Supplementary Online Content

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eMethods

eTable. Summary of SCID newborn screening methods and definitions of T cell lymphopenia in different states

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

***Data collection*:**

The R4S database (http://www.clir-r4s.org/) is a free, open tool that permits de-identified collection and correlation of TREC numbers with flow cytometry data and clinical diagnosis, using diagnostic definitions of SCID and T cell lymphopenia categories common across programs and guidelines, such as the Primary Immunodeficiency Treatment Consortium and the Clinical and Laboratory Standards Institute (Table 1).[1-4] Programs conducting SCID newborn screening were invited to upload data online or to provide equivalent de-identified information in a spreadsheet format.

TREC screening methodologies in each program (also see eTable):

All states performing SCID newborn screening isolated DNA from infant dried blood spots and perform quantitative PCR (qPCR) to enumerate T cell receptor excision circles (TRECs). Amplification of a control segment of genomic DNA was performed (for either all samples or those requiring repeats) to assess sample quality. Absent or low TRECs in samples with adequate amplification of control DNA identified infants at risk for SCID as well as non-SCID conditions characterized by low numbers of circulating naïve T cells. Samples with TRECs below cutoff level and control PCR below cutoff level (eTable 1) required recall of the infant for a new DBS sample.[1] Infants for whom 2 or more samples consistently failed to demonstrate amplification of both TRECs and control DNA required further evaluation. CLSI has published a guide to the methodology of PCR assays for SCID newborn screening.[1] However, individual programs have adapted the test to reflect their own TREC and control PCR reactions, repeat testing strategies and input from immunologists. Program specifics for the interval reported in this study were as follows:

California: As published.[1,5] Flow cytometry was obtained within the SCID newborn screening program at a single contract lab after one positive or 2 incomplete screening results. Designated immunologists interpreted flow results and coordinated further evaluations and care.

Colorado: DBS specimens containing <40 TRECs/μL were repeated for TREC and β-Actin in duplicate using a new punch. Samples that upon repeat had <40 TRECs and >8,000 β-Actin copies/μL were presumptive positive, and patients were referred to a clinical immunologist. Samples with <40 TRECs and <8,000 β-Actin copies were inconclusive, and second dried blood spots were requested. After 2 samples with TRECs <40/μL, a clinical immunologist was contacted to coordinate follow up.

Connecticut: Assay developed by the U. S. Centers for Disease Control and Prevention (CDC). RNaseP was the PCR control. TREC copies <10/μL and RNaseP Cycle threshold (Ct) <28 reflexed to immediate immunological evaluation. TREC cutoffs were ≤30/μL for term and ≤25/μL for preterm infants. Samples with RNaseP Ct ≥28 were unsatisfactory and additional dried blood spots were requested for re-testing. TRECs between 10 and 30/μL required repeat TREC measurement in a new punch before an immunology referral.

Delaware: CDC based assay with RNaseP as the PCR control. Cut-offs were Borderline (17-26 TRECs), Abnormal (4-16 TRECs) and Alert (No Ct – 3 TRECs). RNaseP values out of range were considered invalid. Samples from premature infants (<38 weeks) that were invalid, or had low TRECs, were repeated on a subsequent dried blood spot. Early gestation samples were reported with a qualifier until 38 weeks reached.

Massachusetts: Method as published.[1,6,7]

Michigan: Both full-term and pre-term infants with dried blood spot TRECs ≤7 copies/μL and β-Actin Ct ≤30 referred to a designated immunology clinic for flow cytometry; samples with 7-11 TRECs/μL and β-Actin Ct ≤30 required a repeat sample. If a second dried blood spot also showed ≤11 TRECs/μL, the infant was directed for flow cytometry. The decision to use the same algorithm for both full and pre-term infants was based on experiences prior to and following the implementation.[8]

Mississippi: Dried blood spots were sent to PerkinElmer Genetics, Inc., where the same algorithm as in California was used, except flow cytometry was not conducted within the screening program, instead by the immunologist at the referral center.
Navajo Nation: Dried blood spots were sent to PerkinElmer Genetics, Inc., where the same algorithm as Mississippi was used.
New York: As published.[1,9,10]
Texas: Dried blood spots with initial screen results of <200 TRECs/μL were retested in duplicate. Final average TRECs ≤150 and RNaseP Ct ≤28.5 were reported as Abnormal or Borderline, while infants with undetectable TRECs and RNsseP Ct ≤28.5 were immediately referred to immunologists. All other non-normal results required an additional dried blood spot. Samples with TRECs ≤150 and RNaseP Ct >28.5 were reported as unsatisfactory and repeat specimens requested.
Wisconsin: As published.[1,11,12]

Online references:

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eTable: Summary of SCID newborn screening methods and definitions of T cell lymphopenia in different states

		California	Colorado	Connecticut	Delaware	Massachusetts	Michigan	Mississippi	Navajo Nation	New York	Texas	Wisconsin
Assay protocol[a]		PE[b]	Local	Local	Local	Local	Local	PE	PE	Local	Local	Local
Genomic control		BA	BA	RP	RP	RP	BA	BA	BA	RP	RP	BA
Term infant cutoffs TREC/μL blood (urgent positive)		≤25 (5)	<40	≤30 (10)	<27 (16,3)	<252	≤7	≤25	≤25	<125	≤150	<30
Pre-term or ill infant cutoffs TREC/μL blood		"	"	≤25	"	"	"	"	"	≤200	≤110	<25
Control[c] (copy number/μL blood or Ct)		>10,000	>8,000	Ct <28	Ct <28	≥4,032	Ct ≤30	>10,000	>10,000	Ct <35	Ct ≤28.5	>10,000
Flow cytometry cutoffs for TCL[d]	**T cells/μL**	<1,500	<1,500	<1,500	<1,500	<2,500	<3,505	<2,500	<1,500	*Not defined*	<1,500	<2,500
	$CD4^+/CD45RA^+$ naïve T cells/μL	<50% of T cells	*Not defined*	<50% of T cells	*Not defined*	<50% of T cells	*Not defined*	*Not defined*	*Not defined*	*Not defined*	*Not defined*	*Not defined*

[a]For more detail, see *Methods* in the Supplement and CLSI document[1] (for algorithms of California, Massachusetts, New York and Wisconsin).

[b]Abbreviations: BA, β-actin gene positive control for PCR; Ct, cycle threshold; PE, PerkinElmer Genetics, Inc.; RP, RNase P gene positive control for PCR; TREC, T cell receptor excision circle.

[c]Repeat dried blood spot required if control PCR out of accepted range, whether measured by copy number or Ct.

[d]Test panels included complete and differential blood count, total lymphocytes/μL, and cells/μL for CD3, CD4, CD8 T cells, CD19 B cells, CD16/56 NK cells, CD4/CD45RA+ and CD8/CD45RA+ naïve cells, and CD4/45RO+ and CD8/CD45RO+ memory cells (or equivalent surface markers). For definitions of T cell lymphopenia and criteria for follow-up see Table 4.