Tab 10
Presentation

T-Lymphocyte Defects/Severe Combined Immunodeficiency (SCID)

• Jennifer Puck, M.D.
<table>
<thead>
<tr>
<th><strong>CONDITION</strong></th>
<th><strong>Comment</strong></th>
<th><strong>Genes</strong></th>
<th><strong>Genes (see Suppl Table)</strong></th>
<th><strong>Loci</strong></th>
<th><strong>Genes</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>T Lymphocyte Defects/Severe Combined Immunodeficiency (SCID)</td>
<td></td>
<td>&gt;14 known + unknown</td>
<td></td>
<td>IL2RG, ADA, JAK3, RAG, IL7R, DCLRE1C, etc</td>
<td></td>
</tr>
</tbody>
</table>

*Note: Please reference each statement, listing references below (p.2)*

### Incidence

(Determined by what method(s): Pilot universal screening in Wisconsin<sup>4</sup> found 1/9000 births with T lymphocytopenia, but no true SCID. Minimum incidence of true SCID 1/100,000, calculated from new cases/yr with X-linked mutations in Puck lab.<sup>1</sup> Populations with founder mutations have up to 1/2000.<sup>2</sup> Experts estimate overall SCID incidence ~1/50,000, with higher, but unknown, incidence of T cell defects that are not typical SCID.<sup>1,4,6,8</sup>

### Timing of clinical onset

(Relevance of timing of screening to onset of clinical manifestations) SCID infants’ initial protection by maternal antibodies wanes by 4 mo, leaving them at extreme risk of viral, bacterial & fungal infections causing diarrhea, pneumonia and sepsis. Most SCID infants have frequent, persistent and severe infections by age 7 months. Infections not recognized as signs of an underlying immune disorder may be fatal.<sup>1,9</sup> Vaccine strain rotavirus given by 3 mo causes disease.<sup>10</sup>

### Severity of disease

(Morbidity, disability, mortality, severity) Without treatment, SCID is incompatible with survival beyond early childhood. Delay in diagnosis leads to failure to thrive, meningitis, pulmonary insufficiency, and other debilitating and irreversible conditions. A CDC consensus group recommended development of screening for SCID.<sup>6</sup> Other T-cell defects, e.g. DiGeorge syndrome and profound lymphocytopenias, also carry a risk of severe infections, though some resolve over time.<sup>4,6</sup>

### TEST

(Method, platform). Extract DNA; perform quantitative PCR for T-cell receptor excision circles (TRECs).<sup>1,4,7,11,12</sup> TRECs are circular DNA byproducts of normal T cell maturation.<sup>13</sup> Absence of TRECs indicates lack of normal naive T cells that provide a normal adaptive immune system.<sup>1,13</sup>

### Screening test(s) to be used

(Dried blood spot, physical assessment, other) Dried blood spot DNA assay with quantitative PCR. Note: DNA is the analyte, but this is not a genetic mutation test. SCID cases and other profound T cell defects regardless of disease gene or specific mutation lack TRECs.<sup>1</sup>

### Screening modality

(Physical assessment) Extract DNA; perform quantitative PCR for T-cell receptor excision circles (TRECs).<sup>1,4,7,11,12</sup> TRECs are circular DNA byproducts of normal T cell maturation.<sup>13</sup> Absence of TRECs indicates lack of normal naive T cells that provide a normal adaptive immune system.<sup>1,13</sup>

### Clinical validation

(Preliminary results for clinical validation). Published initial method<sup>12</sup>: 0 to <30 TRECs in DBS at age of diagnosis in 23/23 SCID infants, 0 TRECs in 2 newborn DBS from infants later proven to have SCID. Statewide pilots in WI and MA have validated high-throughput TREC assays.<sup>4</sup>

### Laboratory metrics

(Sensitivity, specificity, indeterminate & false positive rate) Sensitivity: 30/30 true SCID newborn DBS had no TRECs (Puck, unpublished). Very low or undetectable TRECs are also found in cases of clinically significant T lymphocytopenia.<sup>4</sup> Specificity of TREC test is unknown, but all severe T cell defects need medical care with antibiotics, IVIG and close follow-up until resolution or definitive treatment. Detection rate: unknown pending larger pilot trials that find several true SCID cases; population incidence may vary. Inadequate sample (no TRECs, no control genomic PCR): 0.1% in Puck lab collaboration with CA Dept of Public Health (J Puck et al, unpublished), similar in WI<sup>4,11</sup>. Positive predictive value for SCID: unknown, but if SCID incidence is 1/50,000, sensitivity 100%, and false positive rate 0.1% PPV=1/50; some of these would have severe T cell defects and need early intervention.

### Confirmatory testing

(Reliability, availability). Newborn DBS for TREC needed if initial test is inadequate (0 TREC, but 0 control DNA PCR on multiple punches from 1<sup>st</sup> card). Infants with no or low TRECs and positive control PCR need liquid blood flow cytometry, an accurate clinical test for low T cells.<sup>2,3,6,8</sup>

### Risks

(False positives, carrier detection, invasiveness of method, other. Detection or suggestion of other disorders). Call-backs for inadequate samples would engender costs. However, any infant with absent TRECs and positive control DNA PCR may be at high risk of infection.<sup>1,2</sup>
**TREATMENT** | **Comment**
---|---
**Modality** | *(Drug, diet, replacement, transplant)* Hematopoietic cell transplant (HCT) from matched related donor is curative. Other donors: cord blood, matched unrelated donor or parent can be as successful or nearly so, as recent publications from Buckley et al show.\(^1,4,8,9,14,17\) For ADA SCID, enzyme replacement and experimental gene therapy are available.\(^18-20\) IgG antibody infusions and antibiotics are indicated for all infants with low T cells.

**Urgency** | *(How soon after birth is treatment needed)*. HCT by 3.5 months had 95% survival vs. only 66% in older infants with SCID in the Duke series;\(^5,16-17\) morbidity was also less for early HCT.\(^2,3,9,14-17\)

**Efficacy (Benefits)** | *(Extent of prevention of mortality, morbidity)*: Early HCT avoids infections & improves survival & immune reconstitution. Pre-symptomatic treatment means fewer & shorter hospital stays.\(^9,15-17\)

**Availability** | *(Any limits of availability)* 13 large and more small centers for immunodeficiency HCT are now joined in the Primary Immunodeficiency Treatment Consortium (NIAID funded 2009).\(^28\)

**Risks** | *(Potential medical or other ill effects from treatment)*. HCT can have graft vs. host disease, minimized by young age & good donor match. Experimental gene therapy risks leukemia.\(^19\)

---

**KEY REFERENCES**

4. Routes JM et al Statewide newborn screening for severe T-cell lymphopenia. JAMA 2009 302:2465-70

---

**Nat’l DHHS Secretary’s Committee Contact:**
Michele A. Lloyd-Puryear, M.D., Ph.D.
5600 Fishers Lane, Room 19-A-19
Rockville, MD 20857; phone 301-443-1080

**Submission Check list**
☑ 2009 SCID Review Summary
☑ Nomination form
☑ Copy of references not provided in prior nomination

**Contact Information (proponent)**
Jennifer M. Puck, M.D., Dept of Pediatrics, UCSF
513 Parnassus Ave, San Francisco, CA 94143
Email puckj@peds.ucsf.edu Phone 415 476-3181

2003 361:553-60
17. Railey MD et al. Long-term clinical outcome of patients with severe combined immunodeficiency who received related donor bone marrow transplants without pretransplant chemotherapy or post-transplant CVHD prophylaxis. J Pediatr 2009 155:834-40
### Supplementary Table 1. SCID Genotypes.

<table>
<thead>
<tr>
<th>Gene Defect [OMIM number]</th>
<th>Defective Protein, Function</th>
<th>% of SCID&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lymphocyte Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL2RG [#300400]</strong></td>
<td>Common γ-chain (γc) of receptors for IL-2, 4, 7, 9, 15, 21 (X-linked)</td>
<td>45-50</td>
<td>-</td>
</tr>
<tr>
<td><strong>ADA [#102700]</strong></td>
<td>Adenosine deaminase</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td><strong>IL7R [146661]</strong></td>
<td>α chain of IL-7 receptor</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td><strong>JAK3 [600173]</strong></td>
<td>Janus kinase 3 activated by γc</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td><strong>DCLRE1C [#602450]</strong></td>
<td>Artemis, T and B cell antigen receptor rearrangement</td>
<td>&lt;5</td>
<td>-</td>
</tr>
<tr>
<td><strong>RAG1/2 [175615/6]</strong></td>
<td>T and B cell antigen receptor rearrangement</td>
<td>&lt;5</td>
<td>-</td>
</tr>
<tr>
<td><strong>LIG4 [601837]</strong></td>
<td>DNA ligase IV antigen receptor rejoining</td>
<td>rare</td>
<td>-</td>
</tr>
<tr>
<td><strong>CD45 [151460]</strong></td>
<td>Protein tyrosine phosphatase receptor, required for T, B activation by antigen</td>
<td>rare</td>
<td>-</td>
</tr>
<tr>
<td><strong>TCRD, TCRE, TCRZ</strong> [186810/30/80]</td>
<td>CD3 δ, ε, and ζ deficiency with impaired T cell development</td>
<td>rare</td>
<td>-</td>
</tr>
<tr>
<td><strong>LCK [153390]</strong></td>
<td>Lymphocyte tyrosine kinase p56lck, T cell development and activation</td>
<td>rare</td>
<td>-</td>
</tr>
<tr>
<td><strong>FOXN1 [600838]</strong></td>
<td>Forkhead box N1, thymus &amp; hair follicle development (same as nude mouse)</td>
<td>rare</td>
<td>-</td>
</tr>
<tr>
<td><strong>Currently unknown</strong></td>
<td>Unknown, including reticular dysgenesis and congenital anomaly syndromes with SCID</td>
<td>~10</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on Buckley,<sup>3</sup> Cavazzana-Calvo et al.<sup>9</sup> and unpublished estimates (J. Puck). <sup>b</sup> Some patients have substantial numbers of maternally derived T cells at time of diagnosis; such T cells are predominantly post-stimulation memory cells with low or no TREC.

### Supplementary Table 2. Non-SCID T Lymphocyte Defects Detected by Neonatal TREC Screening (Routes et al., 2009).

<table>
<thead>
<tr>
<th>Defect [OMIM #]</th>
<th>Defective Protein, Function</th>
<th>Incidence</th>
<th>T Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion Chr 22q11 (TBX1) [#188400]&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>Complete DiGeorge syndrome, aplasia of thymus</td>
<td>2 per 71000</td>
<td>-/low</td>
</tr>
<tr>
<td><strong>RAC2 [#608203]</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Neutrophil (and lymphocyte) immunodeficiency syndrome&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1 per 71000</td>
<td>low</td>
</tr>
<tr>
<td>Omenn Syndrome&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Erythroderma, adenopathy, eosinophilia, TREC-neg oligoclonal T cells</td>
<td>?</td>
<td>low, normal or high</td>
</tr>
<tr>
<td><strong>Unknown</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Idiopathic T lymphocytopenia</td>
<td>2 per 71000</td>
<td>low</td>
</tr>
<tr>
<td><strong>Unknown</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Extravasation of lymphocytes (chylothorax, etc.)</td>
<td>3 per 71000</td>
<td>low</td>
</tr>
</tbody>
</table>

<sup>a</sup> Routes et al.; <sup>b</sup> Puck, unpublished
EVIDENCE REVIEW: Severe Combined Immunodeficiency (SCID)

Prepared for: ADVISORY COMMITTEE ON HERITABLE DISORDERS IN NEWBORNS AND CHILDREN

Final
01/20/2009 (revised 04/29/2009)

Authors:
Ellen Lipstein, Alixandra A. Knapp, James M. Perrin

Evidence Review Group: Chairperson, James M. Perrin, MD
(MGH Center for Child and Adolescent Health Policy)

Committee Members:

Marsha Browning, MD, MPH, MMSc
(Massachusetts General Hospital)

Ellen Lipstein, MD
(MGH Center for Child and Adolescent Health Policy)

Jennifer Delahaye, BA
(MGH Center for Child and Adolescent Health Policy)

Lisa Prosser, PhD
(University of Michigan)

Nancy Green, MD
(Columbia University)

Denise Queally, JD
(Consumer Representative)

Alex R. Kemper, MD, MPH, MS
(Duke University)

Sienna Vorono, BA
(MGH Center for Child and Adolescent Health Policy; Brown Medical School)

Alixandra Knapp, MS
(MGH Center for Child and Adolescent Health Policy)

This review was made possible by subcontract number SC-07-028 to Massachusetts General Hospital, Center for Child and Adolescent Health Policy under prime contract number HHSP23320045014XI to Altarum Institute, from the Maternal and Child Health Bureau (MCHB) (Title V, Social Security Act), Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services (DHHS).
<table>
<thead>
<tr>
<th>Table of Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Abbreviations used</td>
<td>3</td>
</tr>
<tr>
<td>I. Overview</td>
<td>5</td>
</tr>
<tr>
<td>II. Rationale for review</td>
<td>5</td>
</tr>
<tr>
<td>III. Objectives of review</td>
<td>5</td>
</tr>
<tr>
<td>IV. Main questions</td>
<td>5</td>
</tr>
<tr>
<td>V. Conceptual framework</td>
<td>6</td>
</tr>
<tr>
<td>VI. Methods</td>
<td>7</td>
</tr>
<tr>
<td>VII. Results</td>
<td>9</td>
</tr>
<tr>
<td>VIII. Summary</td>
<td>40</td>
</tr>
<tr>
<td>IX. References</td>
<td>42</td>
</tr>
<tr>
<td>X. Appendix A- SCID evidence tables</td>
<td>50</td>
</tr>
<tr>
<td>XI. Appendix B- Conflict of interest form</td>
<td>70</td>
</tr>
<tr>
<td>XII. Appendix C- Letter and questions sent to SCID experts</td>
<td>76</td>
</tr>
<tr>
<td>XIII. Appendix D- Letter and questions sent to SCID advocacy groups</td>
<td>79</td>
</tr>
</tbody>
</table>
## i. Abbreviations used

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACHDNC</td>
<td>Advisory Committee on Heritable Disorders in Newborns and Children</td>
</tr>
<tr>
<td>ACTB</td>
<td>Gene symbol for beta-actin</td>
</tr>
<tr>
<td>ADA deficiency</td>
<td>Adenosine deaminase deficiency</td>
</tr>
<tr>
<td>BMT</td>
<td>Bone marrow transplant</td>
</tr>
<tr>
<td>B+ SCID</td>
<td>Subgroup of severe combined immunodeficiency in which B lymphocytes are present</td>
</tr>
<tr>
<td>B- SCID</td>
<td>Subgroup of severe combined immunodeficiency in which B lymphocytes are absent</td>
</tr>
<tr>
<td>CD Antigens</td>
<td>Cluster of differentiation, nomenclature of leucocyte molecules</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CIBMTR</td>
<td>Center for International Blood and Marrow Transplant Research</td>
</tr>
<tr>
<td>CMV</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked ImmunoSorbent Assay</td>
</tr>
<tr>
<td>ERG</td>
<td>Evidence review group</td>
</tr>
<tr>
<td>ERT</td>
<td>Enzyme replacement therapy</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte-colony stimulating factor</td>
</tr>
<tr>
<td>GVHD</td>
<td>Graft versus host disease</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>HSCT</td>
<td>Hematopoietic stem cell transplant</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IL-7</td>
<td>Interleukin 7</td>
</tr>
<tr>
<td>IVIG</td>
<td>Intravenous immunoglobulin</td>
</tr>
<tr>
<td>JAK</td>
<td>Janus kinase</td>
</tr>
<tr>
<td>MeSH</td>
<td>National Library of Medicine medical subject heading</td>
</tr>
<tr>
<td>MMRD</td>
<td>Mismatched related donor</td>
</tr>
<tr>
<td>MUD</td>
<td>Matched unrelated donor</td>
</tr>
<tr>
<td>NIAID</td>
<td>National Institute of Allergy and Infectious Diseases</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PHA</td>
<td>Phytohemagglutinin</td>
</tr>
<tr>
<td>RID</td>
<td>Related identical</td>
</tr>
<tr>
<td>SCID</td>
<td>Severe Combined Immunodeficiency</td>
</tr>
<tr>
<td>SCIDA</td>
<td>Severe Combined Immunodeficiency, Athabascan-type</td>
</tr>
<tr>
<td>TREC</td>
<td>T-cell receptor excision circles</td>
</tr>
<tr>
<td>USIDNET</td>
<td>United States Immunodeficiency Network</td>
</tr>
<tr>
<td>XSCID</td>
<td>X-Linked Severe Combined Immunodeficiency</td>
</tr>
<tr>
<td>γc</td>
<td>Common cytokine receptor γ chain</td>
</tr>
</tbody>
</table>
I. Overview
Severe Combined Immunodeficiency (SCID) is a group of disorders characterized by the absence of both humoral and cellular immunity. Reported incidence is approximately 1/100,000 (Chan, Puck 2005, McGhee et al. 2005). Currently at least 15 genes are known to cause SCID when mutated (Puck, SCID Newborn Screening Working Group 2007). Although disease presentation varies (Buckley et al. 1997), as protection from maternal antibodies wanes during the first months of life, infants with SCID develop infections due to both common and opportunistic pathogens (Buckley et al. 1997). Treatment and prevention of infections can prolong life but are not curative actions (Buckley et al. 1999).

Early receipt of hematopoietic stem cell transplant (HSCT), prior to the onset of severe infections, offers the best chance of cure (Buckley et al. 1999). Although family history leads to early detection of some infants, the majority of infants with SCID are not detected until they develop clinical symptoms such as recurrent infections, failure to thrive and infection with opportunistic organisms (Buckley et al. 1997). The apparent value of early HSCT has led to a search for methods for pre-symptomatic identification of infants with SCID, including possible population-based newborn screening.

II. Rationale for Review
The Advisory Committee on Heritable Disorders in Newborns and Children has directed the Evidence Review Group to produce this report for the nominated condition of SCID. SCID has been nominated for the following reasons:

1. Without treatment, SCID leads to death in early childhood.
2. Earlier treatment, particularly before the onset of lung infection, decreases the mortality and morbidity associated with SCID and with HSCT for SCID.
3. Methods to screen infants for SCID, using quantitative PCR for T-cell receptor excision circles (TREC), have been developed.

III. Objectives of review
The objective of this review is to provide information to the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) to guide recommendations regarding screening newborns for SCID. Specifically, the Evidence Review Group’s (ERG) goal was to summarize the evidence available from published studies, as well as critical unpublished data available from key investigators in the field.

IV. Main questions
We sought to answer the following questions, with a particular emphasis on the questions related to screening and the benefits of early treatment.

1. What is the natural history of SCID and are there clinically important phenotypic or genotypic variations?
2. What is the prevalence of SCID and its variations?
3. What methods exist to screen newborns for SCID? How accurate are those methods? What are their sensitivity and specificity? What methods exist to diagnose individuals with positive screens?
4. What benefit does treatment, particularly pre-symptomatic, confer?
5. What are the potential harms or risks associated with screening, diagnosis or treatment?
6. What costs are associated with screening, diagnosis, treatment and the failure to diagnose in the newborn period?

By answering these questions we hoped to provide the ACHDNC with answers to broader questions related to SCID screening. Specifically:

1. Do current screening tests effectively and efficiently identify cases of SCID?
2. Does pre-symptomatic or early symptomatic intervention in newborns or infants with SCID improve health outcomes? In other words, does early identification lead to better outcomes?
3. What is the cost-effectiveness of newborn screening for SCID?
4. What critical evidence appears lacking that may inform screening recommendations for SCID?

V. Conceptual framework
The conceptual framework illustrated below outlines the salient factors when considering newborn screening for any disorder, SCID in the case of this review. Children will develop SCID regardless of whether or not they are screened. The key decision points, therefore, are whether to screen newborns and what treatment to pursue for affected children. At either decision point, children may experience benefits from the decision but also are at risk of adverse effects, including false-positive or false-negative screening results and significant treatment side-effects. The combination of the baseline risk for SCID and the effects of the screening and treatment decisions lead to a state of health.

Figure 1- Conceptual framework.
VI. Methods
This evidence review provides information from two sources: a systematic literature review and interviews with experts in the field of SCID.

A. Systematic literature review
We searched MEDLINE for all relevant studies published over the 20 year period from January 1988 to October 2008. We completed searches combining the National Library of Medicine Medical Subject Heading (MeSH) “severe combined immunodeficiency” with each of the following MeSH terms: epidemiology, incidence, prevalence, disease progression, mass screening, neonatal screening, genetic screening, diagnosis, and therapeutics. In order to capture articles which have not yet been assigned MeSH terms, we also searched the following keywords within the OVID In-Process and Other Non-Indexed Citations database: severe combined immunodeficiency, severe immune deficiency, adenosine deaminase deficiency. We applied human studies and English language limitations to all searches. This search strategy resulted in a total of 725 articles.

All abstracts were reviewed by at least 2 readers (EL, JD, JP). Articles which were non-human data, reviews, editorials or other opinion pieces, case series of <4 patients, only contained adult subjects or did not address one or more of the key questions were eliminated. Disagreements were resolved through discussion with emphasis on inclusion of any potentially useful data. After this process 60 articles remained which were reviewed in detail.

Each article was evaluated, using standardized tools, for the quality of the study design (NHS Centre for Reviews and Dissemination, March 2001) and the quality of the evidence, as it relates to the category of evidence (Pandor et al. 2004, Pollitt et al. 1997). A given article received only one rating per reader for study design, but may have received multiple quality evaluations for the type of evidence. For example, a study that discusses prevalence and natural history would be evaluated for the quality of the evidence in each of those domains.

Data were abstracted from the articles by 2 reviewers (EL, SV). A subset of the papers, approximately 20%, was abstracted by both reviewers in order to check for agreement. There were no significant differences in the data extracted by the reviewers.

B. Interviews with experts
The ERG and the ACHDNC recognize that in a rapidly developing field, such as newborn screening for SCID, there may be crucial evidence that is either not yet ready for publication or has not been published for other reasons. As such, using our literature search and discussions with content experts, we identified a list of key investigators. This list was discussed among the members of the ERG, and additional names were added based on personal knowledge of the field by members of the ERG. This list (Table 1) was approved by the ERG and the experts were contacted via e-mail. Reminder e-mails were sent to non-responders approximately 2 weeks after the initial e-mail.

Experts were sent a letter (appendix C) explaining the purpose of the review, a conflict of interest form (appendix B) and an open-ended survey (appendix C). After receipt of the conflict of interest form and survey, phone interviews were scheduled between experts and members of
the ERG (EL, AK, JP), when clarification of the written survey was needed. The semi-structured phone interviews clarified information provided in the written survey. Information from the surveys and interviews is explicitly provided in this report when such information was not available in the published literature.

