID: Yes, measurement of TRECs using real-time qPCR

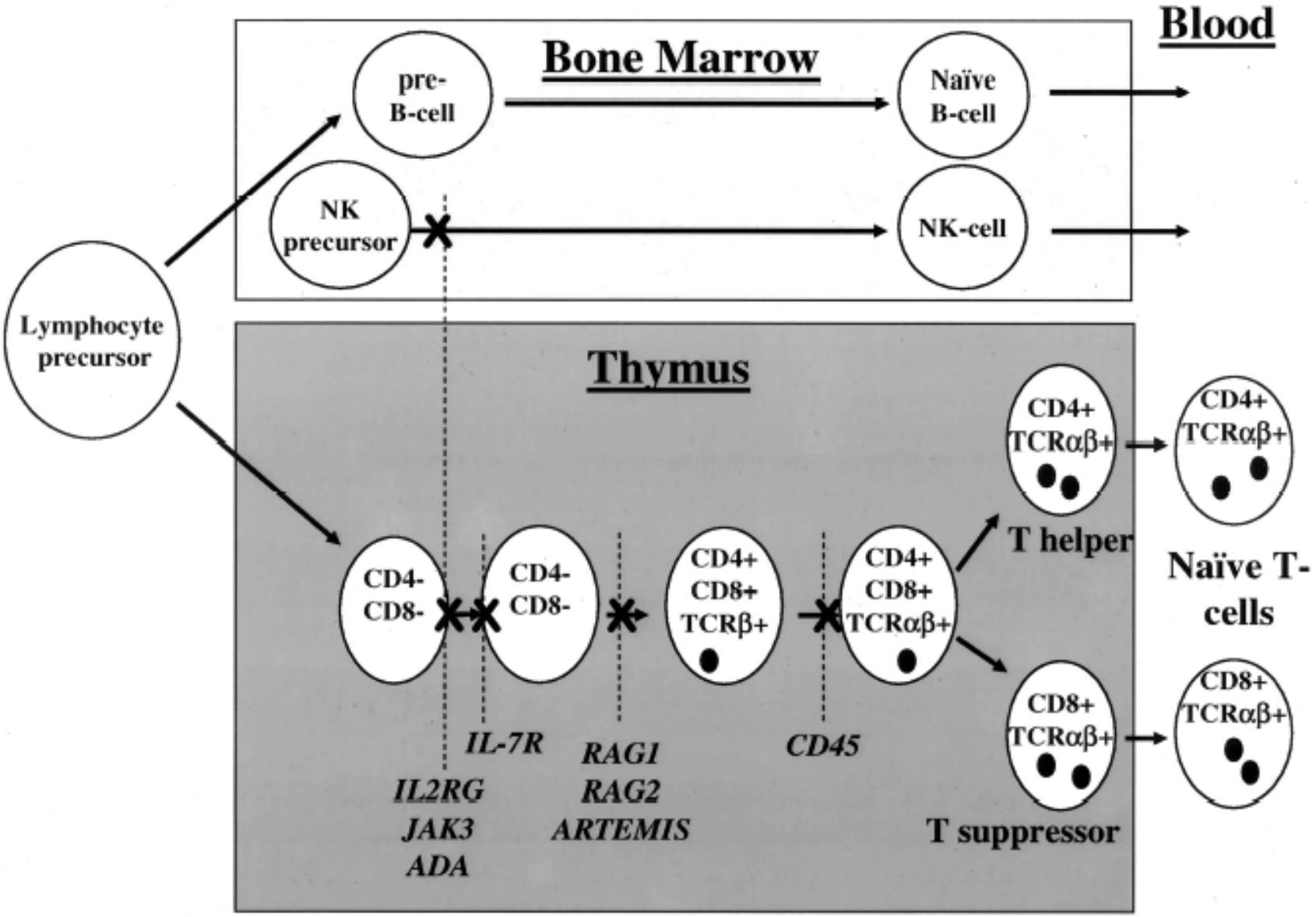
SCID: Benefits of Early Diagnosis



Screening for SCID in Newborns Considerations

- Many genes
- Many mutations in each known gene
- Some genotypes still not known

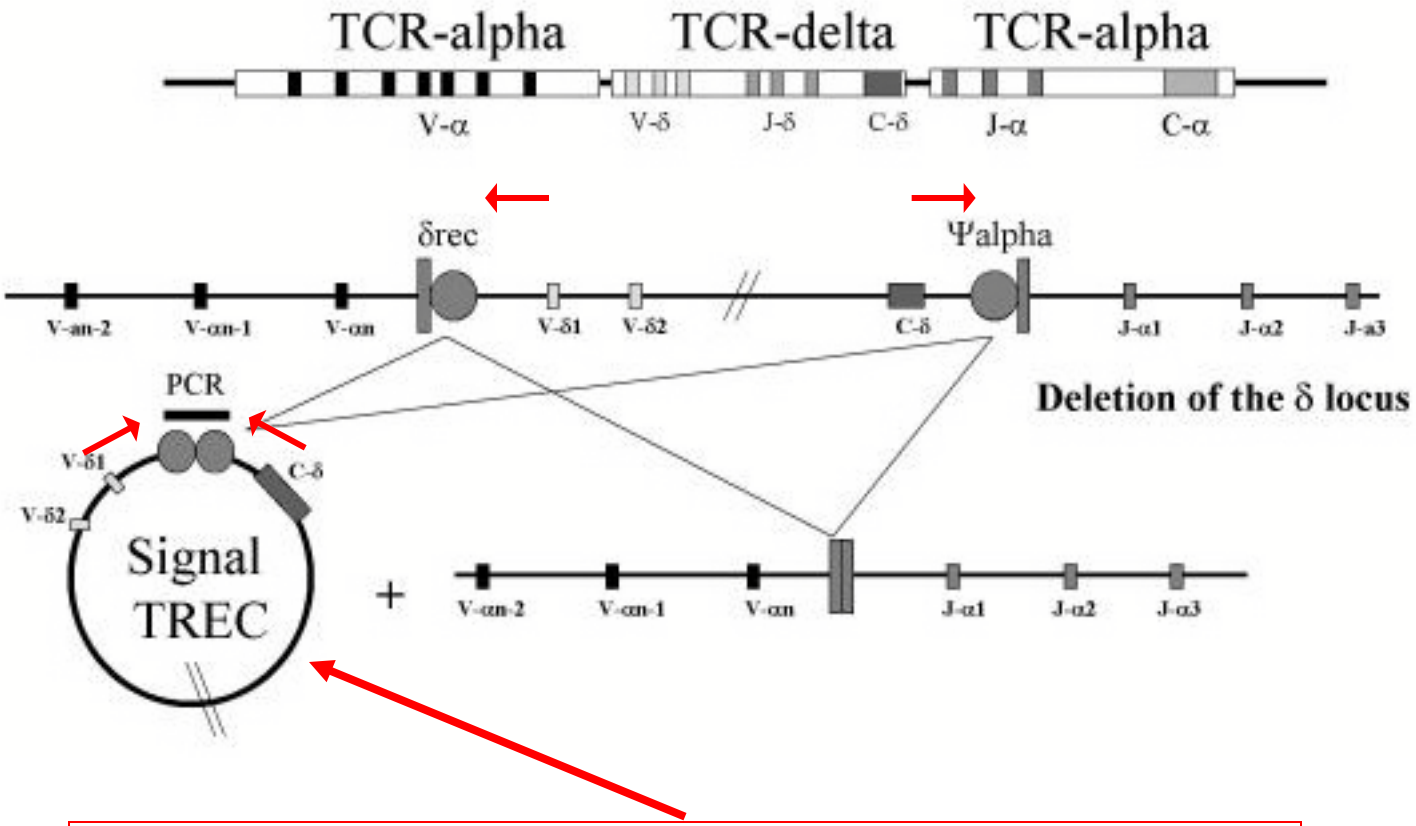
TRECs are reduced in nearly ALL forms of SCID



