

Abstract

Severe combined immunodeficiency (SCID)—also known as “Bubble Boy Disease”—is a group of genetic defects causing blocks in T-cell development, hence causing immune deficiency. SCID babies acquire severe, persistent, recurrent infections shortly after birth, fail to thrive, and rarely reach their first birthday. With prompt diagnosis and treatment before infections, including infections from attenuated vaccines, they can be cured by bone marrow transplant.

We have developed a newborn screening test for SCID based on measurement of T-cell receptor excision circles (TRECs) by real-time qPCR using DNA extracted from newborn screening dried blood spots (DBS). TRECs are by-products generated during T-cell maturation and are consistently absent or present in low numbers in newborns with SCID.

Through a collaboration of Wisconsin Division of Public Health, Wisconsin State Laboratory of Hygiene, Children's Hospital of Wisconsin and Jeffrey Modell Foundation, SCID screening was implemented for all Wisconsin-born infants on January 1, 2008. This pilot study will continue for 2-3 years with the CDC funding (\$500K per year) starting October 1, 2008.

Objectives

Develop a robust SCID screening laboratory protocol, establish a SCID screening algorithm, and demonstrate that SCID screening can be integrated into NBS programs.

Methods

The screening method is based on the absolute measurement of TRECs by real-time qPCR using DNA extracted from 3.2 mm NBS dried blood spots. The copy number of TRECs and β -actin were automatically determined based the standard curve (Figure 1). There are three reporting categories of results and follow up recommendations: normal screening reports, no action taken; inconclusive screening reports, second NBS requested (retesting); and abnormal screening reports, confirmatory test (flow cytometry) and consultation with immunologists recommended on full term newborns (gestation \geq 37 weeks), and tracking the subsequent re-screening results of premature newborns (gestation \leq 36 weeks).

Real-time qPCR to Measure TRECs

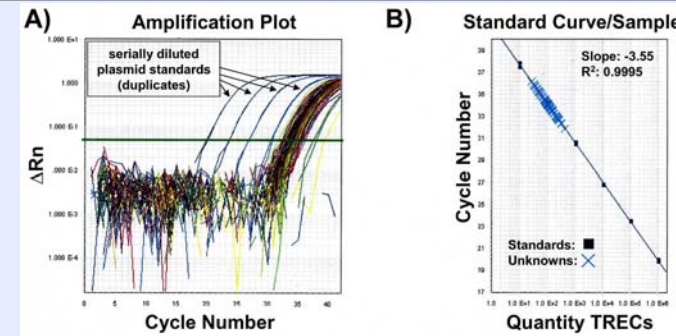
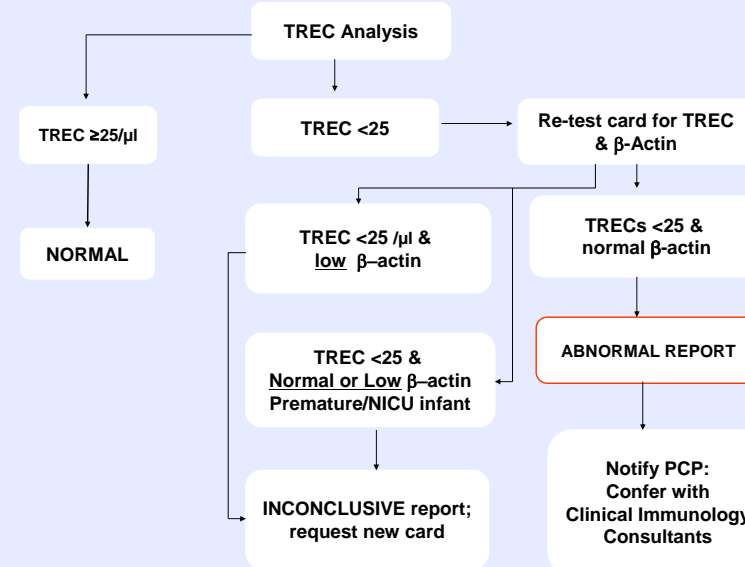


Figure 1. Example of real-time qPCR analysis for TRECs on DNA samples from DBS. A) Arrows indicate serially diluted plasmid standards. B) A standard curve of serially diluted plasmids containing a known copy number of TRECs (solid squares) and unknown DBS samples (crosses). One of the plasmid standards (100 TRECs) is embedded in the unknown DBS samples.

SCID Screening Reporting Algorithm



SCID Screening Results

Number Screened:	47,250 (1/1/08- 8/31/08)
Abnormal Results:	20
– Premature (<37 wks)	11 (0.023%)
– Full term	9 (0.019%)
Inconclusive Results:	76
– Premature (<37 wks)	57 (0.121%)
– Full term	19 (0.040%)

Screening Confirmation Results

- Abnormal Results**
 - 1 DiGeorge Syndrome
 - 1 Downs Syndrome with sepsis at birth
 - 1 Idiopathic T-cell lymphopenia
 - 1 Leukocyte migration defect
 - 4 normal Flow Cytometry results
 - 9 normal results on repeated newborn screening
 - 2 pending cases
 - 1 expired case
- Inconclusive Results**
 - 1 DiGeorge Syndrome
 - 59 normal results on repeated newborn screening
 - 2 pending cases
 - 14 expired cases

Conclusions

We optimized the method of measuring TRECs by real-time qPCR to screen for SCID, which is amenable to NBS throughput. We established screening protocols for testing, reporting and follow-up. Our preliminary experience indicates that screening all newborns for SCID is feasible and results in an acceptable rate of screening false positives. Quantitating the number of TRECs (markers for naïve T-cells) on newborn dry blood spots identifies infants with a variety of primary immunodeficiencies.