Table 1- List of experts contacted and degree of participation

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
<th>Completed written survey</th>
<th>Telephone interview</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mei Baker</td>
<td>Assistant Professor, Department of Pediatrics, School of Medicine and Public Health, Science Advisor, Newborn Screening Program, Wisconsin State Laboratory of Hygiene, University of Wisconsin-Madison</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Barbara Ballard</td>
<td>The SCID Family Network</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Tony (Francisco) Bonilla</td>
<td>Program Director, Clinical Immunology Assistant in Medicine, Assistant Professor, Harvard Medical School and Children’s Hospital, Boston, Massachusetts</td>
<td>✓ *</td>
<td></td>
</tr>
<tr>
<td>Marcia Boyle</td>
<td>President and Founder, Immune Deficiency Foundation</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Rebecca Buckley</td>
<td>Professor of Pediatrics, Professor of Immunology, Duke University Medical Center, Durham, North Carolina</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Anne Comeau</td>
<td>Deputy Director New England Newborn Screening Program University of Massachusetts Medical School</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Lisa Filipovich</td>
<td>Medical Director, Hematology/Oncology Diagnostic Laboratory, Division of Hematology/Oncology, Cincinnati Children's Hospital Medical Center, Ohio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alain Fischer</td>
<td>Professor of Pediatric Immunology and Head of the Pediatric Immunology Department and the INSERM Research Unit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alan P. Knutsen</td>
<td>Professor of Pediatrics, Allergy and Immunology, Director of Pediatric Clinical Immunology Laboratory, Department of Pathology, St Louis University Health Sciences Center</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ronald Laessig</td>
<td>Professor of Population Health Sciences and Pathology at University of Wisconsin</td>
<td>✓ ^</td>
<td></td>
</tr>
<tr>
<td>Edward McCabe</td>
<td>Clinical Biochemical Genetics, Clinical Genetics, Professor and Executive Chair of the UCLA Department of Pediatrics, and Physician-in-Chief of the Mattel Children’s Hospital at UCLA, Los Angeles, California</td>
<td>✓ **</td>
<td></td>
</tr>
</tbody>
</table>
VII. Results

Case definition: For the purpose of this review, Severe Combined Immunodeficiency is defined based on the definition for the PubMed medical subheading. SCID is a “group of rare congenital disorders characterized by impairment of both humoral and cell-mediated immunity, leukopenia, and low or absent antibody levels. It is inherited as an X-linked or autosomal recessive defect.” (U.S. National Library of Medicine and the National Institutes of Health, 2008) Children with SCID universally have extremely low or absent T-cells and may or may not have B-cells. We have included some specific sub-types such as adenosine deaminase deficiency (ADA deficiency), reticular dysgenesis and Omenn syndrome in the definition of SCID because they are characterized by absence of T-cells, but we recognize that some groups consider these disorders distinct from SCID (WHO Scientific Group, 1995).
Table 2- Study design for abstracted articles

<table>
<thead>
<tr>
<th>Study design</th>
<th>Number of papers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental intervention</td>
<td>0</td>
</tr>
<tr>
<td>Cohort study</td>
<td>10</td>
</tr>
<tr>
<td>Case-control study</td>
<td>8</td>
</tr>
<tr>
<td>Case series total</td>
<td>39</td>
</tr>
<tr>
<td>Sample size ≤ 10</td>
<td>11</td>
</tr>
<tr>
<td>Sample size 11 to 50</td>
<td>18</td>
</tr>
<tr>
<td>Sample size ≥ 51</td>
<td>10</td>
</tr>
<tr>
<td>Economic evaluation</td>
<td>1</td>
</tr>
<tr>
<td>Other design</td>
<td>2*</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
</tr>
</tbody>
</table>

*Epidemiologic studies using retrospective record review (Jones et al. 1991) and telephone survey (Boyle & Buckley, 2007)

Evidence review: The remainder of this section of the report is dedicated to presentation of the evidence. Data are organized by key questions. A table indicating the quality of the evidence for that key question leads the section, followed by data from the systematic literature review. Finally, any additional information learned from surveying experts is provided at the end of each section.

A. What is the natural history of SCID and are there clinically important phenotypic or genotypic variations?

Table 3- Quality assessment of abstracted literature pertaining to natural history

<table>
<thead>
<tr>
<th>Genotype/Phenotype correlation</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data from retrospective screening studies in U.S. or similar population.</td>
<td>0</td>
</tr>
<tr>
<td>Data from systematic studies other than whole population screening.</td>
<td>5</td>
</tr>
<tr>
<td>Estimated from the known clinical features of the condition as described for individual cases or short series.</td>
<td>7</td>
</tr>
<tr>
<td><strong>Incidence</strong> (cases per 100,000), average within the U.S.</td>
<td>4</td>
</tr>
<tr>
<td>Data obtained from whole-population screening or comprehensive national surveys of clinically detected cases.</td>
<td>1</td>
</tr>
<tr>
<td>Ia. As in I but more limited in geographical coverage or methodology.</td>
<td>2</td>
</tr>
<tr>
<td>Extrapolated from class I data for non-U.S. populations.</td>
<td>0</td>
</tr>
<tr>
<td>Estimated from number of cases clinically diagnosed in U.S.</td>
<td>1</td>
</tr>
</tbody>
</table>

Adapted from Pandor et al. 2004, Pollitt et al. 1997
The term SCID refers to a group of slightly heterogeneous disorders which are all related by the absence of T-cells in affected individuals. Some subtypes are missing other lymphocyte subsets, such as B-cells or NK-cells, and others have associated non-immune manifestations such as neurocognitive deficits, hearing loss and skeletal abnormalities.

We describe below evidence related to the characteristic presentations of SCID, the natural history (without treatment), subtypes of SCID, and genotype/phenotype data. The main findings are: 1) with the exception of children diagnosed early in life, typically through prenatal testing initiated because of family history, most children are diagnosed after recurrent pulmonary infections or infections with opportunistic organisms; 2) this is true of all SCID subtypes, although the exact timing may vary; and 3) without treatment of the underlying immunodeficiency, children with SCID die in early childhood from infection.

General findings regarding natural history and associated clinical conditions -Systematic literature review

Several studies provide descriptive data regarding symptomatic presentation of children with SCID, almost all of which highlight pulmonary and opportunistic infections, leading to early childhood death, as the key complications of untreated SCID.

A chart review (Deerojanawong et al. 1997) found that all children who did not receive prenatal diagnoses of SCID (13/15) had pulmonary symptoms at presentation. Of 9 that died, 5 were due to pulmonary disease. Moreover, pulmonary infections, most commonly Pneumocystis jirovecii pneumonia (PJP), were the most common presenting sign of SCID, occurring in 10/15 children, at a median age of 4 months. Another case series (Stocks et al. 1999) found 14/18 of the children, over a 20-year time period, had otolaryngological signs or symptoms (including congestion, URI, thrush, oral ulcers, cervical adenopathy, otitis media; mastoiditis) prior to diagnosis with SCID.

An institutional case series (Stephan et al. 1993) of 117 patients treated for SCID between 1970 and 1992, found the median age at diagnosis, which did not change over the time period, was 4.6 months with an average of 2 months delay between clinical manifestations and diagnosis. The primary clinical manifestations included oral candida, skin erythema, diarrhea with growth impairment and interstitial pneumonitis.

Hague et al. (Hague et al. 1994) found that, among 32 children with no family history of SCID, the median age of diagnosis was 7 months, despite the median age of symptom onset being 5 weeks. In this group the median age of first hospital admission was 4 months with 22/32 presenting with respiratory infection, 9/32 with vomiting and diarrhea, 8/32 with candidal infection and 6/32 with failure to thrive.

Two papers discussed physical signs or symptoms that may occur with high prevalence in children with SCID but are not necessarily presenting signs of the disease, including gastroesophageal reflux (GER) (Boeck, Buckley & Schiff 1997) and rashes, particularly due to engraftment of maternal T-lymphocytes (Denianke et al. 2001).
Table 4 - Evaluated literature pertaining to natural history of SCID (alphabetical by author)

<table>
<thead>
<tr>
<th>Study (Author, Date)</th>
<th>Population: Size and age by subgroups</th>
<th>Study type</th>
<th>Key findings</th>
</tr>
</thead>
</table>
| Bertrand et al. 1999 | 178 children total; B+ group: 122, mean age at BMT was 7 months B- group: 56, mean age at BMT was 6.5 months. | Cohort study | • B+ SCID has better prognosis than B- SCID following non-identical BMT.  
• Rate of engraftment not different between groups.  
• B- SCID had higher rates of both early and late death.  
• Deaths in both groups due primarily to infection. |
| Bertrand et al. 2002 | 10 children total; BMT at age 0.5 to 10 months. | Case series | • All children had reticular dysgenesis.  
• All presented with infection in first days of life.  
• HLA-non-identical HSCT resulted in three of the 10 patients surviving.  
• Deaths due primarily to graft failure. |
| Buckley et al. 1997 | 108 children total; Diagnosed at age birth to 21 months. | Case series | • Mean age at diagnosis of SCID was 201 days (6.59 months) and did not differ significantly among different genetic types.  
• 67 (76%) of the 88 families were white, 14 (16%) were black, and 7 (8%) Hispanic.  
• Abnormal serum immunoglobulin concentrations existed in all types of SCID.  
• Mean IgA levels were lowest in γc-deficient, Jak3-deficient and unknown types of SCID.  
• Mean IgM concentrations were lowest in ADA, γc-deficient and Jak3-deficient patients.  
• IgE was normal in all but 2 patients. |
| Buckley et al. 1999 | 89 children total; 22 less than 3.5 months old at transplant, 67 greater than or equal to 3.5 months at transplant. | Case series | • 72 (81%) alive 3 months-16.5 years post-transplant, with a median follow up of 5.6 years.  
• 65 survived greater than 1 year, 38 greater than 5 years and 21 greater than 10 years.  
• All 12 HLA-identical recipients survived.  
• Mean number of T/B/NK-cells varies by genotype; all genotypes had normal average in vitro mitogen response after transplant.  
• Poor B-cell function, with 45 kids requiring IVIG.  
• NK-cell activity low in γc-chain deficiency and JAK3 deficiency, normal in other SCID subtypes. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Children Total</th>
<th>Age/Condition</th>
<th>Methodology</th>
<th>Findings</th>
</tr>
</thead>
</table>
| Cavazzana-Calvo et al. 2007 | 31 children    | 1-42 months old at HSCT, now 10-27 years later.                                | Case series       | - Myeloablation patients more likely to have evidence of donor-derived granulocytes and persistent naïve T-cells, as measured by TREC.  
  - At follow-up, 60% of TREC+ and 45% of TREC- had no clinical manifestations.  
  - Average follow-up time in the TREC+ group was 13 years and in the TREC- was 16 years. |
| Gomez et al. 1995         | 9 children     | 0.5-5 months old (mean 2.8) with BMT for Omenn Syndrome between 1981 and 1989. | Case series       | - Engraftment occurred in 4/5 HLA-identical transplants and 3/4 non-identical transplants.  
  - Full chimerism occurred in all but one of the patients that engrafted.  
  - Clinical manifestations of Omenn syndrome disappeared within days of BMT (likely due to pre-treatment with chemotherapy).  
  - One patient died of CMV 50 days post-transplant, 3 developed interstitial pneumonia.  
  - All survivors except one (who has chronic GVHD) have normal growth rates. |
| Laffort et al. 2004       | 41 children    | at least 10 years after HSCT for SCID.                                         | Case-control study | - 9/41 developed extensive chronic human papilloma virus.  
  - 4 had lesions typical of a rare genodermatosis.  
  - All with human papilloma virus had γc/Adenylate kinase- 3 (AK-3) deficiency, though only 9/18 with γc/JAK3 developed human papilloma virus. |
| Monafo et al. 1992        | 4 children     | (2 sets of siblings); age 20 months, 4 months, 11 months, 24 months at the time of BMT. | Case series       | - Presenting clinical signs of SCID included diarrhea, poor weight gain, oral ulcers, and PJP pneumonia but normal lymphocyte counts.  
  - One child did not present until 1 year of age, with a previously undescribed phenotype.  
  - CD8 cells were virtually absent and mononuclear cells did not proliferate normally in vitro. |
| O'Marcaigh et al. 2001    | 18 children    | Birth to 3 months of age at diagnosis; mean age of 17.5 months at first transplant. | Case series       | - 12/18 developed oral/genital ulcers associated with SCIDA.  
  - 16/18 children were well enough for transplant.  
  - 15/16 achieved engraftment.  
  - 11/16 developed normal T-cell function. |
Roberts et al. 2004
170 total patients; 10 with JAK3 deficiency and 160 with other types of SCID.  
Case series  
- All patients had abnormal B-cell JAK3 dependent interleukin-2 induced signal transducer and activator of transcription 5 (STAT5) phosphorylation.  
- 9/10 patients with JAK3 deficiency were alive and well 4 to 18 years after stem cell transplant.

Rogers et al. 2001
22 children total; 1-51 months old at HSCT; 0.9-18 years old at follow-up; 11 children with ADA deficiency, 11 other SCID types.  
Cohort study  
- No significant difference in full-scale IQ between groups; children with ADA deficiency 2 standard deviations below mean.  
- Significant behavioral differences in children with ADA deficiency: more hyperactivity, higher scores for dysfunction in social, emotional and behavioral domains.  
- Abnormal behavior more evident in older children.  
- Children with ADA deficiency group with no abnormal motor function on gross neurological evaluation.

Genotype specific findings – Systematic literature review
Although as many as 15 different genes (Puck, SCID Newborn Screening Working Group 2007) are associated with SCID, the known phenotypic variation is less extensive. There is some phenotypic variation, as outlined below, in immunoglobulin levels, lymphocyte counts and lymphocyte function. More striking, however, a few phenotypes appear to have distinct characteristics at presentation. Specifically we found evidence supporting very early infections in children with reticular dysgenesis (Bertrand et al. 2002), neurologic symptoms in children with ADA deficiency (Stephan et al. 1993, Honig et al. 2007), and oral/genital ulcers associated with a distinct form of SCID known as SCIDA occurring among Athabascan-speaking Native Americans (O’Marcaigh et al. 2001).

A case series (Buckley et al. 1997) of 108 infants examined the relationship between genotype and immune functional status. These patients ranged from birth to 21 months at diagnosis and children with less severe forms of combined immunodeficiency, such as Omenn syndrome, were excluded. The distribution of genotypes from this series is shown in table 5.
**Table 5- Relative frequencies of different SCID genotypes from Buckley et al. 1997**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SCID Infants* (n=108)</th>
<th>SCID families* (n=88)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>γc deficiency</td>
<td>49</td>
<td>45.4</td>
</tr>
<tr>
<td>ADA deficiency</td>
<td>16</td>
<td>14.8</td>
</tr>
<tr>
<td>Jak3 deficiency</td>
<td>8</td>
<td>7.4</td>
</tr>
<tr>
<td>Autosomal Recessive</td>
<td>21</td>
<td>19.4</td>
</tr>
<tr>
<td>(not ADA or Jak3 deficiency)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reticular Dysgenesis</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Cartilage-hair hypoplasia</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>Unknown</td>
<td>12</td>
<td>11.1</td>
</tr>
</tbody>
</table>

*table depicts genotype according to the 108 individual patients in the first column, and the 88 families of origin in the second column where siblings count as one unit

The authors found abnormal serum immunoglobulin concentrations in all types of SCID. Mean IgA levels were lowest in γc-deficient, Jak3-deficient and unknown types of SCID. Mean IgM concentrations were lowest in ADA, γc and Jak3 deficient patients. IgE was normal in all but 2 patients.

Lymphopenia was present in all categories of SCID but more severe in patients with ADA deficiency. 5 infants had elevated lymphocytes, of which 4 were due to the presence of maternal cells. Lymphocyte subpopulations varied between types of SCID. ADA-deficient children had the lowest mean number of B-cells and γc and Jak3 the highest. Mean number of NK-cells was lowest in ADA, γc, and Jak3 deficient patients. Lymphocyte response to mitogens was diminished in all patients.

**B. What is the prevalence of SCID and its variations?**

Most methods of determining the prevalence and incidence of a disorder depend on case ascertainment. As in other disorders with high risk of early death, the incidence of SCID may be higher than measured via case ascertainment because some patients may die prior to having a definitive diagnosis.

*Incidence and Prevalence-Systematic literature review*

In a study with a primary focus on SCID screening methods, Chan and Puck (Chan, Puck 2005) estimated the annual incidence as a minimum of 1/105,000 live births. This calculation was based on assuming an annual US birthrate of 4,000,000 that all children with XSCID in the U.S. had blood samples sent to the authors’ laboratory for mutation detection (19 in one year) and that XSCID represents approximately half of all cases of SCID. A study (Stephan et al. 1993) of French children with SCID estimated a minimum incidence of 1/100,000 live births. This estimate is based on the number of French children referred to specialized units over a 5 year time period.

Some US populations, namely the Navajo, have a higher prevalence of SCID because of a founder mutation in the Artemis gene. The death records (Jones et al. 1991) of all Native American children in Arizona who died between 1969 and 1978 and Navajo children who died
between 1969 and 1982 in Arizona and New Mexico were reviewed. Subsequently the authors reviewed the hospital records of children who died from SCID, had signs of immunodeficiency or failure to thrive at the time of death or had unknown cause of death. They used this information to determine who may have had SCID. Based on this chart review the estimated prevalence of SCID is 52/100,000 live Navajo births.

**Information from expert interviews**

Dr. Buckley stated that in one year in North Carolina (where the birth rate is approximately 120,000/annually) there were 3 cases of SCID, for an incidence of 1/40,000. However, multi-year data confirming this incidence are not available.

Drs. Buckley and Notarangelo provided the distribution of genotypes among the SCID patients for which they have personally cared. Additionally, Drs. Pai, Bonilla, and Notarangelo provided subtype distribution information from both European and United States immunodeficiency collaboratives. These data corroborated the data from the published literature portion of this evidence review.

**C. What methods exist to screen newborns for SCID? How accurate are those methods? What are their sensitivity and specificity? What methods exist to diagnose individuals with positive screens?**

**Table 6- Quality assessment of abstracted literature pertaining to screening test characteristics**

<table>
<thead>
<tr>
<th>Overall sensitivity and specificity of screening &amp; false-positive rate</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data obtained from screening programs in U.S. population or similar.</td>
<td>0</td>
</tr>
<tr>
<td>Data from systematic studies other than from whole population screening.</td>
<td>3</td>
</tr>
<tr>
<td>Estimated from the known biochemistry of the condition.</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Repeat specimen rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Second-tier testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>Data obtained from screening programs in US population or similar.</td>
</tr>
<tr>
<td>Data from systematic studies other than whole population screening.</td>
</tr>
<tr>
<td>Estimated from the known biochemistry of the condition.</td>
</tr>
</tbody>
</table>

Adapted from Pandor et al. 2004, Pollitt et al. 1997

At least three different methods of screening for SCID have been proposed including 1) lymphocyte counts of whole blood, 2) quantitative polymerase chain reaction (qPCR), and 3) enzyme linked immunosorbent assay (ELISA) of dried blood spots. This section will review the evidence regarding screening. None of the proposed screening methods distinguish the various SCID genotypes and phenotypes.
Table 7- Evaluated literature pertaining to SCID screening studies (alphabetical by author)

<table>
<thead>
<tr>
<th>Study (Author, Date)</th>
<th>Population</th>
<th>Screening method</th>
<th>Sens, spec, PPV, NPV</th>
<th>Type of Evidence</th>
</tr>
</thead>
</table>
| Chan, Puck 2005      | 23 children with SCID, 2 children with non-SCID immunodeficiencies. 242 anonymized newborn screening cards. | • Used DNA amplification of TREC from dried blood spot.  
• Among the children known to have SCID, none had detectable levels of TREC and all had detectable β-actin.  
• The 2 children with non-SCID immunodeficiency had detectable TREC.  
• Had several presumed false-positives in which beta-actin could be amplified but TREC could not.  
• Estimated incidence of SCID to be 1/105,000 births, excluding patients who die before their immunodeficiency is recognized. | *False positive rate: 1.5% from routine nurseries; 5% from special-care nurseries.  
^Sensitivity: 84%-100%  
^Specificity: 97-97.1% | Overall sensitivity and specificity of screening & false-positive rate |
| Hague et al. 1994    | 135 total children, 45 children with SCID, 90 children without SCID. | • Used first available lymphocyte count.  
• SCID children matched to asymptomatic children by age and to children with same presenting symptoms by age.  
• Children with SCID had significantly lower levels of lymphocytes.  
• Unlike the 5 control children with low lymphocyte count, low lymphocyte count persisted in children with SCID. | *False-positive rate: 8%  
^Sensitivity: 86.3%, and  
^Specificity: 94.4% | Overall sensitivity and specificity of screening & false-positive rate |
| Hennewig et al. 2007 | 36 children with rotavirus gastroenteritis; 18 with SCID, 18 without SCID. | Lymphocyte study.  
SCID children were more likely to have:  
• Low white blood cell count: 10/18 vs. 0/18,  
• Eosinophilia: 12/18 vs. 0/18  
• Relative lymphopenia: 17/18 vs. 10/18  
• Absolute lymphopenia: 16/18 vs. 4/18 | ^Sensitivity: 55.6% to 94.4%  
^Specificity: 44.4% to 100%. | Second-tier testing |
|-------------------|-------------------------------------------------|-------------------------------------------------|---------------------|---------------------|
| McGhee et al. 2005 | 13 children with SCID, 183 anonymized dried blood spots, presumed to be from children without SCID. | Dried blood spot study.  
A 2-tiered screening approach in which IL-7 is first measured and only those with elevated IL-7 would have TREC measured, although for this study researchers evaluated each test separately.  
Of the 183 presumed normal children tested for TREC, 14 were undetectable.  
Of the 3 SCID children tested for TREC, all were undetectable. | *Combined specificity of 100% (confidence interval, 97-100%)  
*Combined sensitivity of at least 85% | Overall sensitivity and specificity of screening & false-positive rate |

*Calculation stated in article  
^Our calculation using data provided in article

**Systematic literature review**

**Lymphocyte studies:** A case-control study (Hague et al. 1994) matched children who were both pre- and post-symptomatically diagnosed with SCID to either asymptomatic children (presenting for surgery or bilirubin screening) or children matched by age and presenting symptoms, in order to compare the earliest available lymphocyte counts. Children with SCID had significantly lower levels of lymphocytes. Also, unlike in the 5 control children with low lymphocyte counts, in SCID children the low lymphocyte count persisted. The authors calculated a false-positive rate of 8%, positive predictive value of 86% and negative predictive value of 100% among symptomatic children, and a positive and negative predictive value of 93% for all children. As part of the evidence review, we used all data contained within this article to calculate the sensitivity, 86.3%, and specificity, 94.4%, of “first lymphocyte count” using a cut-off of 2.8x10⁹/l as indicating SCID.

A cohort study (Hennewig et al. 2007) that included 36 children (18 with and 18 without SCID) with rotavirus gastroenteritis, also investigated white blood cell count as a screening tool for SCID. The researchers found that most of the children with SCID were younger than controls.
when rotavirus was diagnosed. They also were more likely to have a low white blood cell count (10/18 vs. 0/18), eosinophilia (12/18 vs. 0/18) and relative lymphopenia (17/18 vs. 10/18) and absolute lymphopenia (16/18 vs. 4/18). From these numbers we calculated sensitivities from 55.6% to 94.4% and specificities from 44.4% to 100%.

**DBS studies:** Two studies published in 2005 evaluated screening methods that utilize dried blood spots. The first (McGhee et al. 2005) evaluated a 2-tiered screening approach in which IL-7 is first measured (Table 8A) and only those with elevated IL-7 would have TREC measured (Table 8B). For the study the researchers evaluated each test separately, rather than using a tiered approach. The investigators tested samples from 13 children with SCID (either dried blood spots [3] collected in immunology clinic or stored serum [10]) and 183 anonymized dried blood spots, presumed to be from children without SCID. They evaluated the levels of T-cell receptor excision circles (TREC) in both groups. However, TREC analysis cannot be done on serum, limiting that portion of the analysis to dried blood spot specimens.

**Table 8A- Tier 1: Elevated IL-7 from McGhee et al. 2005**

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID</td>
<td>11</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>110</td>
<td>114</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>112</td>
<td>127</td>
</tr>
</tbody>
</table>

Authors calculated a specificity of 96.1% for IL-7 and a sensitivity of 85% (confidence interval, 55-98%).

**Table 8B- Tier 2: Detectable TREC from McGhee et al. 2005**

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td>169</td>
<td>14</td>
<td>183</td>
</tr>
<tr>
<td>Total</td>
<td>169</td>
<td>17</td>
<td>186</td>
</tr>
</tbody>
</table>

Authors calculated a specificity of 92.3% for TREC and a sensitivity that “approaches 100%.”

They evaluated the sensitivity and specificity of the proposed 2-tiered approach calculating a combined specificity of 100% (confidence interval, 97-100%) and combined sensitivity of at least 85%.

Among their sample of SCID patients was one child with Omenn syndrome, which is known to have detectable T-cells, and another patient with T-B- SCID of unknown cause that could theoretically have affected the IL-7 system. This high proportion of “unusual types” of SCID led the authors to suggest that the actual sensitivity of IL-7 may be higher than shown in this study.

In the second study (Chan, Puck 2005) investigating screening for SCID using dried blood spots, the sample included 23 children with SCID, 2 children with non-SCID immunodeficiencies, 242 anonymized newborn screening cards.
The researchers used DNA amplification as the screening method in which β-actin served as a control, indicating that DNA could be amplified from a given specimen, i.e., that the specimen was satisfactory for analysis; and TREC amplification served as the screening test.

Among the children known to have SCID, none had detectable levels of TREC and all had detectable β-actin. The 2 children with non-SCID immunodeficiency had detectable TREC.