Genet Med 2004:6(1):16–26.

● = T-cell receptor excision circle (TREC)

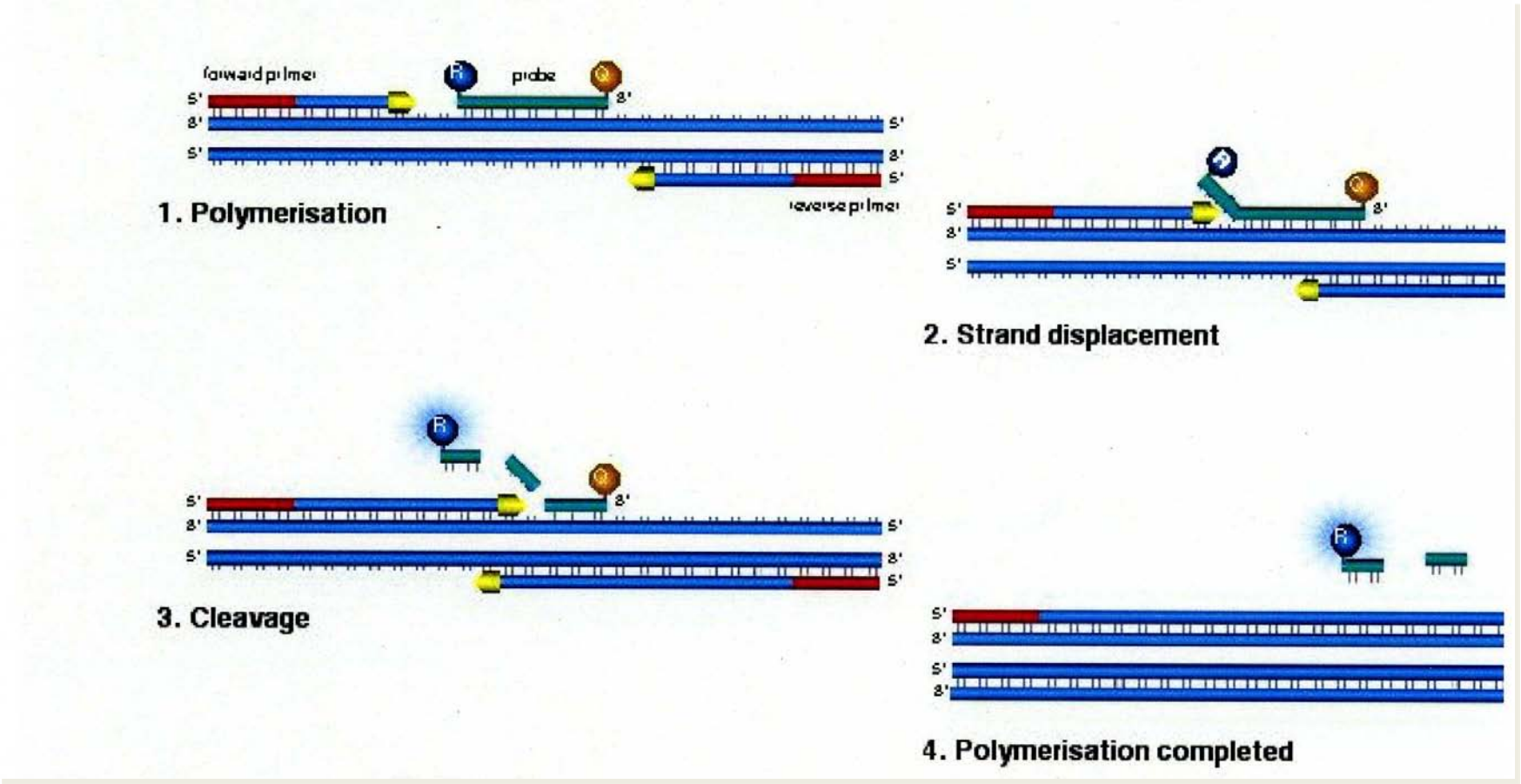
T Cell Receptor Recombination During Development in the Thymus



Generation of T cell receptor excision circles (TRECs) occur in >70% of all new (naïve) T cells and can be detected by PCR

Ponchel *et al. BMC Biotechnology* 2003 **3**:18 doi:10.1186/1472-6750-3-18

TaqMan Probe Real-time qPCR



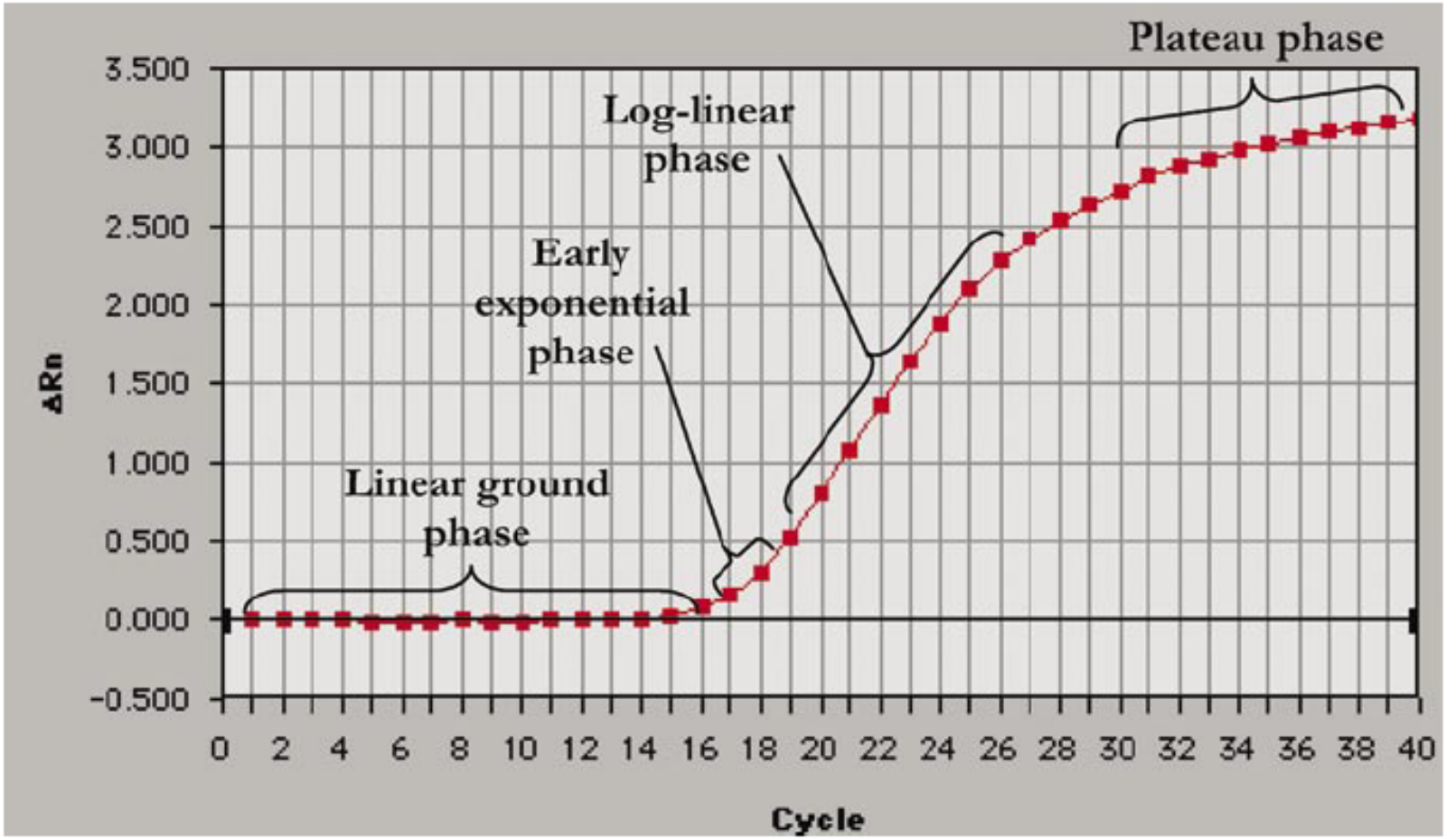


Figure 2. Phases of the PCR amplification curve. The PCR amplification curve charts the accumulation of fluorescent emission at each reaction cycle. The curve can be broken into four different phases: the linear ground, early exponential, log-linear, and plateau phases. Data gathered from these phases are important for calculating background signal, cycle threshold (C_t), and amplification efficiency. Rn is the intensity of fluorescent emission of the reporter dye divided by the intensity of fluorescent emission of the passive dye (a reference dye incorporated into the PCR master mix to control for differences in master mix volume). ΔRn is calculated as the difference in Rn values of a sample and either no template control or background, and thus represents the magnitude of signal generated during PCR. This graph was generated with ABI PRISM SDS version 1.9 software (Applied Biosystems).