The researchers assumed that the anonymized newborn screening cards came from children without SCID. In this group there were 7 (2 from routine nursery, 5 from special-care nursery) cards from which TREC could not be detected. Cards with detectable β-actin but not TREC were presumed to be false-positive. They calculated a false-positive rate of 1.5% among children discharged from routine nurseries and 5% among children discharged from special-care nurseries.

We combined the results from this study, except the cards from which β-actin could not be amplified, and calculated a sensitivity of 84% and specificity of 97.1% for undetectable TREC and sensitivity of 100% and specificity of 97% for TREC <30.

Additional data from expert interviews regarding screening:
Wisconsin implemented universal newborn screening for SCID on January 1, 2008. Their protocol involves TREC quantification. For samples with TREC <25/μl they repeat the testing in duplicate, using two new punches from the same newborn screening sample collection card, and also assess for β-actin. Approximately 1.5% of samples require a second test, and approximately 0.2% require a second newborn screen or confirmatory testing. The table below was provided by Dr. Baker as a summary of their screening reports.

Table 9- Summary of screening categories for Wisconsin’s newborn screening for SCID

<table>
<thead>
<tr>
<th>Reporting Type</th>
<th>Situation</th>
<th>Action Plan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1. TREC ≥ 25 / ul in the first tier test</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2. TREC ≥ 25 / ul in one or both duplicates of the second tier test</td>
<td>None</td>
</tr>
<tr>
<td>Abnormal</td>
<td>TREC &lt; 25 / ul and ACTB &gt; 10,000 / ul in full term babies</td>
<td>1. Phone Primary Care Provider and clinical consultant 2. Recommend confirmatory testing</td>
</tr>
<tr>
<td></td>
<td>TREC &lt; 25 / ul and ACTB &gt; 10,000 / ul in premature infants</td>
<td>1. Phone Primary Care Provider 2. Tracking second NBS*</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>TREC &lt; 25 / ul and ACTB &lt; 10,000 / ul in full term babies</td>
<td>Recommend repeating NBS</td>
</tr>
<tr>
<td></td>
<td>TREC &lt; 25 / ul and ACTB &lt; 10,000 / ul in premature infants</td>
<td>Tracking second NBS*</td>
</tr>
</tbody>
</table>

* The second and third NBS in premature infants is a standard practice in the Wisconsin newborn screening program, and no additional sample is requested for the SCID screening test.
As of December 31, 2008, Wisconsin had screened nearly 70,400 babies, of which 118 babies had inconclusive screens and 232 had abnormal screens (Table 10 and Table 11). To date, they have not identified any children with SCID.

Table 10- SCID screening results (Courtesy of Dr. Mei Baker, presented at the Newborn Screening Symposium, November, 2008; updated January, 2009)

<table>
<thead>
<tr>
<th>Number Screened:</th>
<th>70,397 (01/01/2008-12/31/2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature (&lt;37 weeks)</td>
<td>6487</td>
</tr>
<tr>
<td>Full term</td>
<td>63910</td>
</tr>
<tr>
<td>Abnormal Results:</td>
<td>32 (0.045%)</td>
</tr>
<tr>
<td>Premature (&lt;37 weeks)</td>
<td>20 (0.308%)</td>
</tr>
<tr>
<td>Full term</td>
<td>12 (0.019%)</td>
</tr>
<tr>
<td>Inconclusive Results</td>
<td>118 (0.168%)</td>
</tr>
<tr>
<td>Premature (&lt;37 weeks)</td>
<td>97 (1.50%)</td>
</tr>
<tr>
<td>Full term</td>
<td>21 (0.033%)</td>
</tr>
</tbody>
</table>

Table 11- SCID screening confirmation results (Courtesy of Dr. Mei Baker, presented at the Newborn Screening Symposium, November, 2008; updated January, 2009)

<table>
<thead>
<tr>
<th>Abnormal Results:</th>
<th>Inconclusive Results:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Term</td>
<td>- Full Term</td>
</tr>
<tr>
<td>1 DiGeorge Syndrome</td>
<td>1 Abnormal results on repeated NBS and abnormal Flow Cytometry (Idiopathic T-cell lymphopenia)</td>
</tr>
<tr>
<td>1 Downs Syndrome with sepsis at birth</td>
<td>17 normal results on repeated newborn screening</td>
</tr>
<tr>
<td>1 Idiopathic T-cell lymphopenia</td>
<td>1 pending case</td>
</tr>
<tr>
<td>1 Neutrophil migration defect with RAC2 mutation</td>
<td>2 expired cases</td>
</tr>
<tr>
<td>2 normal Flow Cytometry results</td>
<td>- Premature</td>
</tr>
<tr>
<td>4 normal results on repeated newborn screening</td>
<td>1 DiGeorge Syndrome (36 weeks)</td>
</tr>
<tr>
<td>1 pending case</td>
<td>1 Abnormal results on repeated NBS and abnormal Flow Cytometry (gastrochisis)</td>
</tr>
<tr>
<td>1 expired case</td>
<td>72 normal results on repeated newborn screening</td>
</tr>
<tr>
<td>- Premature</td>
<td>2 pending cases</td>
</tr>
<tr>
<td>1 DiGeorge Syndrome (36 weeks)</td>
<td>21 expired cases</td>
</tr>
<tr>
<td>1 chylous effusions (chylothorax and chylous ascites)</td>
<td></td>
</tr>
<tr>
<td>3 normal Flow Cytometry results</td>
<td></td>
</tr>
<tr>
<td>9 normal results on repeated newborn screening</td>
<td></td>
</tr>
<tr>
<td>4 pending cases</td>
<td></td>
</tr>
<tr>
<td>2 expired cases</td>
<td></td>
</tr>
</tbody>
</table>

Two other investigators have definitive plans for newborn screening trials. In February 2009, Dr. Comeau and colleagues at the New England Newborn Screening Laboratory began a trial of
universal newborn screening for SCID in Massachusetts, with a small pilot program planned in Texas and potential extension to other New England states. Similarly, Dr. Puck will soon begin a screening trial at 2 hospitals on Navajo reservations in New Mexico and Arizona, a site chosen because of the high frequency of SCID in the Navajo population.

All three of these trials use PCR to detect both TREC and DNA from a housekeeping gene as the screening test and control, respectively. However, there are minor variations in the specific methodology currently in practice or planned for each trial. These include variations in specific primers and extraction methods used, as well as the degree of automation.

Additionally, correspondence with topic experts indicated other screening techniques currently under consideration or evaluation. Dr. Pass indicated that work is underway to develop a T-cell immunoassay that could target key T-cell antigens and serve as a quantitative assay. However, this technique is still under development and has not yet, to our knowledge, been validated. Dr. Comeau indicated that her laboratory has developed a multiplex assay that ensures integral quality assurance; TREC's and RNaseP from the same aliquot of blood are measured in the same reaction. Furthermore, the assay is amenable to measuring other markers such as key T-cell antigens and development of multi-analyte profiles for SCID. Proof of principle for the multianalyte assay has been demonstrated; specific non-DNA markers are still in development.

Feasibility/acceptability of screening
We found no articles for inclusion in this review that evaluated the feasibility or acceptability of screening for SCID.

Diagnostic testing
Review of the literature found no evidence that describes any specific diagnostic testing protocol for SCID. We suspect this reflects the time-frame used in the literature search and that diagnostic testing protocols were established prior to 1988.

Articles that make reference to diagnostic testing and the experts with whom we spoke all utilize flow cytometry, which allows for the differentiation and quantification of the various sub-types of white blood cells. As mentioned in conversation with Dr. Puck, the presence of maternal T-cells can confound standard flow cytometry results. However, further testing for T-cell response to mitogens allows for differentiation between the mother’s cells, which do not respond to mitogens in vitro, and the child’s cells.

Additionally, several researchers (Vogt, Puck, Buckley, Notarangelo, Pai and Bonilla) commented on gene sequencing. The current expert opinion seems to be that knowing the specific genotype, with the exception of ADA deficiency, has little clinical significance or implications for treatment. However, the possibility exists that better knowledge of how children with different genotypes respond to treatment may help tailor future treatment.

D. What benefit does treatment, particularly pre-symptomatic, confer?
Numerous studies document that treatment, mainly bone marrow transplant, substantially improves survival. We describe below the evidence regarding the key debates surrounding
forms of treatment and their relative efficacy. We also provide some focus on the evidence regarding early versus later treatment effects.

**Table 12- Quality assessment of abstracted literature pertaining to effectiveness of treatment**

<table>
<thead>
<tr>
<th>Effectiveness of treatment</th>
<th>47</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Well-designed RCTs.</td>
<td>0</td>
</tr>
<tr>
<td>II-1. Well-designed controlled trials with pseudorandomization or no randomization.</td>
<td>0</td>
</tr>
<tr>
<td>II-2. Well-designed cohort studies:</td>
<td>8</td>
</tr>
<tr>
<td>A. prospective with concurrent controls</td>
<td>0</td>
</tr>
<tr>
<td>B. prospective with historical control</td>
<td>1</td>
</tr>
<tr>
<td>C. retrospective with concurrent controls.</td>
<td>7</td>
</tr>
<tr>
<td>II-3. Well-designed case-control (retrospective) studies.</td>
<td>0</td>
</tr>
<tr>
<td>III. Large differences from comparisons between times and/or places with and without intervention</td>
<td>4</td>
</tr>
<tr>
<td>IV. Opinions of respected authorities based on clinical experience, descriptive studies and reports of expert committees.</td>
<td>35</td>
</tr>
</tbody>
</table>

*Adapted from Pandor et al. 2004, Pollitt et al. 1997*

Over the last twenty years, three modes of treatment for SCID have been investigated: allogeneic hematopoietic stem cell transplant (HSCT), the most common subtype being bone marrow transplant (BMT), enzyme replacement therapy (ERT), and gene therapy. While HSCT is the most common treatment for SCID patients, ERT may be used for some patients with ADA deficiency. Lastly, small trials of gene therapy for SCID have been conducted. This section describes the evidence for each method of treatment.

**Hematopoietic stem cell transplant-Systematic literature review**

HSCT has been utilized as a treatment for SCID since its initial patient application in 1968 (Buckley et al. 1999). Since then, many researchers have investigated the efficacy, outcomes for different methods, and the long-term outcomes associated with HSCT. This evidence review focuses on answering the questions pertaining to the benefit of treatment: overall efficacy, efficacy early in life, efficacy for different genotypes and phenotypes of SCID, efficacy based on transplant method, and long-term follow-up including survival and immune reconstitution.

**HSCT in the neonatal period and/or infancy**

We found 2 studies that specifically addressed HSCT in the neonatal period. The first was a cohort study (Myers et al. 2002) and the other was a case series (Kane et al. 2001). In addition, Buckley et al. (Buckley et al. 1999) evaluated a group of patients receiving transplants within the first 3.5 months of life and a large case series from Europe (Antoine et al. 2003) distinguished children by age at transplant.

**Table 13- Abstracted literature pertaining to HSCT in the neonatal period and/or infancy (alphabetical by author)**
<table>
<thead>
<tr>
<th>Study (Author, Date)</th>
<th>Population</th>
<th>Key Findings</th>
<th>Quality of Evidence</th>
</tr>
</thead>
</table>
| Antoine et al. 2003^ | 475 patients (total of 566 transplants); 202 less than 6 months old at transplant, 184 between 6-11 months of age at transplant, from 37 European centers between 1968 and 1999. | - Children transplanted at less than 6 months of age had better 3-year survival rates than older cohorts.  
- Three-year survival with sustained engraftment was 77% for HLA-identical and 54% for HLA-non-identical transplants.  
- Survival has improved over time for both HLA-identical and HLA-non-identical transplant recipients.  
- Myeloablation prior to HLA-non-identical transplant trended towards improving survival among B- children but not B+ children.  
- ADA deficiency (n=51) 3-yr-survival was 81% for matched and 29% for unmatched transplants.  
- Reticular dysgenesis survival (n=12) was 75% for matched and 29% for unmatched transplants. | II-2 C |
| Buckley et al. 1999* | 89 children total; 22 less than 3.5 months old at transplant, 67 greater than or equal to 3.5 months at transplant. | - 72 (81%) alive 3 months-16.5 years post-transplant, with a median follow up of 5.6 years.  
- 65 survived greater than 1 year, 38 greater than 5 years and 21 greater than 10 years.  
- All 12 HLA-identical recipients survived.  
- Mean number of T/B/NK-cells varies by genotype; all genotypes had normal average in vitro mitogen response after transplant.  
- Poor B-cell function with 45 kids requiring IVIG.  
- NK-cell activity low in γc-chain deficiency and JAK3 deficiency, normal in other SCID subtypes. | IV |
| Kane et al. 2001 | 13 children total; transplanted between 7 and 68 days old. | - All patients alive and well 0.5-11.5 years after transplant (median 3 years).  
- 2 children developed chronic GVHD chronic.  
- 3 children required more than one transplant.  
- All children achieved neutrophil engraftment and normal levels of IgA.  
- 7 have normal IgG.  
- 12 have normal IgM.  
- 10/12 have normal neuro-development; 1/12 has trouble with communication and interactive skills, and 1/12 has motor delay. | IV |
| Myers et al. 2002* Cohort study | 21 children total; transplanted prior to 28 days of life (early treatment) and 96 children transplanted at a median age of 190 days (range: 45-516 days) (late treatment). | • 20/21 (95%) early treatment children survived.  
• 71/96 (74%) late treatment children survived.  
• Mean time to significant T-cell function in all early treatment was 33 days and to normal T-cell function was 103 days.  
• Mean TREC value peaked earlier post-transplant for early treatment recipients but the 2 groups were indistinguishable by 5 years.  
• Early transplantation did not have an affect on B-cell function. | II-2 C |

Potential patient overlap of Myers et al. 2002, Buckley et al. 1999

*A subset of patients in Fischer et al. 1990 are also included in Antoine et al. 2003

**Information from expert interviews**

Dr. Buckley shared SCID treatment data with our group. They have transplanted 161 SCID infants over the past 26 years, of which 16 had an HLA-identical (genotypically HLA-identical) donor. The remaining 145 patients received half-matched (HLA-haplo-identical) transplants from their parents. She has documented an overall 26-year survival rate of 125/161 (78%). However, her data shows there is a survival difference when the population is divided by age at transplantation. The survival rate in those transplanted before 3.5 months of age is 96%, whereas the survival rate for those transplanted after later is only 71%. All transplants included in this data were done without pre-transplant chemotherapeutic conditioning or post-transplantation GVHD prophylactic immunosuppressive drugs. The Kaplan-Meier graphs from Dr. Buckley (with permission) for these data are below (Graphs 1A and 1B).

Additionally, Dr. Notarangelo reported that the European experience has also shown that early age at transplant is favored, particularly because that decreases the chance of pre-transplant infection which may negatively impact outcomes.
Graph 1A- Kaplan-Meier plot of 48 children with SCID transplanted at Duke University Medical Center in the first 3.5 months of life

Graph 1B- Kaplan-Meier plot of 113 children with SCID transplanted at Duke University Medical Center after the first 3.5 months of life
Overview of efficacy—large case series of HSCT

We found 3 case series that specifically addressed the efficacy of HSCT over time. In one series, 31 patients undergoing BMT for SCID were evaluated between 1968 and 1992 (van Leeuwen et al. 1994). In a similar large case series (Stephan et al. 1993) 117 patients were followed from 1970 to 1992. A single-center study (Buckley et al. 1999) followed 89 children between 1982 and 1998.

Table 14- Abstracted literature pertaining to efficacy in large case series studies of HSCT (alphabetical by author)

<table>
<thead>
<tr>
<th>Study (Author, Date)</th>
<th>Population</th>
<th>Key findings</th>
<th>Quality of Evidence</th>
</tr>
</thead>
</table>
| Buckley et al. 1999  | Case series | 89 children total; 22 less than 3.5 months old at transplant, 67 greater than or equal to 3.5 months at transplant. | • 72 (81%) alive 3 months-16.5 years post-transplant, with a median follow up of 5.6 years.  
• 65 survived greater than 1 year, 38 greater than 5 years and 21 greater than 10 years.  
• All 12 HLA-identical recipients survived.  
• Mean number of T/B/NK-cells varies by genotype; all genotypes had normal average in vitro mitogen response after transplant.  
• Poor B-cell function with 45 kids requiring IVIG.  
• NK-cell activity low in γc-chain deficiency and JAK3 deficiency, normal in other SCID subtypes. | IV |
| Stephan et al. 1993* | Case series | 117 patients total; treated for SCID between 1970 and 1992; 85 children were treated with BMT. | • Estimated SCID incidence: 1/100-150,000.  
• HLA-identical transplant from a related donor 21/25 (84% survived).  
• Pheno-identical transplant (HLA-genotypically haplo-identical) from related donor 2/5 (40% survived).  
• HLA-haplo-identical transplant without T-cell depletion 0/5 (0% survived).  
• T-cell depleted haplo-identical transplant 28/50 (56% survived).  
• 22 children did not receive any type of transplant and died.  
• 10 received fetal liver transplant, 9 died post transplant. | IV |
van Leeuwen et al. 1994
Case series
31 patients total; 1-94 months old at BMT.
- HLA-identical related 6/10 (60% survived)
- HLA-haplo-identical related: 9/19 (47% survived).
- HLA-matched unrelated: 0/2 (0% survived).
- Major causes of death were graft and respiratory failure.
- All who died of respiratory failure had a lung infection prior to transplant.
- All children with sustained engraftment had evidence of humoral immunity.

* Potential patient overlap with Stephan et al. 1993 and Wijnaendts et al. 1989

**HSCT efficacy in different genotypes and phenotypes of SCID**
Several studies examined HSCT for patients with specific pheno- or genotypes, including: B+ SCID vs. B- SCID, SCIDA, reticular dysgenesis, ADA deficiency, specific Jak3 mutations, and Omenn syndrome. Table 15 summarizes the results of these studies.

**Table 15-Abstracted literature pertaining to HSCT efficacy in different SCID subgroups (alphabetical by author)**

<table>
<thead>
<tr>
<th>Study (Author, Date)</th>
<th>Population</th>
<th>Key findings</th>
<th>Quality of evidence</th>
</tr>
</thead>
</table>
| 1) Albuquerque & Gaspar, 2004 Cohort study | ADA deficiency | 1) Compared 12 children with ADA deficiency to 16 children with other immunodeficiencies, all treated with HSCT.  
• 7/12 children with ADA deficiency had bilateral deafness, compared to 1/16 in the control group. | II-B |
| 2) Honig et al. 2007 Case series | | 2) 12/15 survived, all with developmental delay.  
• 6/12 have significant neurocognitive deficits (learning disabilities, gait problems, hearing deficit, and/or hyperactivity). | IV |
| 3) Rogers et al. 2001 Cohort study | | 3) No significant difference in full-scale IQ between groups; children with ADA deficiency 2 standard deviations below mean.  
• Significant behavioral differences in children with ADA deficiency: more hyperactivity, higher scores for dysfunction in social, emotional and behavioral domains.  
• Abnormal behavior more evident in older children.  
• Children with ADA deficiency group with no abnormal motor function on gross neurological evaluation. | II-2 C |
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Condition</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Bertrand et al. 1999 | Cohort study      | B+ SCID vs. B- SCID | • B+ SCID has better prognosis than B- SCID following non-identical BMT.  
• Rate of engraftment not different between groups.  
• B- SCID had higher rates of both early and late death.  
• Deaths in both groups due primarily to infection. | II-2 C             |
| Bertrand et al. 2002 | Case series       | Reticular Dysgenesis | • All children with reticular dysgenesis.  
• Presented with infection in first days of life.  
• HLA-non-identical HSCT resulted in three of the 10 patients surviving.  
• Deaths due primarily to graft failure. | IV                 |
| Gomez et al. 1995    | Case series       | Omenn syndrome | • Engraftment occurred in 4/5 HLA-identical transplants and 3/4 non-identical transplants.  
• Full chimerism occurred in all but one of the patients that engrafted.  
• Clinical manifestations of Omenn syndrome disappeared within days of BMT (likely due to pre-treatment with chemo.)  
• One patient died of CMV 50 days post-transplant, 3 developed interstitial pneumonia.  
• All survivors except one (who has chronic GVHD) have normal growth rates. | IV                 |
| O'Marcaigh et al. 2001| Case series     | SCIDA     | • 12/18 developed oral/genital ulcers associated with SCIDA.  
• 16/18 children were well enough for transplant.  
• 15/16 achieved engraftment.  
• 11/16 developed normal T-cell function. | IV                 |
| Roberts et al. 2004  | Case series       | Jak3 mutations | • All patients had abnormal B-cell JAK3 dependent interleukin-2 induced signal transducer and activator of transcription 5 (STAT5) phosphorylation.  
• 9/10 patients with JAK3 deficiency were alive and well 4 to 18 years after stem cell transplant. | IV                 |

**HSCT variation in transplant methods**

Multiple studies compare various transplant methods and their outcomes. The main points of discussion surround the degree of matching between the donor and recipient, the extent of myeloablation prior to transplant and the method for T-cell depletion of the donor stem cells. In addition, investigators have considered the source of stem cells and the use of “booster transplants.”

The extent of matching between the donor and recipient is only partially under the control of physicians, as many patients lack an available matched related donor. This results in recipients requiring either a haplo-identical or mismatched transplant. Several studies have looked at the various types of transplants and their efficacy.
<table>
<thead>
<tr>
<th>Study (Author, Date)</th>
<th>Population</th>
<th>Key findings</th>
<th>Quality of evidence</th>
</tr>
</thead>
</table>
| Dalal et al. 2000    | 16 children total, 9 with SCID; all lacked histocompatible siblings or closely matched related donors between 1989 and 1997. | - Mean age at diagnosis of SCID was 7.4 months.  
- Neutrophil engraftment was achieved in all patients at a mean of 15.4 days.  
- Serum IgM and IgA normalized in all patients within a mean of 3.5 and 6 months, respectively.  
- 12/16 patients survived.  
- 2/9 (22%) SCID patients died from GVHD complications. | IV |
| Dal-Cortivo et al. 2004 | 38 children total; 25 children with no potential to exert NK-cell alloreactivity compared to 13 with such potential (defined by donor expression of inhibitory killer immunoglobulin-like receptor (KIR)), all receiving haplo-identical transplants. | - No difference in the rates of engraftment (64% vs. 61.5%).  
- Trend towards lower incidence of grade II-IV acute GVHD in the patients with potential NK-cell alloreactivity (37.5% vs. 50%, p=0.68).  
- One year survival was not significantly different between the two groups (52% vs. 61.5%). | IV |
| Giri et al. 1994     | 11 children total; receiving HLA-non-identical BMT between 1985 and 1992, with a median age at time of BMT of 13 months (range: 3 weeks to 77 months). | - 9 patients engrafted (8 after first BMT, 1 after second BMT).  
- 4/9 developed GVHD.  
- 5 (46%) patients surviving 6-78 months post-BMT. | IV |
| Grunebaum et al. 2006 | Compared recipient outcomes between:  
- related HLA-identical donors (RID, n=13),  
- HLA-matched unrelated donors (MUD, n= 41)  
- HLA-mismatched related donors (MMRD, n=40). | - Median time to transplant was 1 month for RID, 2 months for MMRD and 4 months for MUD.  
- Highest survival was RID (92.3%), followed by MUD (80.5%) and MMRD (52.5%).  
- 0% of RID, 7.3% of MUD and 30% of MMRD had graft failure.  
- No statistical difference between MUD and MMRD lymphocyte subsets and lymphocyte function.  
- Patient sex and presence of B-cells has no impact on survival.  
- No respiratory complications in RID children; respiratory complications occurred in 7.3% of MUD and 35% of MMRD.  
- GVHD most common following MUD BMT (73.1%) or MMRD BMT (45%). | II-2 C |
| Smogorzewska et al. 2000 | 48 children total;  
11 children treated with histocompatible transplants from a sibling;  
37 children treated with T-cell depleted haplo-identical parental bone marrow between 1984 and 1997. | - 100% survival in histocompatible group.  
- 46% survival in haplo-identical group.  
- Mean age at transplant of children who survived: 7.5 months.  
- Mean age at transplant of children who died: 11.4 months.  
- All surviving children recovered T-cell function.  
- Recovery slower for children receiving haplo-identical transplants. | II-2 C |
| Wijnaendts et al. 1989* | 33 children treated with HST between 1972 and 1987 surviving a minimum of 6 months: 18 HLA-identical transplants and 15 HLA-non-identical transplants. | - Development of immune function occurred faster in patients with HLA-identical transplants.  
- Development of T- and B-cell function occurred faster when chemotherapy preceded BMT.  
- Poor B-cell function was observed more frequently when chemotherapy was not used. | IV |

*Potential patient overlap with Stephan et al. 1993 and Wijnaendts et al. 1989*
### Table 17- Abstracted literature pertaining to the role of myeloablation prior to transplant (alphabetical by author)

<table>
<thead>
<tr>
<th>Study (Author, Date)</th>
<th>Population</th>
<th>Key findings</th>
<th>Quality of evidence</th>
</tr>
</thead>
</table>
| **Case series** | 8 patients total, 5 with SCID; Children ineligible for conventional myeloablation due to comorbidities. | - All treated with non-myeloablative chemotherapy prior to transplant.  
- The median duration of admission was 52 days compared to 54 days in a historical cohort of children undergoing conventionally conditioned HSCT  
- 7/8 children survived.  
- At median follow-up of 12 months: all 7 survivors have good recovery of T-cell numbers, 4 patients have normal IgM levels, and 2 patients have normal IgA levels. | IV |
| Rao et al. 2005* | 52 children with primary immunodeficiency; 33 children receiving reduced intensity pre-transplant chemotherapy; 19 receiving myeloablation. | - All children in both groups had primary engraftment.  
- Reduced intensity group: 32/33 were alive at one month and 31/33 alive at one year  
- Myeloablation group: 14/19 were alive at one month and 11/19 alive at one year.  
- At one year: all in the myeloablative group had normal B-cell function, 5 of reduced intensity group still required IVIG.  
- GVHD incidence not different between groups but limited chronic GVHD was more common in myeloablation group.  
- Quality of life, measured by Lansky score, was similar between the groups (97 vs. 94). | III |
| **Case series** | 81 children total; 0.1-17.6 years old, with congenital immunodeficiencies, 20 with SCID. | - All subjects treated with HSCT with reduced-intensity conditioning.  
- Survival rate 84% (68/81) with survival difference between SCID and non-SCID.  
- Increase in reactivation of EBV compared to conventional BMT procedures.  
- HSCT with reduced-intensity conditioning appeared to be well tolerated. | IV |


Other variation in treatment methods

Three studies specifically investigated stem cell sources other than bone marrow. A small study (Knutsen, Wall 2000) which included only 8 children with SCID evaluated transplants using umbilical cord blood derived stem cells and found neutrophil recovery time was the same as that
reported in BMT, while platelet recovery was delayed compared to BMT. A study from Turkey (Arpaci et al. 2008) used peripheral stem cells mobilized by treating donors with G-CSF. 21 patients, of whom 16 had SCID, received a total of 28 haplo- HSCTs. Median age at transplant was 12 months. At last follow-up 8/21 children were alive with the authors believing that delay in transplantation accounted for the poor outcomes compared to other studies. Finally, stem cells from fetal liver (Touraine et al. 2007) were used for transplants in 17 infants; in the 3 patients for whom information was available, they found normal antibody responses several years after transplant.