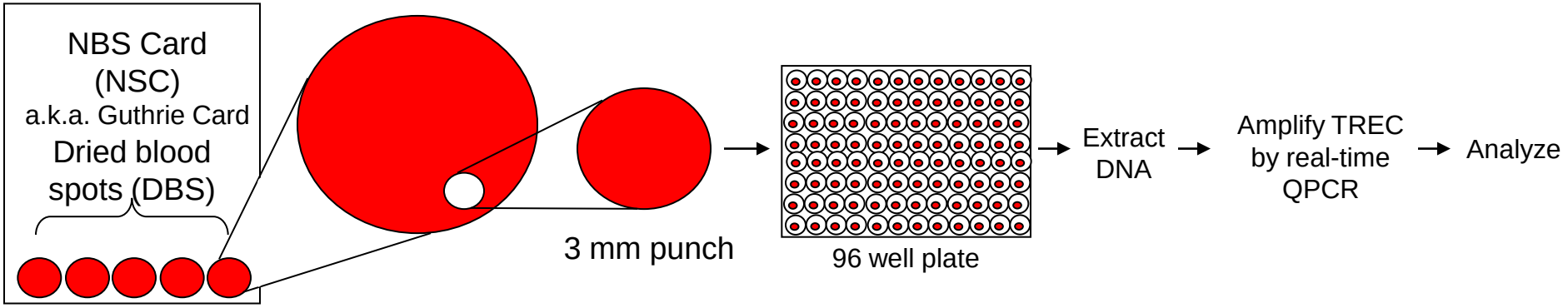
Real-time PCR for mRNA quantitation

Marisa L. Wong and Juan F. Medrano

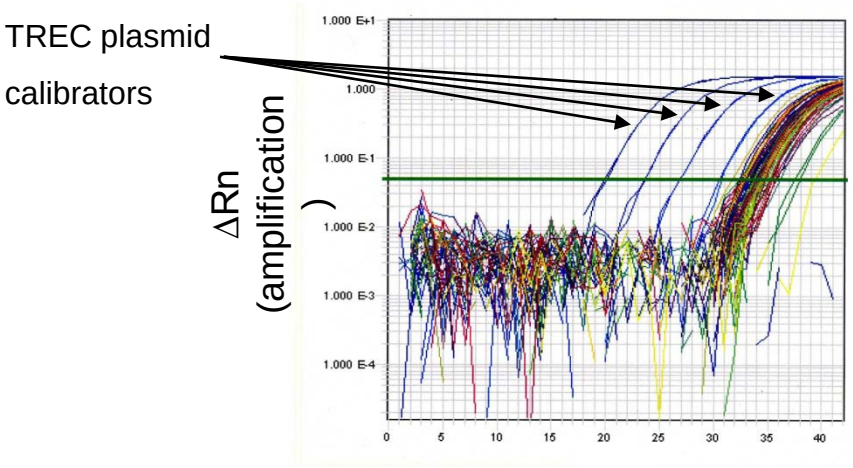
BioTechniques 39:75-85 (July 2005)