One study (Dror et al. 1993) reported on 24 patients who underwent a total of 36 transplants using lectin-treated T-cell-depleted haplo-compatible BMT. Of these 24 children durable T-cell engraftment was achieved in 19, although 6/19 required more than one transplant to achieve engraftment. Pre-transplant conditioning positively affected engraftment, while cell dose, patient’s age, and donor and patient sex had no observable effect on engraftment rates.

For patients that achieve engraftment but not adequate immune function, bone marrow boosts (Kline, Stiehm & Cowan 1996), in which additional stem cells from the same donor were given without pre-treatment, led to increased absolute lymphocyte counts in 5/9, increased mean CD3, CD4 and CD8 counts and improved mean T-cell function. Mentioned prior, the long-term follow up case series (Buckley et al. 1999) also included a total of 20/89 receiving “booster transplants” to overcome poor T/B-cell function or poor engraftment. Of those who received a booster transplant, immune function improved in all but 3.

**HSCT long-term follow up, survival and immune reconstitution**

**Table 18- Abstracted literature pertaining to long-term follow-up and survival after HSCT (alphabetical by author)**

<table>
<thead>
<tr>
<th>Study (Author, Date)</th>
<th>Population</th>
<th>Key findings</th>
<th>Quality of evidence</th>
</tr>
</thead>
</table>
| Antoine et al. 2003 * Case series | 475 patients (total of 566 transplants); 202 less than 6 months old at transplant, 184 between 6-11 months of age at transplant, from 37 European centers between 1968 and 1999. | • Children transplanted at less than 6 months of age had better 3-year survival rates than older cohorts.  
• Three-year survival with sustained engraftment was 77% for HLA-identical and 54% for HLA-non-identical transplants.  
• Survival has improved over time for both HLA-identical and HLA-non-identical transplant recipients.  
• B- SCID (36% 3-yr survival) had a poorer prognosis than B+ SCID (64% 3-yr survival).  
• Myeloablation prior to HLA-non-identical transplant trended towards improving survival among B- children but not B+ children.  
• ADA deficiency (n=51) 3-yr-survival was 81% for matched and 29% for unmatched transplants.  
• Reticular dysgenesis survival (n=12) was 75% for matched and 29% for unmatched transplants. | II-2 C |
<table>
<thead>
<tr>
<th>Study Authors</th>
<th>Study Type</th>
<th>Study Details</th>
<th>Key Findings</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cavazzana-Calvo et al. 2007</td>
<td>Case series</td>
<td>31 children total; 1-42 months old at HSCT, evaluated 10-27 years later.</td>
<td>• 26/31 had T-cell count in normal range for age.</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 30/31 had normal mitogen-induced T-cell proliferation.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 18/31 had normal TREC counts.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Presence of NK-cells not correlated with TREC.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Presence of TREC/naïve T-cells associated with significantly higher t-cell counts and better t-cell mitogen proliferation.</td>
<td></td>
</tr>
<tr>
<td>Fischer et al. 1990 *</td>
<td>Case series</td>
<td>183 patients total; (HLA-identical BMT: 70; HLA-non-identical BMT: 113), mean age at transplantation 7.3 months, from 15 European centers between 1968 and 1989.</td>
<td>• Survival significantly better for HLA-identical (76% survival) than HLA-non-identical transplants (50% survival).</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• SCID phenotype was not associated with difference in survival.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Lung infection before HSCT and absence of a protective environment significantly affected outcome (multivariate analysis).</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• A total of 27% had acute GVHD of grade II or higher and 25% developed chronic GVHD.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 97% survival in those treated since 1983.</td>
<td></td>
</tr>
<tr>
<td>Friedrich, Honig &amp; Muller 2007</td>
<td>Cohort study</td>
<td>32 children total; 7 children with SCID with HLA-identical transplant, 25 children with SCID with HLA-haplo-identical transplant, all at least 10 years out from transplant.</td>
<td>• Most patients had normal and stable T-cell numbers and functions.</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 3 patients’ had decreasing T-cell numbers.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 4 patients’ had decreasing PHA responses (all in HLA-haplo-identical group and no chemotherapy).</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• HLA-haplo-identical with no conditioning had lower levels of naïve CD4+ cells and impaired B-cell functioning.</td>
<td></td>
</tr>
<tr>
<td>Gennery et al. 2001</td>
<td>Case series</td>
<td>30 children total; 1987-1998 using CAMPATH-1MT depleted BMT for SCID.</td>
<td>• 19/30 (63%) children survived longer than 1 year post-BMT; median follow-up of 5.3 years (range 1.33–12 years); 11/30 (37%) died.</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 18/30 showed evidence of successful engraftment (49%).</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• 17/30 had normal immune function following transplantation.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• The overall engraftment rate was 59% (22/37 BMT procedures).</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Most deaths attributed to pre-existing infection.</td>
<td></td>
</tr>
<tr>
<td>Study (Author, Date)</td>
<td>Population</td>
<td>Key findings</td>
<td>Quality of evidence</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>------------</td>
<td>--------------</td>
<td>---------------------</td>
<td></td>
</tr>
</tbody>
</table>
| Haddad et al. 1998   | 193 patients total; from 18 European centers between 1982 and 1993. | • 116 alive with evidence of engraftment 5 months after BMT; 24 later died (20%).
• T-cell function improved during the 2 years after BMT and continued to be better than B-cell function.
• Poor outcomes associated with: absence of T-cell reconstitution, presence of chronic GVHD 6 months after transplant, B- SCID (multivariate analysis).
• At last follow up (median, 6 years after transplant), 93% of survivors had normal T-cell function and 68% had normal B-cell function. | IV |
| Slatter et al. 2008 Cohort study | 36 children total; 2 months to 2 years old, treated with BMT with depletion of T-cells from a non-identical donor and followed for 1-19 years. | • No significant survival difference between children receiving transplants depleted of T-cells using anti-CD52 or anti-CD34 antibodies.
• 5 patients in the anti-CD52 group and 2 in anti-CD34 group developed GVHD. | II-2 C |

*A subset of patients in Fischer et al. 1990 are also included in Antoine et al. 2003*

Table 19- Abstracted literature pertaining to long-term immune reconstitution after HSCT (alphabetical by author)
<table>
<thead>
<tr>
<th>Study</th>
<th>Case series</th>
<th>Total Population</th>
<th>Follow-up Details</th>
<th>Vaccination Details</th>
<th>Growth Details</th>
<th>Other Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mazzolari et al. 2007</td>
<td>Case series</td>
<td>58 children total; treated with HSCT (in-utero to 34 months old at time of transplant).</td>
<td>• 42/58 (72.4%) survived at least 5 years (median follow-up of 132 months). • 85% produced antibodies to tetanus toxoid or hepatitis B vaccines. • 26/28 immunized with Measles, Mumps and Rubella vaccine had evidence of anti-measles antibodies. • Most children had height (77.5%) and weight (82.5%) in the normal range. • At last follow-up, 24/40 required no treatment, 6 required IVIG and/or antibiotics; 5 are on thyroid replacement; 3 receive anti-epileptics; 1 treated for portal hypertension.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patel et al. 2000</td>
<td>Case series</td>
<td>173 total patients; 83 SCID with a mean age of transplant of 0.5 ±0.4 years; 90 healthy controls from less than 1 to 79 years old.</td>
<td>• Non-thymus-dependent cells predominated in the first 100 days after transplant. • 140-180 days after transplant thymus-dependent cells predominated. • After transplant thymus-dependent cells peaked at approximately one year but then diminished over the next 14. • Controls showed a decline in thymus-dependent cells from birth to 79 years old.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vossen et al. 1993</td>
<td>Case series</td>
<td>14 children evaluated; 1-23 years after BMT.</td>
<td>• All children who survived greater than 1 year had some donor T-cell engraftment. • Cellular immunity was quantitatively low but mostly qualitatively normal.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Information from expert interviews**

Consultation with experts pertaining to treatment of SCID corroborated the data from the published literature portion of this evidence review. Experts concurred that major challenges to treating SCID are due to complications prior to diagnosis and treatment (such as infections), and a lack of a uniform approach for treatment. Drs. Buckley and Notarangelo provided information on development of two SCID consortia: United States Immunodeficiency Network (USIDNET) Registry sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) and Center for International Blood and Marrow Transplant Research (CIBMTR). While USIDNET is focused on the registry of primary immune deficiencies, reporting of all BMT procedures to the CIBMTR registry has been mandatory since December. Data from these consortiums may, in the future, provide more data on treatment outcomes for patients with SCID.

Dr. Buckley and Ms Boyle, from the Immune Deficiency Foundation, report there are 15 major and 34 minor centers in the U.S. and Canada currently performing stem cell transplantation for SCID. Drs. Notarangelo, Bonilla and Pai stated that an informal survey performed under the auspices of the NIAID/Rare Diseases workshop identified 34 centers in the United States and Canada that currently perform HSCT for SCID (unpublished data, NIAID May 12-13, 2008 HCT for Primary Immune Deficiency Diseases workshop). Additionally, Ms Ballard from the SCID
Family Network corroborated the information about limited treatment availability in telling us about her son’s transfer from a Washington, DC, area hospital to Duke, in order to receive HSCT for SCID.

**Enzyme replacement therapy—Systematic literature review**

Enzyme replacement therapy has been a therapeutic option for patients with ADA deficiency, a specific type of SCID. Although multiple case studies examining the use of polyethylene glycol-adenosine deaminase (PEG-ADA) have been published, our examination of the literature found only one study meeting criteria for inclusion in this evidence review.

A long-term follow up study by Chan et al (Chan et al. 2005), evaluated outcomes in 9 children treated with PEG-ADA. These children were diagnosed with ADA deficiency at ages ranging from birth to 6.5 years and were followed on intramuscular PEG-ADA for 5-12 years. Some patients also received other treatments. One child died after 9 yrs of PEG-ADA treatment and a failed bone marrow transplant. The remaining 8 children attended school without protective precautions although 2 children have disabilities thought to be related to early, severe infections. Lymphocyte counts (T, B and NK) and function increased significantly following initiation of PEG-ADA and peaked at 1-3 years after treatment initiation. Over time lymphocyte counts and T-cell function, as measured by PHA mitogen stimulation have diminished.

**Gene therapy—Systematic literature review**

Gene therapy using viral vectors has been tried for the treatment of X-linked SCID and ADA deficiency SCID. Two case series of patients treated with gene therapy were included in this evidence review.

In 2002, Hacein-Bey-Abina et al (Hacein-Bey-Abina et al. 2002) published a report of 5 boys with X-linked SCID due to a mutation in the common γc-chain gene who were treated by infusing them with their own bone marrow which had previously been extracted and transduced with a vector containing γc-chain derived from a defective Moloney murine leukemia virus. 4/5 had clinical improvement including resolution of infections, diarrhea and skin lesions. At follow-up 0.7-2.5 years later those 4 were well with normal growth. The fifth patient never had reconstitution of T-cells following gene therapy and underwent a bone marrow transplantation 8 months later. With regards to immunologic function, 3/5 patients had normal T-cell values 3 to 4 months after gene therapy. At last follow up, T-cells from 4/5 patients exhibited normal proliferative responses to in vitro stimulation with phytohemagglutinin and anti-CD3 antibody.

Schmidt et al (Schmidt et al. 2005) reported on a series of 10 patients (5 of whom were included in the Hacein-Bey-Abina paper) with a focus on longer-term follow-up after gene therapy. They found polyclonal T-cell repertoires, in the 9/10 patients who developed normal T-cell counts after treatment, indistinguishable from those of age-matched controls. In long-term follow up, 2/9 developed monoclonal lymphoproliferation 2.5 years post transplant. Additionally, the number of circulating naïve T-cells was similar to that for age-matched controls.
E. Benefits of treatment in screen positive children
We found no evidence related to the benefits of treatment in children who screen positive for SCID. The relative outcomes for early treatment versus late treatment of children with SCID provide evidence of improved outcomes with earlier treatment.

F. Harms of SCID screening
We found no evidence related to the harms associated with screening for SCID.

G. Harms of SCID diagnosis
We found no evidence related to the harms associated with diagnosing SCID.

H. Harms of SCID treatment
We found two papers specifically focused on harms associated with treatment of SCID. The first (Horn et al. 1999) analyzed the rates of auto-immune hemolytic anemia in children undergoing HSCT for SCID. 8/41 children developed auto-immune hemolytic anemia, 3 died from its complications. This was a higher rate of auto-immune hemolytic anemia than previously reported and higher than the rate, in the same institution, among patients undergoing HSCT for non-SCID diseases. Among several potential predictors for the development of auto-immune hemolytic anemia that were analyzed, only peripheral blood as source of stem cells was found to be related to increased risk of developing auto-immune hemolytic anemia.

Work by Hacein-Bey-Abina et al (Hacein-Bey-Abina et al. 2008) documented a total of 4 children (of the 9/10 who had successful treatment with gene therapy) who developed leukemia between 30 and 68 months after gene therapy. All cases were associated with vector insertion near genes associated with cancer development. 3/4 kids were successfully treated with chemotherapy and regained poly-clonal T-cell populations; the 4th child underwent 2 BMTs and ultimately died 60 months after gene therapy and 26 months after leukemia diagnosis.
I. Cost-effectiveness

Table 20- Quality Assessment of abstracted literature pertaining to economic evidence

<table>
<thead>
<tr>
<th>Economic evidence</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Evaluation of important alternative interventions comparing all clinically</td>
<td></td>
</tr>
<tr>
<td>relevant outcomes against appropriate cost measurement and including a clinically</td>
<td></td>
</tr>
<tr>
<td>sensible sensitivity analysis.</td>
<td>0</td>
</tr>
<tr>
<td>II. Evaluation of important alternative interventions comparing a limited</td>
<td></td>
</tr>
<tr>
<td>number of outcomes against appropriate cost measurement, but including a</td>
<td></td>
</tr>
<tr>
<td>clinically sensible sensitivity analysis.</td>
<td>0</td>
</tr>
<tr>
<td>III. Evaluation of important alternative interventions comparing all clinically</td>
<td></td>
</tr>
<tr>
<td>relevant outcomes against inappropriate cost measurement, but including a</td>
<td></td>
</tr>
<tr>
<td>clinically sensible sensitivity analysis.</td>
<td>1</td>
</tr>
<tr>
<td>IV. Evaluation without a clinically sensible sensitivity analysis</td>
<td></td>
</tr>
<tr>
<td>V. Expert opinion with no explicit critical appraisal, based on economic theory</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from NHS Centre for Reviews and Dissemination Report 4, March 2001

We found one study (McGhee, Stiehm & McCabe 2005) that addressed the cost-effectiveness of SCID screening, as it would apply to the United States population. Using a deterministic decision-tree model, comparing universal and targeted screening approaches, the authors assessed the thresholds at which screening would be cost-effective from a health care system perspective. They noted a significant amount of uncertainty in their model parameters due, in part, to the lack of SCID screening studies. Additionally, utility estimates were based on information from patients receiving HSCT for oncologic processes. The authors found that, at a threshold of $100,000 per quality adjusted life year, there is 86% likelihood of screening being cost-effective, but details of the Monte Carlo simulation used to arrive at this estimate were not provided in the paper.

Data from experts regarding cost-effectiveness

None of the experts with whom we had contact provided any specific cost-effectiveness information. However, several (Bonilla, Pai, Notarangelo, and Buckley) had collected some unpublished hospital cost or charge data for patients undergoing HSCT for SCID. These costs or charges provide a cost-estimate that is at least two to three times higher than the cost estimate used by McGhee et al in their base-case analysis. With regard to testing, from the Wisconsin trial and Dr. Puck’s planned trial, estimates of the marginal costs of SCID screening, exclusive of capital expenses associated with new equipment, are $6-9 per child tested for non-automated testing. The Wisconsin cost for diagnostic testing, namely flow cytometry, is approximately $120 per test. These costs are relatively consistent with the base-case analysis costs of testing suggested by McGhee et al.
VIII. Summary

Key findings:
Severe Combined Immunodeficiency (SCID) affects at least 1/100,000 newborns within the United States. However, experts believe that with systematic case-finding the prevalence may be higher (perhaps as high as 1/40,000) due to earlier diagnosis of infants who would otherwise die prior to confirming a diagnosis of SCID. Although several population-based screening trials are underway (Wisconsin) or planned (Massachusetts, Navajo Reservation in Arizona and New Mexico), to date no population-based screening trial has been completed.

Without curative treatment, newborns with SCID develop severe, often opportunistic, infections which lead to early death. Studies indicate that treatment, most commonly with hematopoietic stem cell transplant, is effective in decreasing both the morbidity and mortality associated with SCID. Given the seriousness of SCID, it does not lend itself to randomized controlled trials of treatment. The current evidence supports the notion that earlier treatment leads to better outcomes.

Regarding the key questions:

- **Do current screening tests effectively and efficiently identify cases of SCID?**
  Various screening methods ranging from targeted use of lymphocyte counts in hospitalized children to population based newborn screening using IL-7, TREC or a combination have been studied. Both IL-7 and TREC have been investigated using anonymized dried-blood spots. These studies allow for estimations of sensitivity and specificity, but due to the anonymous nature there is no way to prove which test results are true.

  The state of Wisconsin began a trial of population-based screening for SCID, using low TREC as the marker of SCID, in January 2008. As of December 31, 2008, they had screened 70,397 newborns of which 118 (0.168%) had initially inconclusive results requiring a second newborn screening sample and 32 (0.045%) had abnormal results requiring either a second newborn screening sample or diagnostic testing. The rates for both inconclusive and abnormal results are higher in premature than in full-term infants. As of December 31, 2008, the investigators have not detected any cases of SCID.

- **Does pre-symptomatic or early symptomatic intervention in newborns or infants with SCID improve health outcomes?**
  The most common, and well-studied, treatment modality for SCID is hematopoietic stem cell transplant which appears to be effective in significantly decreasing the morbidity and mortality associated with SCID. Within HSCT there are numerous variables which may contribute to the effectiveness. The most studied of these are the degree of haplotype matching between donor and recipient and the type of pre-conditioning the recipient receives. There is no clear evidence as to the best method for HSCT. However, despite the lack of randomized controlled trials of SCID treatment, the evidence from large case series indicates that HSCT is effective in treating SCID.

  Furthermore, there is evidence, primarily from the early detection of siblings of known SCID cases, that earlier treatment may be more effective. Specifically, the existing evidence
indicates that undergoing HSCT prior to the onset of lung infection has higher likelihood of success.

In addition to HSCT, there is limited evidence that for children with ADA deficiency enzyme replacement may be effective in reconstituting their immune function.

• **What is the cost-effectiveness of newborn screening for SCID?**
The cost-effectiveness data reviewed were very limited and may not reflect current costs of treatment.

• **What critical evidence appears lacking that may inform screening recommendations for SCID?**
We identified several areas with deficient data:
  o **Prevalence of SCID**
    There is limited evidence regarding the true prevalence of SCID. A systematic method of case finding is needed in order to accurately determine the prevalence. Of note, the new consortium of treatment centers (USIDNET) that has recently been established may serve as a method of more systematic case-finding.
  o **Accuracy of screening**
    Initial pilot screening data from Wisconsin suggests that the false-positive rate will be relatively low. However, these data are limited at this time. Data regarding the accuracy of other screening methods, when applied in population-based protocols, are not available.
  o **Feasibility of screening**
    Wisconsin and Massachusetts have been able to implement universal newborn screening for SCID, at least on a pilot basis. Data are needed regarding the ability of other newborn screening laboratories to offer SCID screening.
  o **Acceptability of screening**
    There are no data available regarding consumer or physician acceptance of newborn screening for SCID.
  o **Cost-effectiveness**
    Cost-effectiveness analyses utilizing measured costs and utilities, as well as applicable sensitivity analyses, are needed.
  o **Adequacy of available treatment centers**
    There are no data addressing variation in treatment success between centers or the number of centers in the United States and their capacity to provide treatment for SCID. Data from USIDNET and CIBMTR may, in the future, provide evidence on this topic.
IX. References


Dalal, I., Reid, B., Doyle, J., Freedman, M., Calderwood, S., Saunders, F. & Roifman, C.M. 2000, "Matched unrelated bone marrow transplantation for combined immunodeficiency", *Bone Marrow Transplantation*, vol. 25, no. 6, pp. 613-621.

immunodeficiencies have the potential to exploit donor NK-cell alloreactivity?", *Bone Marrow Transplantation*, vol. 34, no. 11, pp. 945-947.