(www

Overall Analysis Scheme

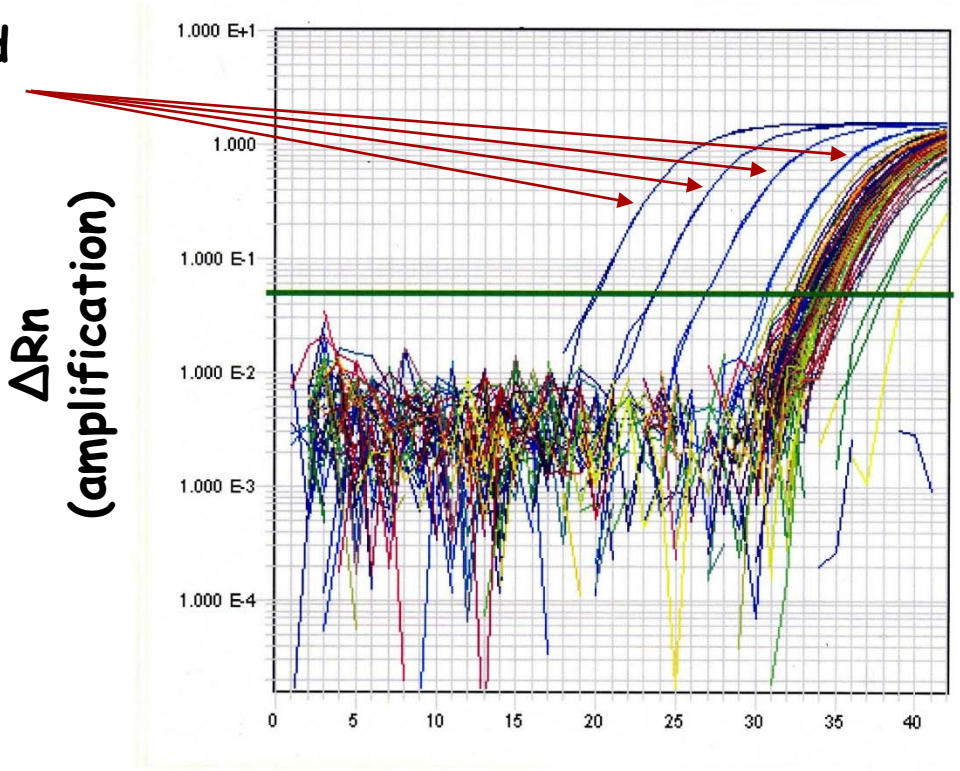


ABI 7900HT Fast Real-Time PCR System

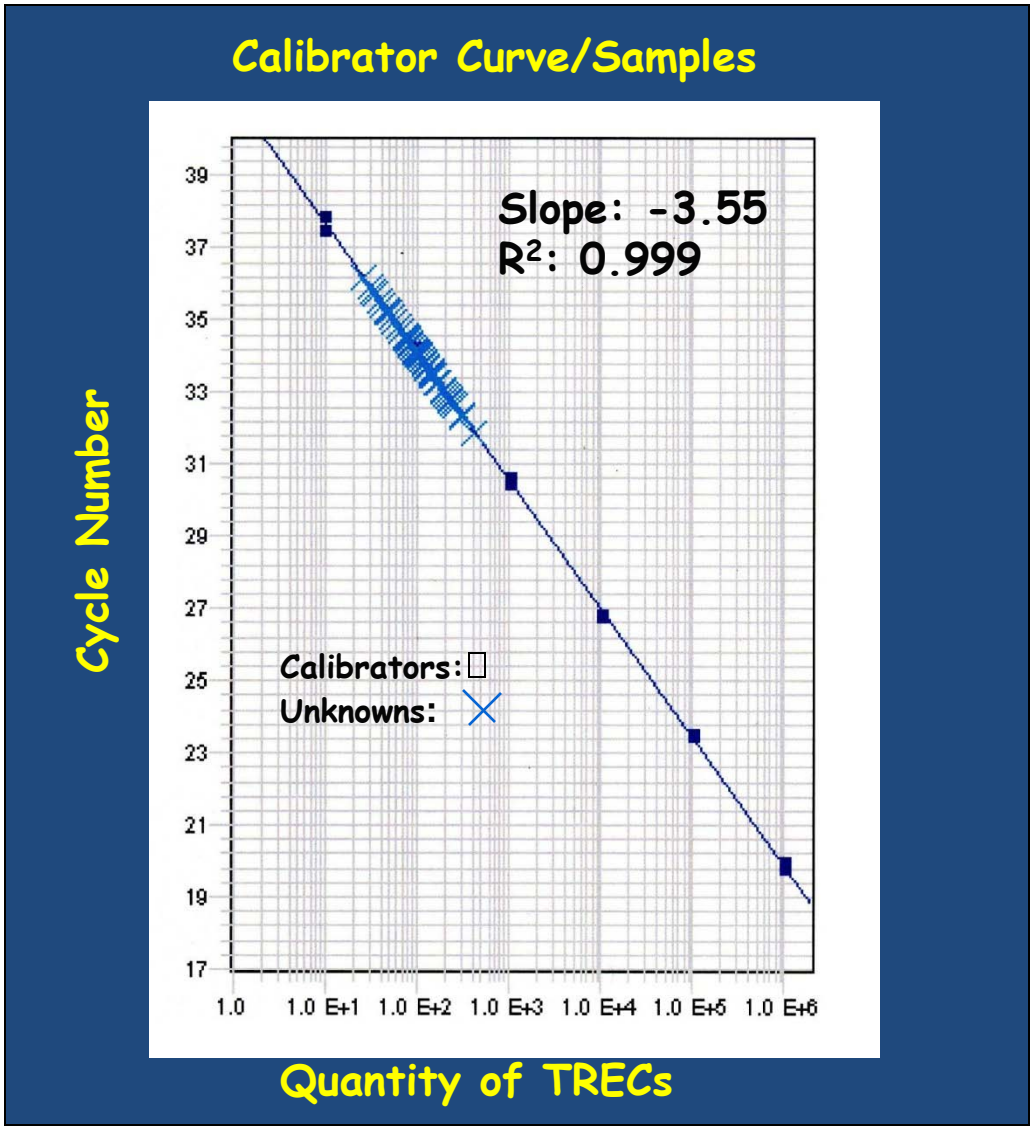


Real-time PCR to Measure TRECs

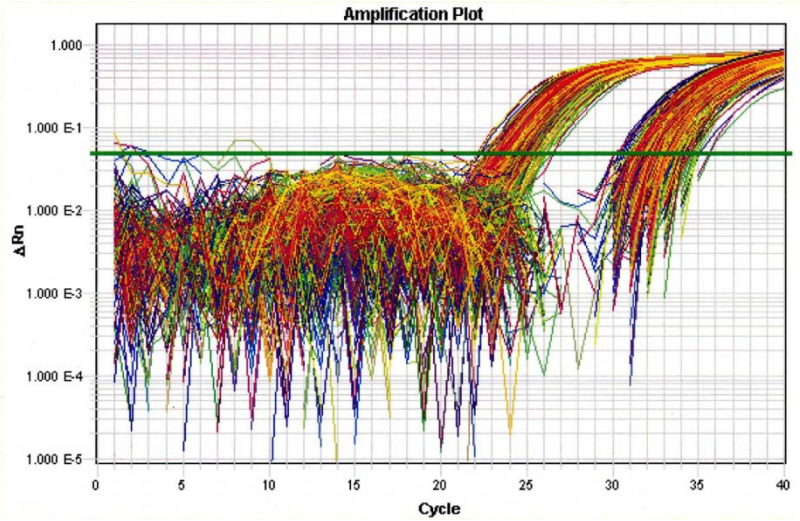
TREC plasmid calibrators

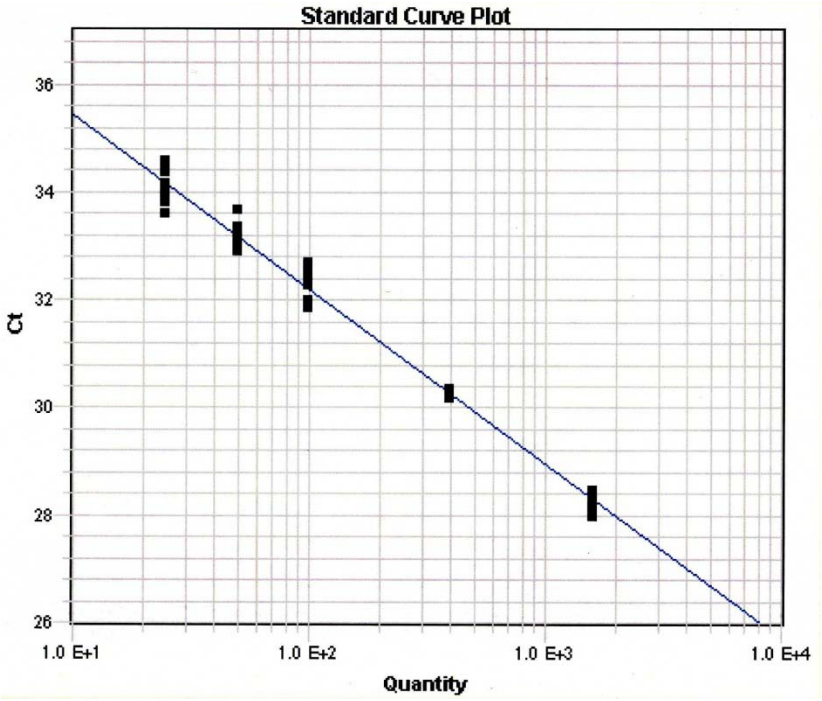


Real-time PCR to Measure TRECs

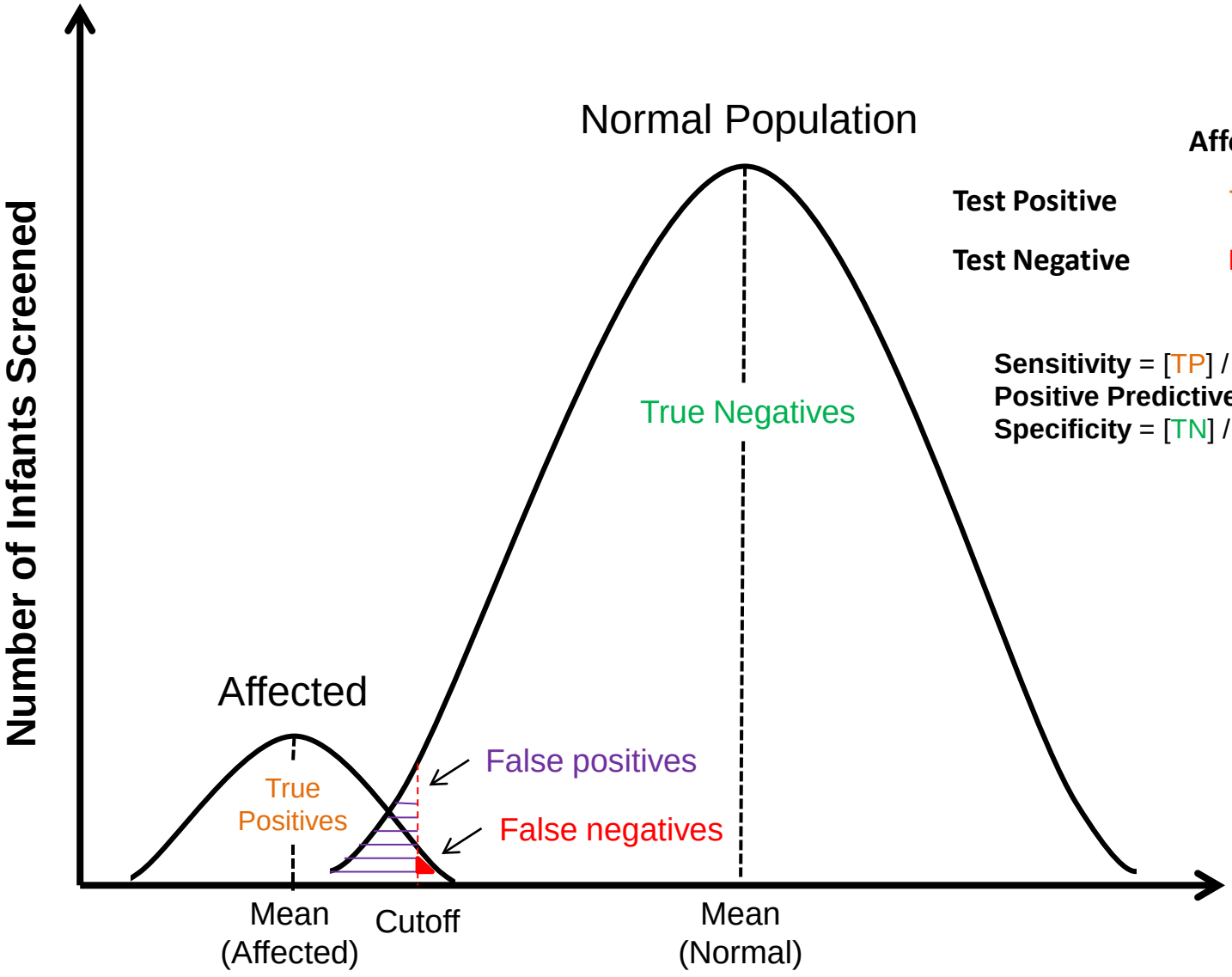


Multiplexing _384-well Plate





Michael Cogley

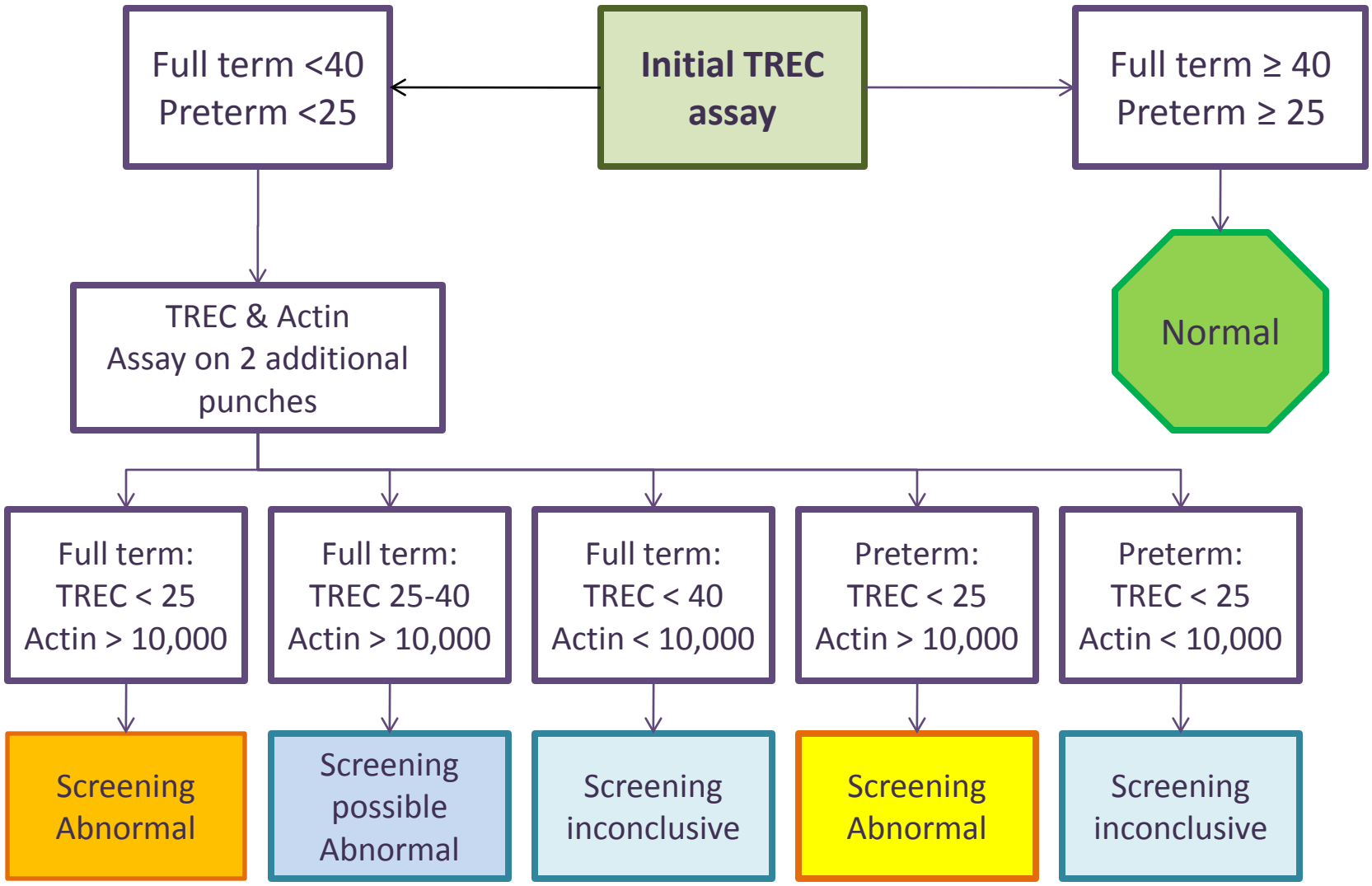


	Affected	Unaffected
Test Positive	TP	FP
Test Negative	FN	TN

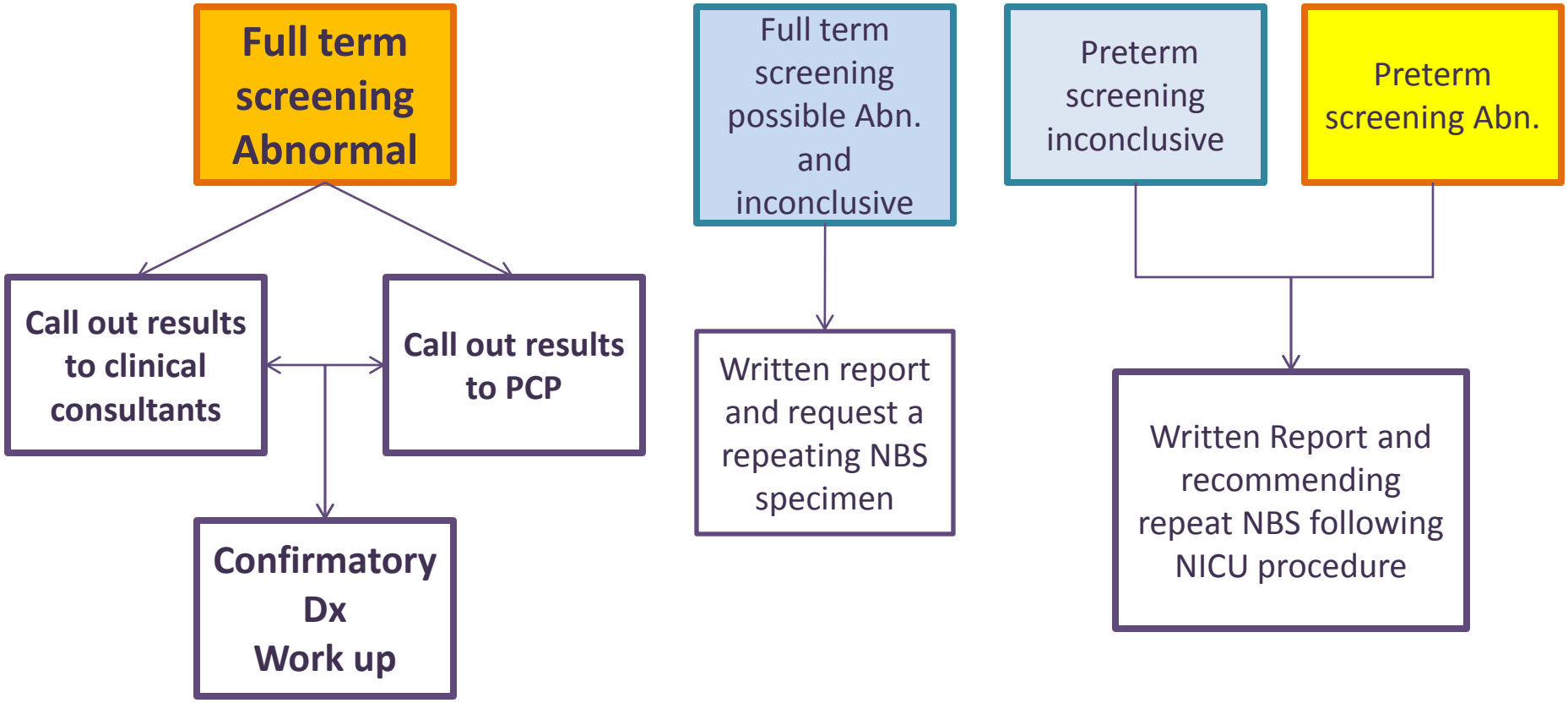
Sensitivity = [TP] / [TP + FN]
Positive Predictive Value = [TP] / [TP + FP]
Specificity = [TN] / [TN + FP]

Anne Atkins

SCID Testing Algorithm



SCID Reporting Algorithm



Special Considerations

- TREC copy numbers
  - Measurement units
  - DNA extraction
  - Calibrators
- TREC assay platform
  - Multiplexing vs. single target
  - 384-well vs. 96-well
- Automation
- QA/QC issues
- Premature Newborns

Wisconsin's Laboratory Experience
TREC Assay Performance in Full Term Babies

- Sensitivity: 100% (No known false negatives reported)
- Positive Predictive Value for T cell lymphopenia: 40-60% (based on Flow results)
- Specificity: > 99%

Conclusions

- The NBS TREC assay allows for high-throughput, population based screening for SCID on a State level.
- The NBS TREC assay is relatively inexpensive and highly reproducible.
- The NBS TREC assay has a low screening positive rate (<0.02%).
- The TREC assay successfully identifies infants with SCID and other T cell related primary immunodeficiency.