46


## X. Appendix A- SCID evidence tables

### Natural History of Disease
(alphabetical by last name of first author)

<table>
<thead>
<tr>
<th>Author(s), Year, Title of Paper &amp; Study Design</th>
<th>Population</th>
<th>Significant Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bertrand, Y., Landais, P., Friedrich, W., Gerritsen, B., Morgan, G., Fasth, A., Cavazzana-Calvo, M., Porta, F., Cant, A., Espanol, T., Muller, S., Veys, P., Vossen, J., Haddad, E. &amp; Fischer, A. 1999, &quot;Influence of severe combined immunodeficiency phenotype on the outcome of HLA- non-identical, T-cell-depleted bone marrow transplantation: a retrospective European survey from the European group for bone marrow transplantation and the European society for immunodeficiency&quot;, <em>The Journal of Pediatrics</em>, vol. 134, no. 6, pp. 740-748.</td>
<td>178 children total; B+ group: 122, mean age at BMT was 7 months B- group: 56, mean age at BMT was 6.5 months.</td>
<td>• B+ SCID has better prognosis than B- SCID following non-identical BMT. • Rate of engraftment not different between groups. • B- SCID had higher rates of both early and late death. • Deaths in both groups due primarily to infection.</td>
</tr>
<tr>
<td>Bertrand, Y., Muller, S.M., Casanova, J.L., Morgan, G., Fischer, A. &amp; Friedrich, W. 2002, &quot;Reticular dysgenesis: HLA- non-identical bone marrow transplants in a series of 10 patients&quot;, <em>Bone Marrow Transplantation</em>, vol. 29, no. 9, pp. 759-762.</td>
<td>10 children total; BMT at age 0.5 to 10 months.</td>
<td>• All children had reticular dysgenesis. • All presented with infection in first days of life. • HLA-non -identical HSCT resulted in three of the 10 patients surviving. • Deaths due primarily to graft failure.</td>
</tr>
<tr>
<td>Boeck, A., Buckley, R.H. &amp; Schiff, R.I. 1997, &quot;Gastroesophageal reflux and severe combined immunodeficiency&quot;, <em>The Journal of Allergy and Clinical Immunology</em>, vol. 99, no. 3, pp. 420-424.</td>
<td>194 children total; age at diagnosis ranged from 3 months to 5 years.</td>
<td>• Significantly increased incidence of gastroesophageal reflux in patients with SCID (20.5%), compared with that reported in the normal population (0.3% to 0.1%, p &lt; 0.001).</td>
</tr>
<tr>
<td>Boyle, J.M. &amp; Buckley, R.H. 2007, &quot;Population prevalence of diagnosed primary immunodeficiency diseases in the United States&quot;, <em>Journal of Clinical Immunology</em>, vol. 27, no. 5, pp. 497-502.</td>
<td>10,005 households were contacted (response rate of 80%)</td>
<td>• 131/10,005 households said that someone had been diagnosed with a primary immunodeficiency. • Specific Primary Immunodeficiency (PID) diagnoses given: 9% SCID, 9% IgG, 26% IgA, 35% Common variable immunodeficiency, 9% chronic granulomatous disease, 13% Alpha gamma. • 4/6773 kids were reported to have a primary immunodeficiency (PID). • Population prevalence of diagnosed PID: 1/1200 for adults, 1/2000 for kids, 1/600 for households.</td>
</tr>
</tbody>
</table>

| Other- Telephone survey |

**Note:** *Case series*

Case series

108 children total; Diagnosed at age birth to 21 months.

- Mean age at diagnosis of SCID was 201 days (6.59 months) and did not differ significantly among different genetic types.
- 67 (76%) of the 88 families were white, 14 (16%) were black, and 7 (8%) Hispanic.
- Abnormal serum immunoglobulin concentrations existed in all types of SCID.
- Mean IgA levels were lowest in γc-deficient, Jak3-deficient and unknown types of SCID.
- Mean IgM concentrations were lowest in ADA, γc-deficient and Jak3-deficient patients.
- IgE was normal in all but 2 patients.


Case series

^Potential patient overlap of Myers et al. 2002, Buckley et al. 1999

89 children total; 22 less than 3.5 months old at transplant, 67 greater than or equal to 3.5 months at transplant.

- 72 (81%) alive 3 months-16.5 years post-transplant, with a median follow up of 5.6 years.
- 65 survived greater than 1 year, 38 greater than 5 years and 21 greater than 10 years.
- All 12 HLA-identical recipients survived.
- Mean number of T/B/NK-cells varies by genotype; all genotypes had normal average in vitro mitogen response after transplant.
- Poor B-cell function with 45 kids requiring IVIG.
- NK-cell activity low in γc-chain deficiency and JAK3 deficiency, normal in other SCID subtypes.


Case series

31 children total; 1-42 months old at HSCT, now 10-27 years later.

- Myeloablation patients more likely to have evidence of donor-derived granulocytes and persistent naïve T-cells, as measured by TREC.
- At follow-up, 60% of TREC+ and 45% of TREC- had no clinical manifestations.
- Average follow-up time in the TREC+ group was 13 years and in the TREC- was 16 years.
<table>
<thead>
<tr>
<th>Study</th>
<th>Case-Control/Case Series</th>
<th>Number of Subjects</th>
<th>Details</th>
</tr>
</thead>
</table>
Among the children known to have SCID, none had detectable levels of TREC and all had detectable β-actin.  
The 2 children with non-SCID immunodeficiency had detectable TREC.  
Had several presumed false-positives in which beta-actin could be amplified but TREC could not.  
Estimated incidence of SCID to be 1/105,000 births, excluding patients who die before their immunodeficiency is recognized. |
| Deerojanawong, J., Chang, A.B., Eng, P.A., Robertson, C.F. & Kemp, A.S. 1997, "Pulmonary diseases in children with severe combined immune deficiency and DiGeorge syndrome", Pediatric Pulmonology, vol. 24, no. 5, pp. 324-330. | Case series *            | 15 children with SCID; 3 weeks to 8 months old. | 13/15 presented with pulmonary findings, while the two others were prenatally diagnosed.  
Median age of pulmonary manifestation was 4 months.  
Pulmonary infection remained a significant cause of morbidity and mortality in those that did not undergo BMT.  
9/13 died; for 5/9, pulmonary disease was the cause of death. |
| Denianke, K.S., Frieden, I.J., Cowan, M.J., Williams, M.L. & McCalmont, T.H. 2001, "Cutaneous manifestations of maternal engraftment in patients with severe combined immunodeficiency: a clinicopathologic study", Bone Marrow Transplantation, vol. 28, no. 3, pp. 227-233. | Case-control study       | 21 children total; 0-16 months old at rash onset and 0-2.1 years of age at BMT. | Divided subjects into two groups comparing cases with evidence of maternal engraftment and rash prior to BMT, to controls that did not have evidence of maternal engraftment or rash pre-BMT but developed rash post BMT.  
The parameters that showed statistical significance between the two groups were psoriasiform hyperplasia (P < 0.04), parakeratosis (P < 0.01), spongiosis (P < 0.04), necrotic keratinocytes (P < 0.04) and lichenoid infiltrate (P < 0.04). |
Full chimerism occurred in all but one of the patients that engrafted.  
Clinical manifestations of Omenn syndrome disappeared within days of BMT (likely due to pre-treatment with chemo.).  
One patient died of CMV 50 days post-transplant, 3 developed interstitial pneumonia.  
All survivors except one (who has chronic GVHD) have normal growth rates. |
<table>
<thead>
<tr>
<th>Case series*</th>
<th>15 children total; Treated with BMT for ADA deficiency, focus on neurologic symptoms.</th>
</tr>
</thead>
<tbody>
<tr>
<td>248 patients total; birth to 15 months old at presentation.</td>
<td></td>
</tr>
<tr>
<td>Other- Cross Sectional review of death records and hospital records, followed by family interviews and genetic segregation analysis</td>
<td></td>
</tr>
<tr>
<td>41 patients total; at least 10 years after HSCT for SCID.</td>
<td></td>
</tr>
<tr>
<td>Case-control study*</td>
<td></td>
</tr>
<tr>
<td>58 children total; treated with HSCT (in-utero to 34 months old at time of transplant).</td>
<td></td>
</tr>
<tr>
<td>Case series*</td>
<td></td>
</tr>
<tr>
<td>12/15 survived, all with developmental delay.</td>
<td></td>
</tr>
<tr>
<td>6/12 have significant neurocognitive deficits (learning disabilities, gait problems, hearing deficit, and/or hyperactivity).</td>
<td></td>
</tr>
<tr>
<td>Cases were spaced evenly both geographically and temporally.</td>
<td></td>
</tr>
<tr>
<td>Minimum SCID incidence rate calculated as 52/100,000 live Navajo births; gene frequency estimated at 2.25%.</td>
<td></td>
</tr>
<tr>
<td>SCID accounted for about 7% of deaths in children under 2.</td>
<td></td>
</tr>
<tr>
<td>Segregation analysis showed that it was not multifactorial inheritance but exact inheritance pattern could not be determined.</td>
<td></td>
</tr>
<tr>
<td>42/58 (72.4%) survived at least 5 years (median follow-up of 132 months).</td>
<td></td>
</tr>
<tr>
<td>85% produced antibodies to tetanus toxoid or hepatitis B vaccines.</td>
<td></td>
</tr>
<tr>
<td>26/28 immunized with Measles, Mumps and Rubella vaccine had evidence of anti-measles antibodies.</td>
<td></td>
</tr>
<tr>
<td>Most children had height (77.5%) and weight (82.5%) in the normal range.</td>
<td></td>
</tr>
<tr>
<td>At last follow-up, 24/40 required no treatment, 6 required IVIG and/or antibiotics; 5 are on thyroid replacement; 3 receive anti-epileptics; 1 treated for portal hypertension.</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Case Study Details</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Monafo, W.J., Polmar, S.H., Neudorf, S., Mather, A. &amp; Filipovich, A.H. 1992, &quot;A hereditary immunodeficiency characterized by CD8+ T lymphocyte deficiency and impaired lymphocyte activation&quot;, Clinical and Experimental Immunology, vol. 90, no. 3, pp. 390-393.</td>
<td>4 children total (2 sets of siblings); age 20 months, 4 months, 11 months, 24 months at the time of BMT.</td>
</tr>
<tr>
<td>Case series</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>O'Marcaigh, A.S., DeSantes, K., Hu, D., Pabst, H., Horn, B., Li, L. &amp; Cowan, M.J. 2001, &quot;Bone marrow transplantation for T-B- severe combined immunodeficiency disease in Athabascan-speaking native Americans&quot;, Bone Marrow Transplantation, vol. 27, no. 7, pp. 703-709.</td>
<td>18 children total; Birth to 3 months of age at diagnosis; mean age of 17.5 months at first transplant.</td>
</tr>
<tr>
<td>Case series</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Case series</td>
<td></td>
</tr>
<tr>
<td>Rogers, M.H., Lwin, R., Fairbanks, L., Gerritsen, B. &amp; Gaspar, H.B. 2001, &quot;Cognitive and behavioral abnormalities in adenosine deaminase deficient severe combined immunodeficiency&quot;, The Journal of Pediatrics, vol. 139, no. 1, pp. 44-50.</td>
<td>22 children total; 1-51 months old at HSCT; 0.9-18 years old at follow-up; 11 children with ADA deficiency, 11 other SCID types.</td>
</tr>
<tr>
<td>Cohort study*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Case series***

*Potential patient overlap with Stephan et al. 1993 and Wijnaendts et al. 1989*

- **Population:** 117 patients total; treated for SCID between 1970 and 1992; 85 children were treated with BMT.
- **Significant Findings:**
  - Estimated SCID incidence: 1/100,000.
  - HLA-identical transplant from a related donor 21/25 (84% survived).
  - Pheno-identical transplant (HLA-genotypically haplo-identical) from related donor 2/5 (40% survived).
  - HLA-haplo-identical transplant without T-cell depletion 0/5 (0% survived).
  - T-cell depleted haplo-identical transplant 28/50 (56% survived).
  - 22 children did not receive any type of transplant and died.
  - 10 received fetal liver transplant, 9 died post transplant.


**Case series**

- **Population:** 18 children total; prenatal to 8 months old at diagnosis.
- **Significant Findings:**
  - Children presented with many ENT findings including congestion, URI, thrush, oral ulcers, cervical adenopathy, otitis media; mastoiditis.
  - Greater than 70% of this series had an otolaryngological presentation prior to diagnosis.

---

**Screening Test Characteristics**

(Alphabetical by last name of first author)

<table>
<thead>
<tr>
<th>Author(s), Year, Title of Paper &amp; Study Design</th>
<th>Population</th>
<th>Significant Findings</th>
</tr>
</thead>
</table>
  - Used DNA amplification of TREC from dried blood spot.
  - Among the children known to have SCID, none had detectable levels of TREC and all had detectable β-actin.
  - The 2 children with non-SCID immunodeficiency had detectable TREC.
  - Had several presumed false-positives in which beta-actin could be amplified but TREC could not.
  - Estimated incidence of SCID to be 1/105,000 births, excluding patients who die before their immunodeficiency is recognized. |
  - Used first available lymphocyte count.
  - SCID children matched to asymptomatic children by age and to children with same presenting symptoms by age.
  - Children with SCID had significantly lower levels of lymphocytes.
  - Unlike the 5 control children with low lymphocyte count, low lymphocyte count persisted in children with SCID. |

Cohort study*

<table>
<thead>
<tr>
<th>Population</th>
<th>Significant Findings</th>
</tr>
</thead>
</table>
| 36 children with rotavirus gastroenteritis; 18 with SCID, 18 without SCID. | • Lymphocyte study.  
  - SCID children were more likely to have:  
    - Low white blood cell count: 10/18 vs. 0/18,  
    - Eosinophilia: 12/18 vs. 0/18  
    - Relative lymphopenia: 17/18 vs. 10/18  
    - Absolute lymphopenia: 16/18 vs. 4/18 |


Case-control study

<table>
<thead>
<tr>
<th>Population</th>
<th>Significant Findings</th>
</tr>
</thead>
</table>
| 13 children with SCID, 183 anonymized dried blood spots, presumed to be from children without SCID. | • Dried blood spot study.  
  - A 2-tiered screening approach in which IL-7 is first measured and only those with elevated IL-7 would have TREC measured, although for this study researchers evaluated each test separately.  
  - Of the 183 presumed normal children tested for TREC, 14 were undetectable.  
  - Of the 3 SCID children tested for TREC, all were undetectable |

* Study conducted outside the United States

<table>
<thead>
<tr>
<th>Author(s), Year, Title of Paper &amp; Study Design</th>
<th>Population</th>
<th>Significant Findings</th>
</tr>
</thead>
</table>
  - 7/12 children with ADA deficiency had bilateral deafness, compared to 1/16 in the control group |
  - The median duration of admission was 52 days compared to 54 days in a historical cohort of children undergoing conventionally conditioned HSCT  
  - 7/8 children survived.  
  - At median follow-up of 12 months: all 7 survivors have good recovery of T-cell numbers, 4 patients have normal IgM levels, and 2 patients have normal IgA levels |


**Case series**

*A subset of patients in Fischer et al. 1990 are also included in Antoine et al. 2003*

<table>
<thead>
<tr>
<th>475 patients (total of 566 transplants); 202 less than 6 months old at transplant, 184 between 6-11 months of age at transplant, from 37 European centers between 1968 and 1999.</th>
</tr>
</thead>
</table>

- Children transplanted at less than 6 months of age had better 3-year survival rates than older cohorts.
- Three-year survival with sustained engraftment was 77% for HLA-identical and 54% for HLA-non-identical transplants.
- Survival has improved over time for both HLA-identical and HLA-non-identical transplant recipients.
- B- SCID (36% 3-yr survival) had a poorer prognosis than B+ SCID (64% 3-yr survival).
- Myeloblation prior to HLA-non-identical transplant trended towards improving survival among B- children but not B+ children.
- ADA deficiency (n=51) 3-yr-survival was 81% for matched and 29% for unmatched transplants.
- Reticular dysgenesis survival (n=12) was 75% for matched and 29% for unmatched transplants.


**Case series**

<table>
<thead>
<tr>
<th>21 children total, 16 with SCID; Overall median age 12 months (range: 2.7 months to 12.5 years).</th>
</tr>
</thead>
</table>

- 21 kids received 28 haplo-identical HSCT.
- The rate of engraftment was 66%.
- Of the 12 patients with engraftment, 7 (58%) had no signs of GvHD.
- 8 of 21 patients were still alive at last follow up; median follow up was 167 days (range: 14-2,204 days).
- Transplant related mortality was 14.2% (3/21).
- Projected 6-year-survival was 32% for all patients and 29.76% for SCID patients.
- Causes of death were infection, GVHD, VOD and bleeding.


**Cohort study**

<table>
<thead>
<tr>
<th>178 children total; B+ group: 122, mean age at BMT 7 months B- group: 56, mean age at BMT 6.5 months.</th>
</tr>
</thead>
</table>

- B+ SCID has better prognosis than B- SCID following non-identical BMT.
- Rate of engraftment not different between groups.
- B- SCID had higher rates of both early and late death.
- Deaths in both groups due primarily to infection.
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Journal Title</th>
<th>Case Study Type</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bertrand, Y., Muller, S.M., Casanova, J.L., Morgan, G., Fischer, A. &amp; Friedrich, W.</td>
<td>2002</td>
<td>Bone Marrow Transplantation</td>
<td>Case series</td>
<td>10 children total; BMT at age 0.5 to 10 months. • All children with reticular dysgenesis. • Presented with infection in first days of life. • HLA-non-identical HSCT resulted in three of the 10 patients surviving. • Deaths due primarily to graft failure.</td>
</tr>
<tr>
<td>Borghans, J.A., Bredius, R.G., Hazenberg, M.D., Roelofs, H., Jol-van der Zijde, E.C., Heidt, J., Otto, S.A., Kuijpers, T.W., Fibbe, W.E., Vossen, J.M., Miedema, F. &amp; van Tol, M.J.</td>
<td>2006</td>
<td>Blood</td>
<td>Case-control study*</td>
<td>192 patients total; 19 children with SCID 1 month to 33 months old at time of BMT (follow-up now 5-32 years post BMT) compared to 173 healthy controls. • Median number of T-cells was lower than in healthy matched controls: 11 SCID patients had normal T-cell counts. • 8 SCID patients who had low t-cell counts at last follow-up also had low counts early (1-4 years after BMT). • Predictors of low T-cell count included NK+ SCID, B- SCID, earlier transplant. • No significant difference in late clinical outcomes found between patients with and without “good” immune reconstitution.</td>
</tr>
<tr>
<td>Brugnoni, D., Airo, P., Malagoli, A., Cattaneo, R., Pennacchio, M. &amp; Porta, F.</td>
<td>1998</td>
<td>Blood</td>
<td>Case series</td>
<td>8 children treated with BMT from unrelated donors. • Number of activated T-cells decreased in the months after BMT. • Proliferative response to PHA initially decreased and then increased to normal by 18 months post-transplant.</td>
</tr>
<tr>
<td>Buckley, R.H., Schiff, S.E., Schiff, R.I., Markert, L., Williams, L.W., Roberts, J.L., Myers, L.A. &amp; Ward, F.E.</td>
<td>1999</td>
<td>The New England Journal of Medicine</td>
<td>Case series</td>
<td>89 children total; 22 less than 3.5 months old at transplant, 67 greater than or equal to 3.5 months at transplant. • 72 (81%) alive 3 months-16.5 years post-transplant, with a median follow up of 5.6 years. • 65 survived greater than 1 year, 38 greater than 5 years and 21 greater than 10 years. • All 12 HLA-identical recipients survived. • Mean number of T/B/NK-cells varies by genotype; all genotypes had normal average in vitro mitogen response after transplant. • Poor B-cell function with 45 kids requiring IVIG. • NK-cell activity low in γc-chain deficiency and JAK3 deficiency, normal in other SCID subtypes.</td>
</tr>
</tbody>
</table>

*Potential patient overlap of Myers et al. 2002, Buckley et al. 1999

<table>
<thead>
<tr>
<th>Case series*</th>
<th>31 children total; 1-42 months old at HSCT, evaluated 10-27 years later.</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 26/31 had T-cell count in normal range for age.</td>
<td></td>
</tr>
<tr>
<td>• 30/31 had normal mitogen-induced T-cell proliferation.</td>
<td></td>
</tr>
<tr>
<td>• 18/31 had normal TREC counts.</td>
<td></td>
</tr>
<tr>
<td>• Presence of NK-cell NK-cells not correlated with TREC.</td>
<td></td>
</tr>
<tr>
<td>• Presence of TREC/naïve T-cells associated with significantly higher t-cell counts and better t-cell mitogen proliferation.</td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Case series</th>
<th>9 children total; birth to 6.5 years old at ADA deficiency diagnosis, and study done 5-12 years after starting PEG-ADA treatment.</th>
</tr>
</thead>
<tbody>
<tr>
<td>• One child died after 9 yrs of PEG-ADA treatment and a failed bone marrow transplant.</td>
<td></td>
</tr>
<tr>
<td>• Remaining 8 children attended school without protective precautions.</td>
<td></td>
</tr>
<tr>
<td>• 2 children have disabilities likely due to early infection.</td>
<td></td>
</tr>
<tr>
<td>• Lymphocyte counts (T, B and NK) and function increased significantly following initiation of PEG-ADA and peaked at 1-3 years after treatment initiation.</td>
<td></td>
</tr>
<tr>
<td>• Lymphocyte counts and T-cell function, as measured by PHA mitogen stimulation diminished overtime.</td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Case series*</th>
<th>16 children total, 9 with SCID; all lacked histocompatible siblings or closely matched related donors between 1989 and 1997.</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Mean age at diagnosis of SCID was 7.4 months.</td>
<td></td>
</tr>
<tr>
<td>• Neutrophil engraftment was achieved in all patients at a mean of 15.4 days.</td>
<td></td>
</tr>
<tr>
<td>• Serum IgM and IgA normalized in all patients within a mean of 3.5 and 6 months, respectively.</td>
<td></td>
</tr>
<tr>
<td>• 12/16 patients survived.</td>
<td></td>
</tr>
<tr>
<td>• 2/9 (22%) SCID patients died from GVHD complications.</td>
<td></td>
</tr>
<tr>
<td>Author(s)</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>Dal-Cortivo, L., Ouachee-Chardin, M., Hirsch, I., Blanche, S., Fischer, A., Cavazzana-Calvo, M. &amp; Caillat-Zucman, S.</td>
<td>2004, &quot;Does haplo-identical transplantation in children with primary immunodeficiencies have the potential to exploit donor NK-cell alloreactivity?&quot;, <em>Bone Marrow Transplantation</em>, vol. 34, no. 11, pp. 945-947.</td>
</tr>
<tr>
<td>Diaz de Heredia, C., Ortega, J.J., Diaz, M.A., Olive, T., Badell, I., Gonzalez-Vicent, M. &amp; Sanchez de Toledo, J.</td>
<td>2008, &quot;Unrelated cord blood transplantation for severe combined immunodeficiency and other primary immunodeficiencies&quot;, <em>Bone Marrow Transplantation</em>, vol. 41, no. 7, pp. 627-633.</td>
</tr>
<tr>
<td>Dror, Y., Gallagher, R., Wara, D.W., Colombe, B.W., Merino, A., Benkerrou, M. &amp; Cowan, M.J.</td>
<td>1993, &quot;Immune reconstitution in severe combined immunodeficiency disease after lectin-treated, T-cell-depleted haplocompatible bone marrow transplantation&quot;, <em>Blood</em>, vol. 81, no. 8, pp. 2021-2030.</td>
</tr>
<tr>
<td>Fischer, A., Landais, P., Friedrich, W., Morgan, G., Gerritsen, B., Fasth, A., Porta, F., Griscelli, C., Goldman, S.F. &amp; Levinsky, R.</td>
<td>1990, &quot;European experience of bone-marrow transplantation for severe combined immunodeficiency&quot;, <em>Lancet</em>, vol. 336, no. 8719, pp. 850-854.</td>
</tr>
</tbody>
</table>

\*A subset of patients in Fischer et al. 1990 are also included in Antoine et al. 2003

Cohort study*

32 children total; 7 children with SCID with HLA-identical transplant, 25 children with SCID with HLA-haplo-identical transplant, all at least 10 years out from transplant.

- Most patients had normal and stable T-cell numbers and functions.
- 3 patients’ had decreasing T-cell numbers.
- 4 patients’ had decreasing PHA responses (all in HLA-haplo-identical group and no chemotherapy).
- HLA-haplo-identical with no conditioning had lower levels of naïve CD4+ cells and impaired B-cell functioning.


Case series*

30 children total; 1987-1998 using CAMPATH-1MT depleted BMT for SCID.

- 19/30 (63%) children survived longer than 1 year post-BMT; median follow-up of 5.3 years (range 1.33–12 years); 11/30 (37%) died.
- 18/30 showed evidence of successful engraftment (49%).
- 17/30 had normal immune function following transplantation.
- The overall engraftment rate was 59% (22/37 BMT procedures).
- Most deaths attributed to pre-existing infection.


Case series*

11 children total; receiving HLA-non-identical BMT between 1985 and 1992, with a median age at time of BMT of 13 months (range: 3 weeks to 77 months).

- 9 patients engrafted (8 after first BMT, 1 after second BMT).
- 4/9 developed GVHD.
- 5 (46%) patients surviving 6-78 months post-BMT.

- Engraftment occurred in 4/5 HLA-identical transplants and 3/4 non-identical transplants.
- Full chimerism occurred in all but one of the patients that engrafted.
- Clinical manifestations of Omenn syndrome disappeared within days of BMT (likely due to pre-treatment with chemo.)
- One patient died of CMV 50 days post-transplant, 3 developed interstitial pneumonia.
- All survivors except one (who has chronic GVHD) have normal growth rates.


Case series*

9 children total; 0.5-5 months old (mean 2.8) with BMT for Omenn Syndrome between 1981 and 1989.

- All survivors except one (who has chronic GVHD) have normal growth rates.

Cohort study*

Compared recipient outcomes between:
- related HLA-identical donors (RID, n=13),
- HLA-matched unrelated donors (MUD, n= 41)
- HLA-mismatched related donors (MMRD, n=40).

- Median time to transplant was 1 month for RID, 2 months for MMRD and 4 months for MUD.
- Highest survival was RID (92.3%), followed by MUD (80.5%) and MMRD (52.5%).
- 0% of RID, 7.3% of MUD and 30% of MMRD had graft failure.
- No statistical difference between MUD and MMRD lymphocyte subsets and lymphocyte function.
- Patient sex and presence of B-cells has no impact on survival.
- No respiratory complications in RID children; respiratory complications occurred in 7.3% of MUD and 35% of MMRD.
- GVHD most common following MUD BMT (73.1%) or MMRD BMT (45%).


Case series*

5 children total; 1-11 months old at time of gene therapy.

- After infusion, 4/5 had clinical improvements.
- 3/5 patients had normal T-cell values 3 to 4 months post gene therapy.
- At last follow up (between 10 and 30 months post gene therapy for each patient), T-cells from 4/5 patients exhibited normal proliferative responses to in vitro simulation with phytohemagglutinin and anti-CD3 antibody.
- Serum IgG, IgA, and IgM levels were within age related normal range in 3 patients at 25, 21, and 13 months post gene therapy.


Case series*

4 children total; 1-11 months old at time of gene therapy.

- 4 children (of the 9/10 who had successful treatment with gene therapy) developed leukemia between 30 and 68 months after gene therapy.
- All cases of leukemia were associated with vector insertion near genes associated with cancer development.
- 3/4 children were successfully treated with chemotherapy and regained polyclonal t-cell populations.
- The 4th child underwent 2 BMTs and died 60 months after gene therapy, 26 months after leukemia diagnosis.

Case series*

193 patients total; from 18 European centers between 1982 and 1993.

- 116 alive with evidence of engraftment 5 months after BMT; 24 later died (20%).
- T-cell function improved during the 2 years after BMT and continued to be better than B-cell function.
- Poor outcomes associated with: absence of T-cell reconstitution, presence of chronic GVHD 6 months after transplant, B-SCID (multivariate analysis).
- At last follow up (median, 6 years after transplant), 93% of survivors had normal T-cell function and 68% had normal B-cell function.


Case series*

15 children total; Treated with BMT for ADA deficiency, focus on neurologic symptoms.

- 12/15 survived, all with developmental delay.
- 6/12 have significant neurocognitive deficits (learning disabilities, gait problems, hearing deficit, and/or hyperactivity).


Case-control study

41 patients with SCID; treated with HSCT.

- 8/41 (19.5%) developed auto-immune hemolytic anemia (AIHA); one case occurred pre-transplant, others were a median of 8 months post-transplant.
- 3 patients died from AIHA complications.
- Peripheral blood as source of stem cells was found to be related to increased risk of developing auto-immune hemolytic anemia.


Case series*

13 children total; transplanted between 7 and 68 days old.

- All patients alive and well 0.5-11.5 years after transplant (median 3 years).
- 2 children developed chronic GvHD chronic.
- 3 children required more than one transplant.
- All children achieved neutrophil engraftment and normal levels of IgA.
- 7 have normal IgG.
- 12 have normal IgM.
- 10/12 have normal neuro-development; 1/12 has trouble with communication and interactive skills, and 1/12 has motor delay.
<table>
<thead>
<tr>
<th>Study</th>
<th>Authors</th>
<th>Year</th>
<th>Description</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kline, R.M., Stiehm, E.R. &amp; Cowan, M.J.</td>
<td>1996</td>
<td>&quot;Bone marrow 'boosts' following T-cell-depleted haplo-identical bone marrow transplantation&quot;, Bone Marrow Transplantation, vol. 17, no. 4, pp. 543-548.</td>
<td>9 patients total; 8 with SCID and 1 aplastic anemia.</td>
<td>• Bone marrow “boost” increased absolute lymphocyte counts in 5/9, also increased mean CD3, CD4 and CD8 counts and improved mean T-cell function. • The 2 patients who did not show complete recovery after “boost” were on chronic steroid therapy. • 8/9 patients are alive and well 0.8 to 6.2 years (median 2.9) years after “boost”.</td>
</tr>
<tr>
<td>Knutsen, A.P. &amp; Wall, D.A.</td>
<td>2000</td>
<td>&quot;Umbilical cord blood transplantation in severe T-cell immunodeficiency disorders: two-year experience&quot;, Journal of Clinical Immunology, vol. 20, no. 6, pp. 466-476.</td>
<td>8 children with SCID; 2 weeks to 8 years old.</td>
<td>• Transplanted using umbilical cord blood derived stem cells. • Neutrophil recovery time was the same as that reported in BMT. • Platelet recovery was delayed compared to BMT. • 6/8 children alive and well 18 to 24 months after transplant. • All patients had 100% chimerism.</td>
</tr>
<tr>
<td>Laffort, C., Le Deist, F., Favre, M., Caillat-Zucman, S., Radford-Weiss, I., Debre, M., Fraitag, S., Blanche, S., Cavazzana-Calvo, M., de Saint Basile, G., de Villartay, J.P., Giliani, S., Orth, G., Casanova, J.L., Bodemer, C. &amp; Fischer, A.</td>
<td>2004</td>
<td>&quot;Severe cutaneous papillomavirus disease after haemopoietic stem-cell transplantation in patients with severe combined immune deficiency caused by common gamma c cytokine receptor subunit or JAK-3 deficiency&quot;, Lancet, vol. 363, no. 9426, pp. 2051-2054.</td>
<td>41 patients total; at least 10 years after HSCT for SCID.</td>
<td>• 9/41 developed extensive chronic human papilloma virus. • 4 had lesions typical of a rare genodermatosis. • All with human papilloma virus had γc/Adenylate kinase-3 (AK-3) deficiency, though only 9/18 with γc/JAK3 developed human papilloma virus.</td>
</tr>
<tr>
<td>Mazzolari, E., Forino, C., Guerci, S., Imberti, L., Lanfranchi, A., Porta, F. &amp; Notarangelo, L.D.</td>
<td>2007</td>
<td>&quot;Long-term immune reconstitution and clinical outcome after stem cell transplantation for severe T-cell immunodeficiency&quot;, The Journal of Allergy and Clinical Immunology, vol. 120, no. 4, pp. 892-899.</td>
<td>58 children total; treated with HSCT (in-utero to 34 months old at time of transplant).</td>
<td>• 42/58 (72.4%) survived at least 5 years (median follow-up of 132 months). • 85% produced antibodies to tetanus toxoid or hepatitis B vaccines. • 26/28 immunized with Measles, Mumps and Rubella vaccine had evidence of anti-measles antibodies. • Most children had height (77.5%) and weight (82.5%) in the normal range. • At last follow-up, 24/40 required no treatment, 6 required IVIG and/or antibiotics; 5 are on thyroid replacement; 3 receive anti-epileptics; 1 treated for portal hypertension.</td>
</tr>
</tbody>
</table>
| Myers, L.A., Patel, D.D., Puck, J.M. & Buckley, R.H. 2002, "Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival", *Blood*, vol. 99, no. 3, pp. 872-878. | 21 children total; transplanted prior to 28 days of life (early treatment) and 96 children transplanted at a median age of 190 days (range: of 45-516 days) (late treatment). | - 20/21 (95%) early treatment children survived.  
- 71/96 (74%) late treatment children survived.  
- Mean time to significant T-cell function in all early treatment was 33 days and to normal T-cell function was 103 days.  
- Mean TREC value peaked earlier post-transplant for early treatment recipients but the 2 groups were indistinguishable by 5 years.  
- Early transplantation did not have an affect on B-cell function. |
| O'Marcaigh, A.S., DeSantes, K., Hu, D., Pabst, H., Horn, B., Li, L. & Cowan, M.J. 2001, "Bone marrow transplantation for T-B- severe combined immunodeficiency disease in Athabascan-speaking native Americans", *Bone Marrow Transplantation*, vol. 27, no. 7, pp. 703-709. | 18 children total; Birth to 3 months of age at diagnosis; mean age of 17.5 months at first transplant. | - 12/18 developed oral/genital ulcers associated with SCIDA.  
- 16/18 children were well enough for transplant.  
- 15/16 achieved engraftment.  
- 11/16 developed normal T-cell function. |
| Patel, D.D., Gooding, M.E., Parrott, R.E., Curtis, K.M., Haynes, B.F. & Buckley, R.H. 2000, "Thymic function after hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency", *The New England Journal of Medicine*, vol. 342, no. 18, pp. 1325-1332. | 173 total patients; 83 SCID with a mean age of transplant of 0.5 ±0.4 years; 90 healthy controls from less than 1 to 79 years old. | - Non-thymus-dependent cells predominated in the first 100 days after transplant.  
- 140-180 days after transplant thymus-dependent cells predominated.  
- After transplant thymus-dependent cells peaked at approximately one year but then diminished over the next 14.  
- Controls showed a decline in thymus-dependent cells from birth to 79 years old. |
- Reduced intensity group: 32/33 were alive at one month and 31/33 alive at one year  
- Myeloablation group: 14/19 were alive at one month and 11/19 alive at one year.  
- At one year: all in the myeloablative group had normal B-cell function, 5 of reduced intensity group still required IVIG.  
- GVHD incidence not different between groups but limited chronic GVHD was more common in myeloablative group.  
- Quality of life, measured by Lansky score, was similar between the groups (97 vs. 94). |
<table>
<thead>
<tr>
<th>Study Authors</th>
<th>Population</th>
<th>Outcomes</th>
</tr>
</thead>
</table>
  • 9/10 patients with JAK3 deficiency were alive and well 4 to 18 years after stem cell transplant. |
| Rogers, M.H., Lwin, R., Fairbanks, L., Gerritsen, B. & Gaspar, H.B. 2001, "Cognitive and behavioral abnormalities in adenosine deaminase deficient severe combined immunodeficiency", The Journal of Pediatrics, vol. 139, no. 1, pp. 44-50. | 22 children total; 1-51 months old at HSCT; 0.9-18 years old at follow-up; 11 children with ADA deficiency, 11 other SCID types. | • No significant difference in full-scale IQ between groups; children with ADA deficiency 2 standard deviations below mean.  
  • Significant behavioral differences in children with ADA deficiency: more hyperactivity, higher scores for dysfunction in social, emotional and behavioral domains.  
  • Abnormal behavior more evident in older children.  
  • Children with ADA deficiency group with no abnormal motor function on gross neurological evaluation. |
  • 7/9 maintained polyclonal lymphocyte population up to 5 years after treatment, and 2/9 developed monoclonal lymphoproliferation 2.5 years post transplant.  
  • Circulating naïve T-cells were similar to that of age-matched controls. |
  • 5 patients in the anti-CD52 group and 2 in anti-CD34 group developed GVHD. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Author(s)</th>
<th>Year</th>
<th>Study Design</th>
<th>Patient Details</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case series*</td>
<td>Stephan, J.L., Vlekova, V., Le Deist, F., Blanche, S., Donadieu, J., De Saint-Basile, G., Durandy, A., Griscelli, C. &amp; Fischer, A.</td>
<td>1993</td>
<td>117 patients total; treated for SCID between 1970 and 1992; 85 children were treated with BMT.</td>
<td>• Estimated SCID incidence: 1/100-150,000. • HLA-identical transplant from a related donor 21/25 (84% survived). • Pheno-identical transplant (HLA-genotypically haplo-identical) from related donor 2/5 (40% survived). • HLA-haplo-identical transplant without T-cell depletion 0/5 (0% survived). • T-cell depleted haplo-identical transplant 28/50 (56% survived). • 22 children did not receive any type of transplant and died. • 10 received fetal liver transplant, 9 died post transplant.</td>
<td></td>
</tr>
<tr>
<td>Case series*</td>
<td>Touraine, J.L., Plotnick, H., Roncarolo, G.M., Bacchetta, R. &amp; Gebuhrer, L.</td>
<td>2007</td>
<td>19 infants and 2 fetuses.</td>
<td>• Stem cells from fetal liver were used for transplants. • 14/19 had evidence of engraftment and immunological reconstitution. • In the 3 patients for whom information was available, there was normal antibody responses several years after transplant.</td>
<td></td>
</tr>
<tr>
<td>Case series*</td>
<td>van Leeuwen, J.E., van Tol, M.J., Joosten, A.M., Schellekens, P.T., van den Bergh, R.L., Waaijer, J.L., Oudeman-Gruber, N.J., van der Weijden-Ragas, C.P., Roos, M.T. &amp; Gerritsen, E.J.</td>
<td>1994</td>
<td>31 patients total; 1-94 months old at BMT.</td>
<td>• HLA-identical related 6/10 (60% survived) • HLA-haplo-identical related: 9/19 (47% survived). • HLA-matched unrelated: 0/2 (0% survived). • Major causes of death were graft and respiratory failure. • All who died of respiratory failure had a lung infection prior to transplant. • All children with sustained engraftment had evidence of humoral immunity.</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Subjects</td>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
<td>---------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veys, P., Rao, K. &amp; Amrolia, P. 2005, &quot;Stem cell transplantation for congenital immunodeficiencies using reduced-intensity conditioning&quot;, Bone Marrow Transplantation, vol. 35 Suppl 1, pp. S45-7.</td>
<td>81 children total; 0.1-17.6 years old, with congenital immunodeficiencies, 20 with SCID.</td>
<td>All subjects treated with HSCT with reduced-intensity conditioning.  Survival rate 84% (68/81) with survival difference between SCID and non-SCID.  Increase in reactivation of EBV compared to conventional BMT procedures.  HSCT with reduced-intensity conditioning appeared to be well tolerated.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waruiru, C., Slatter, M.A., Taylor, C., Ramesh, V., Flood, T.J., Abinun, M., Cant, A.J. &amp; Gennery, A.R. 2007, &quot;Outcome of hematopoietic stem cell transplantation in severe combined immune deficiency with central nervous system viral infection&quot;, The Pediatric Infectious Disease Journal, vol. 26, no. 2, pp. 129-133.</td>
<td>8 patients total; median age of 3 months old (range: 6 weeks to 7 years), 7 are children with SCID treated with HSCT.</td>
<td>All children with central nervous system viral infections prior to HSCT.  Neurologic manifestations of infection were present in 4 patients before HSCT.  Magnetic resonance image abnormalities ranged from cerebral atrophy to multifocal diffuse mass lesions.  Of 6 patients who had an electroencephalographic examination, most showed slow wave activity.  Only 3 children remain alive.  5/8 children died in first 200 days after HSCT from neurologic sequelae or disseminated infection.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wijnaendts, L., Le Deist, F., Griscelli, C. &amp; Fischer, A. 1989, &quot;Development of immunologic functions after bone marrow transplantation in 33 patients with severe combined immunodeficiency&quot;, Blood, vol. 74, no. 6, pp. 2212-2219.</td>
<td>33 children treated with HST between 1972 and 1987 surviving a minimum of 6 months: 18 HLA-identical transplants and 15 HLA-non-identical transplants.</td>
<td>Development of immune function occurred faster in patients with HLA-identical transplants.  Development of T- and B-cell function occurred faster when chemotherapy preceded BMT.  Poor B-cell function was observed more frequently when chemotherapy was not used.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Study conducted outside the United States
<table>
<thead>
<tr>
<th>Author(s), Year, Title of Paper &amp; Study Design</th>
<th>Population</th>
<th>Significant Findings</th>
</tr>
</thead>
</table>
| McGhee, S.A., Stiehm, E.R. & McCabe, E.R. 2005, "Potential costs and benefits of newborn screening for severe combined immunodeficiency", *The Journal of Pediatrics*, vol. 147, no. 5, pp. 603-608. | N/A | • At a threshold of $100,000/QALY there is 86% likelihood of screening being cost-effective; this would be true with 61.2% false-neg rate, 3.2% false-positive rate; test cost of about $15, incidence of 1:125,000 and treatment costs of $1.35 million.  
• Assuming base case scenario, the cost to identify and treat each case would be $485,000; implementation of screening would cost $23,920,000 and save 760 life-years per year of screening. |
XI. Appendix B- Conflict of interest form

MGH Center for Child and Adolescent Health Policy

MassGeneral Hospital
for Children™

BACKGROUND INFORMATION
AND
CONFIDENTIAL CONFLICT OF INTEREST DISCLOSURE
For Newborn Screening Evidence Working Group Reviews

NAME: _____________________________ TELEPHONE: ____________

ADDRESS: ___________________________________________________
____________________________________________________

EMAIL ADDRESS: ____________________________________________

CURRENT EMPLOYER: _________________________________________

ROLE:  Staff member______ Proposer______ Consultant______
    Company representative_____ Principal investigator_____  
    Parent group_____  State health department_____  
    Other (specify)________

(Please make all that apply.)

There are two parts to this form, Part I Background Information, and Part II Confidential Conflict of Interest Disclosure. Complete both parts, sign and date this form on the last page, and return the form to the responsible staff member for the MGH Center for Child and Adolescent Health Policy Newborn Screening Evidence Working Group program. Retain a copy for your records.
PART I BACKGROUND INFORMATION

INSTRUCTIONS

Please provide the information requested below regarding relevant organizational affiliations, government service, public statements and positions, research support, and additional information (if any). Information is "relevant" if it is related to -- and might reasonably be of interest to others concerning -- your knowledge, experience, and personal perspectives regarding the subject matter and issues to be addressed by the evidence team efforts in general or for a specific screening proposal. If some or all of the requested information is contained in your curriculum vitae, you may if you prefer simply attach your CV to this form, supplemented by additional responses or comments below as necessary.

I. ORGANIZATIONAL AFFILIATIONS. Report your relevant business relationships (as an employee, owner, officer, director, consultant, etc.) and your relevant remunerated or volunteer non-business relationships (e.g., professional organizations, trade associations, public interest or civic groups, etc.).

II. GOVERNMENT SERVICE. Report your relevant service (full-time or part-time) with federal, state, or local government in the United States (including elected or appointed positions, employment, advisory board memberships, military service, etc.).

III. RESEARCH SUPPORT. Report relevant information regarding both public and private sources of research support (other than your present employer), including sources of funding, equipment, facilities, etc.

IV. PUBLICATIONS. Please list all publications related to the evidence topic or the work of the Evidence Working Group. Please separate peer-reviewed from other publications.

V. PUBLIC STATEMENTS AND POSITIONS. List your relevant articles, testimony, speeches, etc., by date, title, and publication (if any) in which they appeared, or provide relevant representative examples if numerous. Provide a brief description of relevant positions of any organizations or groups with which you are closely identified or associated.

VI. ADDITIONAL INFORMATION. If there are relevant aspects of your background or present circumstances not addressed above that might reasonably be construed by others as affecting your judgment in matters within the specified evidence reviews and therefore might constitute an actual or potential source of bias, please describe them briefly.
PART II CONFIDENTIAL CONFLICT OF INTEREST DISCLOSURE

INSTRUCTIONS

It is essential that the work of the Evidence Working Group and its advisors used in the development of reports for the Advisory Committee on Heritable Disorders and Genetic Diseases of Newborns and Children (ACHDGDNC) not be compromised by any significant conflict of interest. For this purpose, the term "conflict of interest" means any financial or other interest which conflicts with the service of the individual because it (1) could significantly impair the individual's objectivity or (2) could create an unfair competitive advantage for any person or organization. Except for those situations in which the working group determines that a conflict of interest is unavoidable and promptly and publicly discloses the conflict of interest, no individual can be participate in the development of evidence reports if the individual has a conflict of interest that is relevant to the functions to be performed.

The term "conflict of interest" means something more than individual bias. There must be an interest, ordinarily financial, that could be directly affected by the work of the committee.

Conflict of interest requirements are objective and prophylactic. They are not an assessment of one's actual behavior or character, one's ability to act objectively despite the conflicting interest, or one's relative insensitivity to particular dollar amounts of specific assets because of one's personal wealth. Conflict of interest requirements are objective standards designed to eliminate certain specific, potentially compromising situations from arising, and thereby to protect the individual, the other members of the working group and its advisors, the ACHDGDNC, and the public interest. The individual, the committee, and the institution should not be placed in a situation where others could reasonably question, and perhaps discount or dismiss, the work of the group simply because of the existence of conflicting interests.

The term "conflict of interest" applies only to current interests. It does not apply to past interests that have expired, no longer exist, and cannot reasonably affect current behavior. Nor does it apply to possible interests that may arise in the future but do not currently exist, because such future interests are inherently speculative and uncertain. For example, a pending formal or informal application for a particular job is a current interest, but the mere possibility that one might apply for such a job in the future is not a current interest.

The term "conflict of interest" applies not only to the personal interests of the individual but also to the interests of others with whom the individual has substantial common financial interests if these interests are relevant to the functions to be performed. Thus, in assessing an individual's potential conflicts of interest, consideration must be given not only to the interests of the individual but also to the interests of the individual's spouse/partner and minor children, the individual's employer, the individual's business partners, and others with whom the individual has substantial common financial interests. Consideration must also be given to the interests of those for whom one is acting in a fiduciary or similar capacity (e.g., being an officer or director of a corporation, whether profit or nonprofit, or serving as a trustee).
Much of the work of the Evidence Working Group involves scientific and technical studies and assistance for the ACHDGDNC. Related activities may include, for example: assessing prevalence, testing, and treatment data; defining research needs, priorities, opportunities and agendas; addressing questions of human health promotion and assessment; and assessing the state of scientific or technical knowledge on particular genetic diseases. Such activities frequently address scientific and technical issues that are sufficiently broad in scope that they do not implicate specific financial interests or conflict of interest concerns.

However, where such activities address more specific issues having significant financial implications -- e.g., judging the quality of the evidence related to a specific disease or disorder, making recommendations to agencies regarding specific research needs, etc. -- careful consideration must be given to possible conflict of interest issues with respect to the role of staff, consultants, and advisors who will be used by the Evidence Working Group in the development of reports to be provided to the ACHDGDNC.

The overriding objective of the conflict of interest inquiry in each case is to identify whether there are interests – primarily financial in nature – that could impair the individual's objectivity or could create an unfair competitive advantage for any person or organization. The fundamental question in each case is does the individual, or others with whom the individual has substantial common financial interests, have identifiable interests that could be directly affected by the outcome of the Evidence Working Group activities?

The application of these concepts to specific scientific and technical studies and assistance projects must necessarily be addressed in each case on the basis of the particular facts and circumstances involved. The questions set forth below are designed to elicit information from you concerning possible conflicts of interest that are relevant to the functions to be performed by the Evidence Working Group and your relationship to the group.

1. **FINANCIAL INTERESTS.** (a) Taking into account stocks, bonds, and other financial instruments and investments including partnerships (but excluding broadly diversified mutual funds and any investment or financial interests valued at less than $10,000), do you or, to the best of your knowledge others with whom you have substantial common financial interests, have financial investments that could be affected, either directly or by a direct effect on the business enterprise or activities underlying the investments, by the outcome of the Evidence Working Group and its reports to the ACHDGDNC?

   (b) Taking into account real estate and other tangible property interests, as well as intellectual property (patents, copyrights, etc.) interests, do you or, to the best of your knowledge others with whom you have substantial common financial interests, have property interests that could be directly affected by the outcome of the Evidence Working Group and its reports to the ACHDGDNC?

   (c) Could your employment or self-employment (or the employment or self-employment of your spouse/partner), or the financial interests of your employer or clients (or the financial interests of
your spouse's employer or clients) be directly affected by the outcome of the Evidence Working Group and its reports to the ACHGDNC?