Development of a routine newborn screening protocol for severe combined immunodeficiency

Mei W. Baker, MD,[a,b] William J. Grossman, MD, PhD,[c] Ronald H. Laessig, PhD,[a] Gary L. Hoffman, BS,[a] Charles D. Brokopp, DrPH,[a] Daniel F. Kurtycz, MD,[a] Michael F. Cogley, BS,[a] Thomas J. Litsheim, BS,[a] Murray L. Katcher, MD, PhD,[b,d] and John M. Routes, MD[c] *Madison and Milwaukee, Wis*

Background: Severe combined immunodeficiency (SCID) is characterized by the absence of functional T cells and B cells. Without early diagnosis and treatment, infants with SCID die from severe infections within the first year of life.
Objective: To determined the feasibility of detecting SCID in newborns by quantitating T-cell receptor excision circles (TRECs) from dried blood spots (DBSs) on newborn screening (NBS) cards.
Methods: DNA was extracted from DBSs on deidentified NBS cards, and real-time quantitative PCR (RT-qPCR) was used to determine the number of TRECs. Positive controls consisted of DBS from a 1-week-old $T^-B^-NK^+$ patient with SCID and whole blood specimens selectively depleted of naive T cells.
Results: The mean and median numbers of TRECs from 5766 deidentified DBSs were 827 and 708, respectively, per 3.2-mm punch (~3 μL whole blood). Ten samples failed to amplify TRECs on initial analysis; all but 1 demonstrated normal TRECs and β-actin amplification on retesting. No TRECs were detected in either the SCID or naive T-cell–depleted samples, despite the presence of normal levels of β-actin.
Conclusions: The use of RT-qPCR to quantitate TRECs from DNA extracted from newborn DBSs is a highly sensitive and specific screening test for SCID. This assay is currently being used in Wisconsin for routine screening infants for SCID.
(J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

J Allergy Clin Immunol. 2009; 124:522-7

Statewide Newborn Screening for Severe T-Cell Lymphopenia

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Gary L. Hoffman, BS
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Mei W. Baker, MD

Context A newborn blood screening (NBS) test that could identify infants with a profound deficiency of T cells may result in a reduction in mortality.

Objective To determine if quantitating T-cell receptor excision circles (TRECs) using real-time quantitative polymerase chain reaction on DNA extracted from dried blood spots on NBS cards can detect infants with T-cell lymphopenia in a statewide program.

Design, Setting, and Participants Between January 1 and December 31, 2008, the Wisconsin State Laboratory of Hygiene screened all infants born in Wisconsin for T-cell lymphopenia by quantitating the number of TRECs contained in a 3.2-mm punch (approximately 3 μL of whole blood) of the NBS card. Flow cytometry to enumerate the number of T cells was performed on full-term infants and preterm infants when they reached the equivalent of at least 37 weeks' gestation with TREC values of less than 25/μL. Infants with T-cell lymphopenia were evaluated by a clinical immunologist.

Main Outcome Measures The number of infants with TREC values of less than 25/μL with T-cell lymphopenia confirmed by flow cytometry.

Results Exactly 71 000 infants were screened by the TREC assay. Seventeen infants aged at least 37 weeks' gestation had at least 1 abnormal TREC assay (TREC values < 25/μL), 11 of whom had samples analyzed by flow cytometry to enumerate T cells. Eight infants demonstrated T-cell lymphopenia. The causes of the T-cell lymphopenia included DiGeorge syndrome (n=2), idiopathic T-cell lymphopenia (n=2), extravascular extravasation of lymphocytes (n=3), and a *Rac2* mutation (n=1). The infant with the *Rac2* mutation underwent successful cord blood transplantation.

Conclusion In a statewide screening program, use of the TREC assay performed on NBS cards was able to identify infants with T-cell lymphopenia.

JAMA. 2009;302(22):2465-2470 www.jama.com

JAMA 2009; 302:2465-70

Implementing Routine Testing for Severe Combined Immunodeficiency within Wisconsin's Newborn Screening Program

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SYNOPSIS

Severe combined immunodeficiency (SCID) is the result of genetic defects that impair normal T-cell development. SCID babies typically appear normal at birth, but acquire multiple life-threatening infections within a few months. Early diagnosis and treatment with a bone-marrow transplant markedly improves long-term outcomes.

On January 1, 2008, the newborn screening (NBS) program in Wisconsin became the first in the world to routinely test all newborns for SCID. A real-time quantitative polymerase chain reaction assay measures T-cell receptor excision circles (TRECs), which are formed during the maturation of normal T-cells. A lack or very low number of TRECs is consistent with T-cell lymphopenia. The development and validation of the TREC assay and the results of the first year of screening have been published. This article describes the process used to add SCID to the NBS panel, the establishment of follow-up capacity, and the integration of SCID screening into routine NBS workflows. The development of this expanded NBS program is described so that other states might benefit from the processes used in Wisconsin.

Public Health Reports 2010; 125:88-95

Newborn Screening for Severe Combined Immunodeficiency; The Wisconsin Experience (2008–2011)

James W. Verbsky · Mei W. Baker · William J. Grossman · Mary Hintermeyer · Trivikram Dasu · Benedetta Bonacci · Sreelatha Reddy · David Margolis · James Casper · Miranda Gries · Ken DeSantes · Gary L. Hoffman · Charles D. Brokopp · Christine M. Seroogy · John M. Routes

Received: 25 July 2011 / Accepted: 6 October 2011

Abstract Severe combined immunodeficiency is a life-threatening primary immune deficiency characterized by low numbers of naïve T cells. Early diagnosis and treatment of this disease decreases mortality. In 2008, Wisconsin began newborn screening of infants for severe combined immunodeficiency and other forms of T-cell lymphopenia by the T-cell receptor excision circle assay. In total, 207,696 infants were screened. Seventy-two infants had an abnormal assay. T-cell numbers were normal in 38 infants, abnormal in 33 infants, and not performed in one infant, giving a positive predictive value for T-cell lymphopenia of any cause of 45.83% and a specificity of 99.98%. Five infants with severe combined immunodeficiency/severe T-cell lymphopenia requiring hematopoietic stem cell transplantation or other therapy were detected. In summary, the T-cell receptor excision circle assay is a sensitive and specific test to identify infants with severe combined immunodeficiency and severe T-cell lymphopenia that leads to life-saving therapies such as hematopoietic stem cell transplantation prior to the acquisition of severe infections.

J Clin Immunol. 2012 Feb;32(1):82-8