(d) Taking into account research funding and other research support (e.g., equipment, facilities, industry partnerships, research assistants and other research personnel, etc.), could your current research funding and support (or that of your close research colleagues and collaborators) be directly affected by the outcome of the Evidence Working Group and its reports to the ACHGDNC?

(e) Could your service create a specific financial or commercial competitive advantage for you or others with whom you have substantial common financial interests?

If the answer to all of the above questions under FINANCIAL INTERESTS is either "no" or "not applicable," check here _____ (NO).

If the answer to any of the above questions under FINANCIAL INTERESTS is "yes," check here ____ (YES), and briefly describe the circumstances on the last page of this form.

2. OTHER INTERESTS. (a) Is the central purpose of the project for which this disclosure form is being prepared a critical review and evaluation of your own work or that of your employer?

(b) Do you have any existing professional obligations (e.g., as an officer of a scientific or engineering society) that effectively require you to publicly defend a previously established position on an issue that is relevant to the functions to be performed by the Evidence Working Group?

(c) To the best of your knowledge, will your participation in this committee activity enable you to obtain access to a competitor's or potential competitor's confidential proprietary information?

(d) If you are or have ever been a U.S. Government employee (either civilian or military), to the best of your knowledge are there any federal conflict of interest restrictions that may be applicable to your service in connection with this committee activity?

If the answer to all of the above questions under OTHER INTERESTS is either "no" or "not applicable," check here _____ (NO).

If the answer to any of the above questions under OTHER INTERESTS is "yes," check here ____ (YES), and briefly describe the circumstances on the last page of this form.

EXPLANATION OF "YES" RESPONSES:
During your period of service in connection with the activity for which this form is being completed, any changes in the information reported, or any new information, which needs to be reported, should be reported promptly by written or electronic communication to the responsible staff member.

_______________________________  ________________________  
YOUR SIGNATURE              DATE

Reviewed by:  ___________________________  ________________________
PRINCIPAL INVESTIGATOR    DATE
XII. Appendix C- Letter and questions sent to SCID experts

Dear (name of specialist),

You have been identified as a key subject matter expert in the field of Severe Combined Immunodeficiency (SCID). As such, we are requesting input from you concerning the viability and efficacy of newborn screening for this condition.

As you likely know, the Secretary of the Health and Human Services Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) has the responsibility to make evidence-based recommendations regarding newborn screening. The Committee has recently been given the task of evaluating evidence for heritable diseases that have been formally nominated for neonatal screening. The advisory committee has in turn asked an Evidence Review Workgroup, based in the MGH Center for Child and Adolescent Health Policy, to develop systematic reviews of evidence to help the committee in its deliberations.

We are currently in the process of conducting a review of Severe Combined Immunodeficiency (SCID) and are writing to request your help with this process. Specifically, we would like to know if you are available to share your experience and expertise regarding the current state of SCID screening, diagnosis, and treatment. Participating in this project would involve answering a short set of questions which ask about your clinical experience with SCID (see attachment). After providing responses to these inquiries, we will likely want to contact you by phone, so that we may clarify some of your answers or seek more information about certain topics.

Our goal in contacting you is to have access to the most recent and up to date information available regarding SCID. In all of its activities, the Evidence Review Group has maintained sensitivity to issues of conflict of interest and the importance of limiting bias in the development of evidence. Should you agree to discuss your work with us, we will discuss with you at that time our approach to confidentiality, transparency, conflict of interest, and our requirements for public reporting.

If you are willing to help in this crucial review process, please return your answers to the attached questions by Friday August 29th, along with a signed copy of the attached conflict of interest form. Additionally, in case we need clarification regarding your responses, please send us a list of times between September 2nd and September 12th when you would be available to discuss you answers over the phone. You may send all of these materials to Project Manager Alix Knapp by email (aaknapp@partners.org) or confidential fax (617-726-1886).

We appreciate your unique insight into this condition. We thank you in advance for participation and look forward to hearing from you.
Sincerely,

Marsha Fearing Browning, M.D., M.P.H., MMSc.
Medical Genetics, MassGeneral Hospital for Children

James M Perrin, M.D.
Professor of Pediatrics, Harvard Medical School
Director, Division of General Pediatrics
Director, MGH Center for Child & Adolescent Health Policy
Associate Chair for Research, MassGeneral Hospital for Children

Key Questions:

A. Diagnosis
   1. Do you have any data regarding the distribution of the different phenotypes/genotypes of SCID?
   2. At the time of diagnostic evaluation, what processes are used to determine genotype?
   3. Describe the most effective process to phenotype patients and issues that may confound the diagnosis:

B. Treatment
   1. If you are currently involved in the treatment of patients with SCID, what is your treatment protocol for SCID in patients either diagnosed clinically or through newborn screening?
   2. What are the major challenges to treating SCID?
   3. What unique outcomes data for the treatment of SCID can you provide?
   4. From your perspective, how many treatment centers in the U.S. exist that can provide appropriate treatment for SCID?
   5. What estimates (insight?) do you have regarding the cost of treatment for SCID or ways of measuring those costs?

C. Screening
   If you currently participate in a newborn screening protocol for SCID and/or you participate in a “clinical laboratory” screening program for SCID please answer the following:

Describe your screening protocol, including:
   1. Describe your screening protocol, including:
a. What is your first tier test (e.g., fluorometry, PCR, etc)?
b. What, if anything is your second tier test (i.e., testing on the same screening sample if the first tier test is abnormal)?
c. What subsequent testing do you perform on a new sample from an individual with an abnormal first tier/second tier test (i.e., third tier testing)?

2. What are the advantages and disadvantages of your screening protocol?
   a. If your screening protocol has been implemented, please describe your experience, including:
   b. When screening began
   c. The number of children who have been screened
   d. The total number of children that had a positive screening test that required a diagnostic evaluation
   e. The number of cases identified. If possible, please characterize the immune phenotype or genotype of these cases. How many were SCID? How many were other types of immunodeficiencies?
   f. The cost of screening. Because costs can be determined so many different ways, please outline what is included in your calculation.

D. Additional Comments:
Please submit any additional comments that may contribute towards implementation of newborn screening for this disease.
XIII. Appendix D- Letter and questions sent to SCID advocacy groups

Dear Name of Advocacy Group,

As you likely know, the Secretary of the Health and Human Services Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children (ACHDGDNC) is responsible for making evidence-based recommendations regarding what tests to include in newborn screening. The Committee has the responsibility to evaluate evidence for heritable diseases that have been formally nominated for neonatal screening. The advisory committee asked an Evidence Review Workgroup, based in the MGH Center for Child and Adolescent Health Policy, to develop systematic reviews of evidence to help the committee in its deliberations. As a parent of a child with a genetic disorder, I am working as a member of the Evidence Review Workgroup to gather views and evidence from parents and advocacy groups.

We are currently conducting a review of Severe Combined Immunodeficiency (SCID) and are writing to request your help with this endeavor. Specifically, we would like to know if you are available to share your experience and expertise as a parent regarding the current state of SCID screening, diagnosis, and treatment. Participating in this project would involve answering a short set of questions about your experience with SCID. After providing responses to these inquiries, you may also be contacted by phone, so that we may clarify some of your answers or seek more information about certain topics.

Our goal in contacting you is to have access to the most recent and up to date information available regarding SCID. In all of its activities, the Evidence Review Group has maintained sensitivity to issues of conflict of interest and the importance of limiting bias in the development of evidence. Should you agree to discuss your experience with us, we will discuss with you at that time our approach to confidentiality and transparency.

If you can help in this review process, please return your answers to the questions we have provided by Friday, December 19th, along with a signed copy of the attached conflict of interest form. Additionally, in case we need clarification regarding your responses, please send us a list of times between January 6th and 9th, 2009, when you would be available to discuss your answers over the phone. You may send all of these materials to Project Manager Alixandra Knapp by email (aaknapp@partners.org) or fax (617-726-1886).

Thank you very much for your time. We look forward to hearing from you.
Sincerely,

Denise Queally  
Consumer Perspective  
Evidence Review Group

James M. Perrin, MD  
Professor of Pediatrics

Key Questions:

Please answer the following questions based on your experiences with families of children with SCID.

I. Screening
   - What do you know about screening children for SCID? Do you have information about how effective it is?
   - Do you or your group recommend particular screening approaches? If so, which, and why?

II. Diagnosis
   - Can you provide any information regarding the distribution or rates of the different physical or clinical characteristics of SCID?
   - What information can you provide about the relationship between any gene findings (especially, by screening in infancy) and the child’s clinical course?
   - Are there benefits to early identification and diagnosis of SCID? If so, what are they?

III. Treatment
   - How effective is treatment for SCID? What is done and how well does it work?
   - How many treatment centers exist in the United States, and how do you learn of them?
   - What are your experiences with any potential harms, physical or non-physical, associated with treatment?

IV. Other Information
   - Is there anything else about SCID that you would like to share with us?
Development of a routine newborn screening protocol for severe combined immunodeficiency

Mei W. Baker, MD, William J. Grossman, MD, PhD, Ronald H. Laessig, PhD, Gary L. Hoffman, BS, Charles D. Brokopp, DrPH, Daniel F. Kurtycz, MD, Michael F. Cogley, BS, Thomas J. Litsheim, BS, Murray L. Katcher, MD, PhD, and John M. Routes, MD

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 determine 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 naïve 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 naïve 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 2009;124:522-7.)

Key words: Dried blood spots, hematopoietic stem cell transplantation, newborn screening, real-time quantitative PCR, severe combined immunodeficiency, T-cell receptor excision circles

The goal of newborn screening (NBS) is to identify presymptomatic newborns with potentially serious or fatal disorders that can be successfully treated, leading to significant reductions in morbidity and mortality. The 45-year history of NBS demonstrates that it is an extremely successful and cost-efficient public health undertaking and provides useful information in the field of preventive medicine. Routine NBS began in the 1960s with a single disorder, phenylketonuria, and grew to a core panel of 29 disorders as recommended by the American College of Medical Genetics. As knowledge of the causes of genetic disorders increases, detection technologies advance, and better treatment regimens emerge, more diseases will be added to the NBS panel.

Severe combined immunodeficiency (SCID) was recognized as a disorder that meets the criteria for inclusion in NBS in a Centers for Disease Control and Prevention 2004 conference entitled “Applying Public Health Strategies to Primary Immunodeficiency Diseases.” Criteria include infants who are asymptomatic at birth, serious medical consequences without treatment, availability of confirmatory tests and effective treatment, and improved outcomes with early intervention. The National Advisory Committee of Heritable Disorders in Newborns and Children has selected SCID as the focus of an evidentiary review regarding recommendations for NBS.

Severe combined immunodeficiency is a group of disorders caused by more than a dozen single-gene defects. All known gene mutations cause a defect in the development of normal naive T cells, leading to combined cellular and humoral immunodeficiency. It is estimated that as many as 50% of infants die because they are not diagnosed early enough to implement life-saving therapies. Consequently, the true incidence of SCID is unknown, but it is estimated at 1 in 66,000 live births.

Severe combined immunodeficiency is a pediatric medical emergency. Early diagnosis and treatment of SCID by hematopoietic stem cell transplantation (HSCT) is essential to prevent death and re-establish a normal functional immune system. Infants with SCID typically appear healthy at birth. Undiagnosed, these infants eventually develop severe life-threatening infections with a 100% mortality rate, usually within the first year of life. If HSCT is performed within the first 3.5 months of life, before the development of life-threatening infections, infants with SCID have upwards of a 95% long-term survival rate. In contrast, infants with SCID who undergo HSCT after 3.5 months, long-term survival drops dramatically to 60% to 70%, illustrating the importance of early diagnosis and treatment.
Because most SCID cases are sporadic without a family history, the majority of infants with SCID would clearly benefit from a NBS program that would allow early detection before the development of life-threatening infections. Several screening methods have been suggested, including a complete blood count on all newborns, IL-7 immunoassay, luminex multiplex assay of T-cell antigentic markers, and quantitation of T-cell receptor excision circles (TRECs) on dried blood spots (DBSs).

To date, the quantitation of TRECs by real-time quantitative PCR (RT-qPCR) is the most investigated method and appears to be compatible with the state NBS programs using DBS in the NBS card as the primary specimen. Normal T-cell differentiation in the thymus is characterized by the rearrangement of the T-cell receptor genes leading to the joining of the V, D, and J gene segments of the T-cell receptor. During this process of T-cell receptor rearrangement, small, episomal pieces of DNA are generated, which are known as TRECs. One specific TREC, the $\delta$Rec-$\delta$Jα TREC, is produced from approximately 70% of all T cells that express the $\alpha/\beta$ T-cell receptor. Seminal studies by Douek et al. established a method for the enumeration of the $\delta$Rec-$\delta$Jα TRECs by RT-qPCR using DNA isolated from PBMCs. These investigators further demonstrated that quantitation of TRECs by RT-qPCR serves as an excellent surrogate marker for the number of naive T cells that recently have emigrated from the thymus. Subsequently, Chan and Puck showed that the RT-qPCR TREC assay could be performed by using DNA extracted from DBSs on newborn specimen collection cards (Guthrie cards), and that all forms of SCID, regardless of the molecular defect, were characterized by low numbers of detectable TRECs. One potential drawback to this approach was the reported relatively high rate of false-positive results (1.4%), which could make this method untenable for widespread, population-based NBS for SCID. We report here our experience in further optimizing this method. Our data demonstrate that the TREC assay is amenable to routine high-throughput NBS and that it can be implemented within ongoing NBS programs.

**METHODS**

**Institutional review and approval**

The protocol to use deidentified NBS specimen collection cards from the Wisconsin State NBS program was approved by the University of Wisconsin Health Sciences Institutional Review Board.

**Screened samples**

Deidentified DBSs used for this assay development were punched from NBS cards submitted to the Wisconsin State Laboratory of Hygiene NBS Laboratory. Blank filter paper NBS collection cards spotted with peripheral blood from a T$^{-}$B$^{-}$NK$^{+}$ patient with SCID (pre-HSCT) were used as a positive control for the TREC assay (ie, low or 0 TRECs present). In addition, filter paper NBS cards were also spotted with peripheral blood from an adult subject and depleted of naïve T cells (CD3$^{+}$CD45RA$^{+}$) by staining cells with CD45RA–fluorescein isothiocyanate, followed by negative selection using an Aria Cell Sorter (Becton, Dickinson and Co, Rutherford, NJ). This whole blood sample was demonstrated to be >95% depleted of CD3$^{+}$CD45RA$^{+}$ naïve T cells by fluorescence-activated cell sorting before spotting on NBS cards as determined by flow cytometry. In addition, NBS cards were spotted with blood reconstituted with different CD45RA:CD45RO ratios as outlined.

**Reconstitution of whole blood with different CD45RA:CD45RO ratios**

PBMCs were isolated from normal donors and stained with CD3-Phycoerythrin y7, CD45RA–fluorescein isothiocyanate, and CD45RO–Allophycocyanin (BD Biosciences, San Jose, Calif). Greater than 90% pure populations of naïve (CD45RA$^{hi}$CD45RO$^{hi}$) and memory (CD45RO$^{hi}$CD45RA$^{lo}$) T lymphocytes were obtained through the use of a 4-laser Aria high-speed cytometer sorter (BD Biosciences). Isolated naïve and memory T cells were mixed at varying ratios (from 10:1 to 1:10) in plasma and then added back to a mixture of red blood cells, neutrophils, eosinophils, and monocytes to achieve a final concentration that approximated the original complete blood count. The mixture of cells was reanalyzed on the Forcyte Hematology Analyzer (Oxford Science, Oxford, Conn) to ensure final white blood cell (WBC) count and differential counts were similar to the original CBC (initial WBC count/differential: WBC count 7,340 mL, neutrophils [51.8%], lymphocytes [23.4%], monocytes [9.1%], eosinophils [7.6%], hematocrit [40.2%]; postreconstitution representative WBC count/differential: WBC count 7,600 mL, neutrophils [53.3%], lymphocytes [20.7%], monocytes [12.3%], eosinophils [6.7%], hematocrit [42%]). All samples were coded and blinded to the personnel who performed the RT-qPCR TREC assay. TREC numbers were determined by RT-qPCR. The mean and SE were plotted for each CD45RA:CD45RO ratio using Prism GraphPad (GraphPad Software, Inc, La Jolla, Calif).

**DNA extraction**

In an effort to maximize DNA yield and template integrity from DBS, we used the Generation DNA Purification Solution and Generation DNA Elution Solution (Qiagen, Valencia, Calif). This method of DNA extraction was chosen because it does not use column purification or salt precipitation to isolate DNA and results in a higher DNA yield from DBSs. DNA extraction was performed in a 96-well plate format. A 3.2-mm disk of DBS from each NBS card was punched into a MicroAmp Optical 96-Well Reaction Plate well (Applied Biosystems, Foster City, Calif) using a standard MultiPuncher (PerkinElmer, Waltham, Mass) and washed twice with 90 μL Purification Solution, followed by washing once with 90 μL of Elution Solution. A total of 24 μL autoclaved sterile water was then added to each well, and the plate was chilled in a −20°C freezer for a minimum 15 minutes before being heated at 99°C for 25 minutes. To assess whether cross-contamination occurred among the different wells in a 96-well plate, a plain filter paper disk was placed into a well after every seventh DBS. TRECs were not detected in any wells containing a blank filter paper disk, indicating a lack of sample cross-contamination (data not shown).

**TABLE I. Sequences of the primers and probes**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREC forward primer</td>
<td>5’-CAC ATC CCT TTC AAC CAT GCT-3’</td>
</tr>
<tr>
<td>TREC reverse primer</td>
<td>5’-GCC AGC TGC AGG GTT TAG G-3’</td>
</tr>
<tr>
<td>β-Actin forward primer</td>
<td>5’-ATT TCC CTC TCA GGC ATG GA-3’</td>
</tr>
<tr>
<td>β-Actin Reverse Primer</td>
<td>5’-CGT CAC ACT TCA TGA TGG AGT TG-3’</td>
</tr>
<tr>
<td>TREC probe</td>
<td>6-FAM-ACA CCT CTG GTT TTT GTA AAG GTG CCC ACT-3’-TAMRA</td>
</tr>
<tr>
<td>β-Actin probe</td>
<td>6-VIC-GTG GCA TTC ACG AAA CTA-3’ TAMRA</td>
</tr>
</tbody>
</table>

**Abbreviations used**

- DBS: Dried blood spot
- HSCT: Hematopoietic stem cell transplantation
- NBS: Newborn screening
- NK: Natural killer cell
- PBMCs: Peripheral blood mononuclear cells
- RT-qPCR: Real-time quantitative PCR
- SCID: Severe combined immunodeficiency
- TREC: T-cell receptor excision circle
- WBC: White blood cell
RT-qPCR

Real-time quantitative PCR for detecting TRECs and β-actin was performed in a total volume of 20 μL containing 1X TaqMan Gene Expression Master Mix (Applied Biosystems), 0.5 μmol/L TREC primers, 0.25 μmol/L β-actin primers, 0.15 μmol/L TaqMan probes, and 0.8 μL 1% BSA (New England Biolabs, Ipswich, Mass). A total of 8 μL DNA extract was used for the TREC assay, and 1 μL was used in the β-actin assay. The DNA sequence of the primers and probes is listed in Table I. The primers and probe for TREC amplification were identical to those previously reported by Douek et al.13 The reactions were carried out on an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems) and underwent 1 cycle of 2 minutes at 50°C, 1 cycle of 5 minutes at 95°C and 40 cycles of 30 seconds at 95°C and 60 seconds at 60°C. For data collection, a fixed cycle threshold (Ct) was set at the point where PCR amplification is still in the exponential phase. The Ct is the number of cycles at which the amplification plot, representing the fluorescence emission of the report dye, passes a fixed threshold. Serially diluted plasmids containing a known copy number of TRECs (kindly provided by Dr Daniel Douek) and β-actin were used to generate a calibration curve; the copy

FIG 1. Representative RT-qPCR TREC assay on DNA samples from a 3.2-mm DBS. A, Arrows indicate some of 10-fold diluted plasmid standards ranging 1,000,000 to 10 copies of TRECs. B, A standard curve of serially diluted plasmids containing a known copy number of TRECs (solid squares) and 82 unknown samples (crosses). A 100-copy TREC standard is embedded with unknown DBS samples.

FIG 2. Results of initial RT-qPCR TREC assay on 5766 NBS cards. A, TREC copy number distribution in 5766 deidentified 3.2-mm DBSs. The mean is 827, and the median is 708. B, The number of samples with a TREC copy number ≤150 per 3.2-mm DBS. Sixty-one samples (1% of the total) have less than 75 TRECs per 3.2-mm DBS.
1.4% of DBS specimens (Table II). The assay is currently being used to screen all newborns in Wisconsin. The population-wide screening will provide a better estimate of the frequency of specimens with undetectable TRECs. We have successfully optimized the method of measuring TRECs from NBS DBSs by enhancing the yield of DNA extraction and increasing the amplification efficiency of the RT-qPCR. In addition, by retesting NBS cards that were low for TRECs on our initial screening test, we were able to reduce markedly the screening positive rate using this protocol. The TREC assay is reliable, reproducible, inexpensive, and easy to use. Importantly, the TREC assay is highly sensitive for detecting abnormally low numbers of TRECs by enhancing the yield of DNA extraction and increasing the amplification efficiency of the RT-qPCR. In addition, by retesting NBS cards that were low for TRECs on our initial screening test, we were able to reduce markedly the screening positive results using this protocol. The TREC assay is highly sensitive and specific for identifying DBSs that did not contain TRECs.

RESULTS

The first step of the SCID assay protocol is to isolate the DNA optimally from 3.2-mm DBSs for RT-qPCR. To accommodate the need for a high-throughput and yet inexpensive procedure for a NBS program, we used commercially available reagents (see Methods) to extract DNA from DBS. DNA extraction methodology and the RT-qPCR for TRECs were initially optimized by using DBS from 4020 deidentified full-term infants (≥37 weeks of gestation, and data not shown). Fig 1 demonstrates an example of a RT-qPCR TREC assay.

Using the DNA extraction methodology and RT-qPCR for TRECs that were optimized, we performed the RT-qPCR assay on 5766 deidentified serial NBS cards obtained over a 4-week collection period. A TREC cutoff level was established at 75 TRECs per 3.2-mm DBS. Fig 2, A shows the distribution of TREC copy numbers in all 5766 NBS specimens. A total of 61 specimens (~1% of the analyzed specimens) had a value below 75 TRECs per 3.2-mm DBS. Ten of those 61 specimens (<0.2%) did not exhibit TRECs in initial RT-qPCR TREC assays (Fig 2, B). All of 61 specimens were retested by using 2 separate 3.2-mm punches from a different area of the DBS specimen card. Sixty of 61 specimens had TRECs greater than the cutoff (75 TRECs per 3.2-mm DBS). Only 1 of the original 5766 specimens did not have TRECs amplified. It should be noted that β-actin amplification was observed on all 10 specimens that did not have detectable TRECs in initial RT-qPCR TREC assays. These results demonstrated that our optimized method of DNA extraction and RT-qPCR for TRECs and retesting protocol markedly reduced the previously reported predicted screening positive rate of 1.4% of DBS specimens (Table II).12 The assay is currently being used to screen all newborns in Wisconsin. The population-wide screening will provide a better estimate of the frequency of specimens that contain undetectable TRECs. The optimized TREC assay must be sensitive and specific for the absence of TRECs in DBS. To assess this, we spotted several NBS cards with whole blood from a recently identified T+B−NK+ patient with SCID lacking detectable T cells. We also spotted NBS cards with blood from a normal control that was selectively depleted of all CD3+CD45RA− T cells (ie, naive T cells) by flow-cytometry cell sorting. NBS cards spotted with blood from an infant with SCID or depleted of naive T cells were then randomly inserted into several routine runs of deidentified normal NBS cards. In all cases, we correctly identified NBS cards spotted with SCID blood or blood depleted of naive T cells. In each case, these NBS cards had no detectable TRECs with normal amplification of the β-actin gene. These data indicate that the TREC assay is highly sensitive and specific in identifying DBSs that did not contain TRECs.

We also determined the linearity of the TREC assay performed on DBSs. To address this issue, pure populations (>90%) of naive (CD45RA bright) and memory (CD45RO bright) T cells were obtained from peripheral blood of normal donors by cell sorting, mixed at varying ratios, and spotted on newborn screening cards for TREC analysis (see Methods). The data presented are the average TREC ± SEM (each ratio cohort represents 4-8 individual TREC analyses).

DISCUSSION

We have successfully optimized the method of measuring TRECs from NBS DBSs by enhancing the yield of DNA extraction and increasing the amplification efficiency of the RT-qPCR. In addition, by retesting NBS cards that were low for TRECs on our initial screening test, we were able to reduce markedly the screening positive results using this protocol. The TREC assay is reliable, reproducible, inexpensive, and easy to use. Importantly, the TREC assay is highly sensitive for detecting abnormally low numbers of TRECs by clearing 99.98% of all specimens using only the first submitted NBS specimen collection cards. The rate of positive TREC assays reported herein (no more than 1 in 5766 NBS cards) was substantially less than the positive rate of 1.4% reported by Chan and Puck.12 The reasons for this discrepancy are not known but could be a result of differences in the method of DNA extraction.

### Table II. Comparison of published data.

<table>
<thead>
<tr>
<th></th>
<th>Chan and Puck</th>
<th>Wisconsin data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine NBS samples</td>
<td>140</td>
<td>5766</td>
</tr>
<tr>
<td>Average TRECs/DBS</td>
<td>510</td>
<td>827</td>
</tr>
<tr>
<td>Median TRECs/DBS</td>
<td>Not provided</td>
<td>708</td>
</tr>
<tr>
<td>Samples without TREC</td>
<td>2 in 140</td>
<td>1 in 5766</td>
</tr>
</tbody>
</table>

**Figure 3.** TREC determination from DBS with varying CD45RA:CD45RO ratios. Pure populations (>90%) of naive (CD45RA bright) and memory (CD45RO bright) T cells were obtained from peripheral blood by cell sorting, mixed at varying ratios, and spotted on newborn screening cards for TREC analysis (see Methods). The data presented are the average TREC ± SEM (each ratio cohort represents 4-8 individual TREC analyses).
extraction from the DBS, differences in the quantity and quality of DNA template used in the RT-qPCR, differences in the age and mode of storage of the NBS card, and our use of BSA in the RT-qPCR. Regardless of the reasons for the different results, we believe that any properly equipped laboratory can replicate the optimized TREC assay with appropriately trained personnel.

In newborns with SCID, all known gene mutations result in a profound defect in T-cell production, and subsequently lead to undetectable or low TRECs in the peripheral blood. Thus, it is most likely that the TREC assay performed on NBS cards will accurately identify infants with SCID regardless of the genetic defect. However, in some cases of SCID, the specific genetic mutation may lead to a protein that has partial activity, such as certain mutations in the recombination activating genes 1 and 2, leading to Omenn syndrome. Such hypomorphic mutations may result in leaky SCID with the production of sufficient naive T cells to be missed by the TREC assay. Infants with SCID and maternally engrafted T cells or Omenn syndrome may also not be profoundly lymphopenic. However, nearly all the T cells in these infants are memory T cells (CD45RO<sup>+</sup>). On the basis of our data provided in Fig 3, we would predict that such infants would be identified by the TREC assay. In addition, there are conditions such as congenital infections that may result in the marked expansion of CD45RO<sup>+</sup> T cells. In these situations, because of a paucity of CD45RA<sup>+</sup> T cells, the number of TRECs in the DBS may be extremely low, leading to a false-positive result. However, our data demonstrate that only 1 in 5766 NBS cards exhibited a TREC number below the cut-off value (<75 TRECs per 3.2-mm DBS). Therefore, we predict that a false-positive TREC assay caused by the expansion of CD45RO<sup>+</sup> T cells will be a rare occurrence when prospective studies are performed. Finally, there are some primary immunodeficiencies that are not SCID but are characterized by decreased numbers of T cells. For example, newborns with DiGeorge syndrome (22q11 deletion) may have sufficient T-cell lymphopenia to be identified by the TREC assay. Thus, we expect that the use of the TREC assay will not be entirely specific for SCID, nor may it be sufficiently sensitive to identify every infant born with SCID.

Although we have markedly improved the TREC assay for NBS, the assay does have some shortcomings that need to be rectified. The disadvantages of the current protocol are that DNA extraction involves multiple manual steps and a separate reference gene amplification. An alternative DNA extraction method and a multiplexing TRECs assay are currently under investigation in our laboratory, with promising results. In addition, we are also working on automating the assay procedure for both DNA extraction and RT-qPCR for TRECs.

We have demonstrated that the TREC assay is highly sensitive and specific for detecting abnormally low numbers of TRECs in DNA extracted from NBS cards. These advantages as well as the low cost ($6.00/sample) make the TREC assay amenable to statewide NBS programs to detect infants with SCID. On the basis of data from the deidentified specimens, the Secretary of the Wisconsin Department of Health and Family Services granted permission for a pilot program to start routine prospective screening (using identified specimens) effective on January 1, 2008.

We thank Dr Daniel Douek for supplying TREC plasmid and DNA sequence information.

This paper is dedicated to the memory of Dr. Ronald Laessig (April 1940-March 2009).

Clinical implications: A sensitive, specific NBS test for SCID is available that will enable NBS programs to facilitate earlier diagnosis and improve treatment of affected infants.

REFERENCES


## Statewide Newborn Screening for Severe T-Cell Lymphopenia

John M. Routes; William J. Grossman; James Verbsky; et al.


http://jama.ama-assn.org/cgi/content/full/302/22/2465

<table>
<thead>
<tr>
<th>Supplementary material</th>
<th>eFigure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><a href="http://jama.ama-assn.org/cgi/content/full/302/22/2465/DC1">http://jama.ama-assn.org/cgi/content/full/302/22/2465/DC1</a></td>
</tr>
</tbody>
</table>

### Correction

Contact me if this article is corrected.

### Citations

Contact me when this article is cited.

### Topic collections

Pediatrics; Neonatology and Infant Care; Screening; Genetics; Genetic Counseling; Testing/Therapy; Immunology; Immunology, Other

Contact me when new articles are published in these topic areas.

---

Subscribe

[http://jama.com/subscribe](http://jama.com/subscribe)

Permissions

permissions@ama-assn.org

[http://pubs.ama-assn.org/misc/permissions.dtl](http://pubs.ama-assn.org/misc/permissions.dtl)

Email Alerts

[http://jamaarchives.com/alerts](http://jamaarchives.com/alerts)

Reprints/E-prints

reprints@ama-assn.org

Downloaded from www.jama.com at Duke University on December 13, 2009
INFANTS WITH SEVERE T-CELL LYMPHOPENIA, including severe combined immunodeficiency (SCID), often appear normal at birth and have no family history of immunodeficiency. Consequently, many infants with severe T-cell deficiencies are not identified until life-threatening infections occur. This is an important issue because the long-term prognosis of infants with SCID and other serious immunodeficiencies is markedly improved if the diagnosis is made early, before the onset of serious infections. For example, hematopoietic stem cell transplantation is more successful when performed in the first 3 months of life. Administration of attenuated vaccines that are recommended in early infancy and which can cause serious infection in infants with T-cell lymphopenia can also be avoided.

Prior studies indicated that the enumeration of T-cell receptor excision circles (TRECs) by real-time quantitative polymerase chain reaction (RT-qPCR) on DNA extracted from routine newborn blood screening (NBS) cards could identify infants with SCID regardless of genetic cause. T-cell receptor excision circles are small, episomal pieces of DNA that are formed during the differentiation of T cells in the thymus as a result of the rearrangement of T-cell receptor genes. One particular TREC, the δRec-ψα TREC, is produced by approximately 70% of all T cells that express the α/β T-cell receptor. Quantitation of the δRec-ψα TREC by RT-qPCR using DNA isolated from peripheral blood serves as a surrogate marker for the number of naive T cells, which are T cells that have not responded to antigen. Therefore, the TREC assay using DNA isolated from NBS cards should identify any infant with profound T-cell lymphopenia and not just infants with SCID.

In a large-scale pilot study using nearly 6000 anonymized, stored NBS cards, we recently reported optimization of the TREC assay on NBS cards and adapted it as a high-throughput assay for potential use statewide as a screen for severe T-cell lymphopenia. In this study, we present data from 2008 to determine whether the TREC assay performed on NBS cards was able to identify infants with T-cell lymphopenia.

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
performed on NBS cards can detect T-cell lymphopenia in newborn infants.

**METHODS**

**Study Population**

All infants born in Wisconsin between January 1 and December 31, 2008, were included in the study. The secretary of the Wisconsin Department of Health and Family Services approved an administrative rule that added screening for SCID and severe T-cell lymphopenia using the TREC assay effective on January 1, 2008. According to Wisconsin state law, because screening for SCID and severe T-cell lymphopenia was part of routine NBS, the screening performed during 2008 was not considered research and no institutional review board approval for the expanded NBS was needed. Prior research to develop and evaluate the TREC assay was conducted under an exemption obtained from the University of Wisconsin–Madison Institutional Review Board pursuant to 45 CFR 46.101. The study protocol and consent procedure performed apart from the NBS for T-cell lymphopenia was approved by the institutional review board of Children's Hospital of Wisconsin. Wisconsin allows parents to opt out of NBS of their infant only for religious reasons. Wisconsin and Massachusetts are the only states to perform such screening. Massachusetts began screening infants in February 2009.

**Algorithm for Use of TREC Assay to Detect T-Cell Lymphopenia in Newborn Infants**

The TREC analysis was performed as previously described. Briefly, NBS cards were obtained, a 3.2-mm disk was punched from the dried blood spot, DNA was extracted, and RT-qPCR of TRECs and β-actin were performed. The TREC values were normalized to μL of blood based on the estimation that each punch contained 3 μL of blood. An algorithm of TREC analysis for NBS for SCID is shown in the eFigure (available at http://www.jama.com).

Using results from our prior study, a cutoff value was established at less than 25 TRECs/μL, representing the lowest 1.1% of all samples tested. This cutoff detected all blinded NBS cards spotted with blood from infants with SCID. All NBS cards with a TREC level of at least 25/μL were considered normal and no further analysis was performed. An NBS card with a TREC value of less than 25/μL led to a second round of analysis for TRECs and β-actin, which were performed with 2 new 3.2-mm punches from the same NBS card. The β-actin evaluation was performed in parallel with repunching to ensure the presence and integrity of the DNA extracted from the NBS card. If the TREC result remained less than 25/μL and the β-actin level was low, an inconclusive report was issued and a new NBS card was requested. If the TREC values remained less than 25/μL and the β-actin levels were normal, confirming DNA template integrity, an abnormal report was issued and the primary care physician was contacted. At this point, the primary care physician could either request a confirmatory flow cytometry screening test to validate the diagnosis of T-cell lymphopenia, which is the option recommended by the NBS program, or obtain a new NBS card for a repeat TREC assay. An abnormal TREC assay on the second NBS card resulted in a confirmatory flow cytometry screening test. All inconclusive TREC assays were repeated with new NBS cards until either a normal or abnormal result was obtained. All screened infants that had an abnormal TREC assay and low numbers of naïve T cells by flow cytometry were referred to a clinical immunologist (J.M.R., W.J.G., or J.V.) for a complete evaluation.

During the initial weeks of the statewide screening program, NBS cards from a few premature infants were found to have low TREC numbers with normal β-actin levels, but T-cell enumeration by flow cytometry failed to demonstrate T-cell lymphopenia. These results were consistent with the previously reported high-false positive rate of NBS tests in the premature infant population. Based on this experience, all preterm infants (<37 weeks’ gestation) with abnormal or inconclusive TREC assays have their TREC levels monitored until the infant reaches the equivalent of 37 weeks’ gestation; then full-term criteria are applied (eFigure). This practice is the standard operating procedure for all premature infants tested in the Wisconsin NBS program.

**Flow Cytometry Evaluation**

A limited confirmatory flow cytometry screening test was developed that enumerated the total number of CD3 T cells, CD3 CD4 T cells, CD3 CD8 T cells, CD3 CD4 CD45RO memory CD4 T cells, CD3 CD8 CD45RO memory CD8 T cells, CD3 CD56 natural killer cells, and CD19 B cells. One to 2 mL of whole blood collected in sodium heparin tubes was stained with antibodies to CD3-FITC, CD4-PE, CD8-PE, and CD3-APC (N901; Beckmann Coulter, Fullerton, California), and analyzed on a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, New Jersey).

**RESULTS**

**Results of NBS TREC Assay**

The overall distribution of the number of TRECs per microliter of whole blood extracted from dried blood spots in 2008 is shown in Figure 1. The mean number of TRECs was 225/μL and the median was 186/μL. Exactly 71,000 infants were screened in Wisconsin in 2008; 64,397 infants (90.7%) were full-term and 6603 infants (9.3%) were preterm (Figure 2). Inconclusive reports were issued on 23 full-term infants (0.032% of all infants tested) and 96 preterm infants (0.135% of all infants tested, 1.46% of premature infants). Thus, the rate of required repeat testing was 0.168%. Of the full-term infants with inconclusive results, 19 infants had normal TREC assays on repeat testing, 2 infants died in the perinatal period, 1 infant was not retested at the parent’s request, and 1 infant had an abnormal TREC test result. Of the 96 preterm infants with inconclusive results, repeat TREC assays were normal in 72 infants, 22 infants died, 1 infant had an abnormal TREC assay at
37 weeks' gestation, and 1 infant received care in a neighboring state and was diagnosed with DiGeorge syndrome.

Twelve full-term infants (0.017% of all infants tested) and 23 preterm infants (0.032% of all infants tested, 0.35% of all preterm infants tested) had abnormal TREC assays on the initial NBS. Of the 23 preterm infants who had an abnormal test result, repeat testing produced a normal test result in 12 infants, 3 infants had a normal lymphocyte enumeration by flow cytometry while still premature, 3 infants had an abnormal result at the equivalent age of 37 weeks' gestation, and 5 infants died.

**Evaluation of Infants With Abnormal TREC Test Results**

In total, 17 infants that reached 37 weeks' gestation had at least 1 abnormal TREC assay (12 full-term infants had abnormal results on the initial screen, 1 full-term infant and 1 premature infant had...
an initial inconclusive test result and had an abnormal result on repeat testing, 3 premature infants had an initial abnormal result that remained abnormal when achieving the equivalent of 37 weeks' gestation (Figure 2). In 4 infants, a repeat TREC assay using a new NBS card was performed and the test result was normal, 1 infant died of causes unrelated to immunodeficiency, and 1 infant was not tested at the parent's request. Therefore, 11 infants at the equivalent of 37 or more weeks' gestation with abnormal TREC assays were analyzed by flow cytometry to determine lymphocyte subset numbers. Three of the infants had normal T-cell numbers on flow cytometric analysis. Flow cytometric analysis of the remaining 8 infants demonstrated T-cell lymphopenia, which can be grouped into known disorders of T cells, disorders of lymphocyte extravasation, and idiopathic T-cell lymphopenia (TABLE).

**Known Disorders of T-Cell Lymphopenia**

Two infants with T-cell lymphopenia determined by flow cytometry were diagnosed with DiGeorge syndrome (22q11.2 microdeletion syndrome), a disorder known to be associated with T-cell lymphopenia (Table). One infant was found to have a high percentage of phenotypically unusual lymphocytes that did not express CD3, TCRαβ, CD4, CD8, CD19, CD34, CD56, CD117, or Flt3 (CD135), but did express CD7, CD16, CD38, and CD45. This infant's peripheral blood was analyzed longitudinally and the atypical cells decreased over time, but the T-cell lymphopenia persisted, likely due to the lack of thymic tissue present in infants with DiGeorge syndrome.

**Disorders Associated With Extravasation of Lymphocytes From the Vascular Space**

Three infants with abnormal TREC analysis had comorbidities that resulted from lymphocyte extravasation from the vascular space (1 infant with a congenital chylothorax, 1 infant with gastroschisis, and 1 infant with meconium aspiration, pulmonary hypertension, and chyloperitoneum) (Table). In all cases, the T-cell lymphopenia resolved with appropriate medical therapy.

**Idiopathic Causes of T-Cell Lymphopenia**

Three infants were found to have idiopathic T-cell lymphopenia (Table). The first infant presented with low numbers of T cells and a high percentage of phenotypically unusual cells within the lymphoid gate, with the same surface markers as those found in the infant with DiGeorge syndrome. Due to the presence of a cleft palate, testing was performed for a 22q11.2 microdeletion and was negative. Apart from a cleft palate, this infant did not have the other characteristic features of DiGeorge syndrome. The population of unusual cells in the lymphocyte gate decreased over time, but unlike the infant with DiGeorge syndrome, there was a concomitant normalization of peripheral T-cell numbers.

The second was a male infant who had very low numbers of CD4 and CD8 T cells but normal numbers of natural killer cells and B cells. There were increased numbers of CD45RO memory T cells, which are T cells that previously responded to antigen. Further immunological evaluation demonstrated

---

**Table. Etiologies of T-Cell Lymphopenia (n = 8 Infants)**

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>TREC, Copies/µL</th>
<th>CD3 (2300-7000/µL)</th>
<th>CD4 (1700-5300/µL)</th>
<th>CD8 (400-700/µL)</th>
<th>CD3RO, % (600-1900/µL)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Known disorders</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DiGeorge syndrome (22q11)</td>
<td>5-19</td>
<td>664</td>
<td>520</td>
<td>144</td>
<td>16</td>
<td>771</td>
</tr>
<tr>
<td>DiGeorge syndrome (22q11)</td>
<td>3-16</td>
<td>1211</td>
<td>936</td>
<td>275</td>
<td>20</td>
<td>1046</td>
</tr>
<tr>
<td><strong>Disorders of lymphocyte extravasation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chylothorax</td>
<td>6-17</td>
<td>887</td>
<td>699</td>
<td>189</td>
<td>15</td>
<td>529</td>
</tr>
<tr>
<td>Chyloperitoneum</td>
<td>5-6</td>
<td>361</td>
<td>250</td>
<td>111</td>
<td>40</td>
<td>403</td>
</tr>
<tr>
<td>Gastroschisis</td>
<td>6-15</td>
<td>1172</td>
<td>1132</td>
<td>566</td>
<td>20</td>
<td>481</td>
</tr>
<tr>
<td><strong>Idiopathic T-cell lymphopenias</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic T-cell lymphopenia</td>
<td>15-23</td>
<td>551</td>
<td>477</td>
<td>73</td>
<td>20</td>
<td>422</td>
</tr>
<tr>
<td>Rac2 mutation</td>
<td>0-4</td>
<td>411</td>
<td>390</td>
<td>22</td>
<td>49</td>
<td>1342</td>
</tr>
</tbody>
</table>

Abbreviation: TREC, T-cell receptor excision circle.

*Note:* Normal ranges for lymphocyte subpopulations shown in parentheses.

1. Negative for 22q11 microdeletion.
normal serum immunoglobulins, normal tetanus and diphtheria responses, normal lymphocyte proliferation to mitogens, negative human immunodeficiency virus PCR, normal adenosine deaminase and purine nucleoside phosphorylase analysis, and normal radiation sensitivity studies. Interestingly, this infant’s older sister, who was born before the institution of the Wisconsin NBS program for T-cell lymphopenia, also exhibited CD4 T-cell lymphopenia with increased numbers of CD4 memory T cells and normal numbers of natural killer cells and B cells. At 18 months, her CD8 T-cell count was elevated and an abnormally high percentage of the CD8 T cells were activated. Similar to his sister, the boy also has developed an increased number of activated CD8 T cells over time. The sister has evidence of immunodysregulation with diffuse mild inflammatory bowel disease and immunodeficiency with treatment-resistant esophagitis and vaginitis due to Candida albicans, recurrent skin abscess formation, recurrent otitis media, pylonephritis, and borderline failure to thrive. The increased numbers of activated CD8 T cells and memory CD4 T cells in the sister is consistent with the clinical picture of immunodysregulation and recent or persistent infections. The sister is currently in preparation for hematopoietic stem cell transplantation. The boy is being closely monitored for evidence of immunodeficiency or immunodysregulation.

The final infant with idiopathic T-cell lymphopenia was a male born at term to nonconsanguineous parents. Lymphocyte subset analysis showed low numbers of B cells and decreased numbers of CD4 and CD8 T cells, but no evidence of increased numbers of activated T cells or memory T cells. The infant was followed up closely by the clinical immunologist because of persistent T-cell lymphopenia. Within the first 8 weeks of life, this infant was admitted for omphalitis and a paratracheal abscess. Evaluation for neutropenia, chronic granulomatous disease, leukocyte adhesion deficiency 1, and leukocyte adhesion deficiency 2 were all negative. Subsequent workup for other etiologies causing a neutrophil defect demonstrated a G→A substitution in exon 3 at codon 57 in the Rac2 gene, resulting in the substitution of Asp57 to Asn(D57N), which is the same mutation of Rac2 in the only other case previously reported. This mutation was not present in the infant’s parents.

**COMMENT**

In this study, we present the results of the first statewide NBS program to our knowledge to detect T-cell lymphopenia by enumerating TRECs by RT-qPCR using DNA extracted from dried blood spots of NBS cards. Only 17 of 64 397 infants older than 37 weeks’ gestation screened by the TREC assay (approximately 0.026%) had TREC values below the cutoff limit (<25 TREC/µL blood). Eleven infants subsequently underwent a confirmatory flow cytometry screening test, which confirmed the presence of T-cell lymphopenia in 8 infants.

Some of the causes of T-cell lymphopenia, such as extravasation of T cells outside the vascular compartment, DiGeorge syndrome, or idiopathic T-cell lymphopenia, were consistent with the immunological findings known to be associated with these disorders. Importantly, 1 of 2 infants with DiGeorge syndrome did not have a heart defect at birth or other stigmata associated with DiGeorge syndrome. The diagnosis was made by a clinical immunologist, who evaluated the infant after flow cytometry confirmed the diagnosis of T-cell lymphopenia.

One case of T-cell lymphopenia identified by the TREC assay was unexpected. The TREC assay identified an infant with a mutation of Rac2, the same mutation in Rac2 as has previously been reported. The protein translated by this mutant form of Rac2 (D57N) functions in a dominant negative manner and inhibits neutrophil migration and the production of superoxide by neutrophils in response to physiological stimuli. Consequently, the clinical manifestations of the immunodeficiency in both cases was predominantly characterized by a defect in neutrophil function with recurrent pyogenic infections. Recent studies demonstrate the importance of Rac1 and Rac2 in T-cell development. Mice deficient in both Rac1 and Rac2 exhibit a severe developmental block in T-cell maturation in the thymus. The mutant D57N Rac2 protein substantially inhibits the normal activity of the highly homologous Rac1 protein. Therefore, we hypothesize that the abnormal function of both Rac1 and Rac2 proteins led to T-cell lymphopenia in this infant. This infant received a cord blood transplantation and is currently engrafted and clinically well at age 12 months. The ability of the TREC assay to identify an infant with a Rac2 mutation and T-cell lymphopenia illustrates the power of this test to detect rare but life-threatening immunodeficiencies apart from SCID.

In comparison with full-term infants, the finding that more premature infants had either abnormal or inconclusive test results was not unexpected and in agreement with a prior study. There are several likely reasons for this result. Frequently, in very low birth weight premature infants, blood for the NBS card is obtained through an intravenous catheter. Although this is an acceptable practice, such blood may be diluted or contain a substance that may inhibit the RT-qPCR, resulting in an abnormal or inconclusive test result. Additionally, the cutoff value for an abnormal TREC assay was based on the distribution curve obtained on full-term infants, which is likely different in premature infants and may vary with gestational age. With additional data on TREC values in premature infants, it may be possible to derive a different cutoff value for premature infants compared with full-term infants. Importantly, the standard operating procedure for all premature infants in the Wisconsin NBS program adequately addresses the problem associated with abnormal or inconclusive TREC test results in premature infants.

©2009 American Medical Association. All rights reserved.
In 2006, a task force evaluated the potential inclusion of SCID in US state NBS programs. Criteria used for inclusion of specific diseases in state NBS programs include: (1) the incidence of the disease should be at least 1:100,000 live births; (2) the disease must result in serious morbidity or mortality; (3) early diagnosis and treatment should significantly improve the prognosis; (4) the disorder must not be readily detected at birth by a routine physical examination; (5) a sensitive, specific, and inexpensive test must exist that uses existing NBS cards; and (6) a confirmatory expensive test must exist that uses existing NBS cards. Based on these and other criteria, the task force concluded that SCID should not be included in US NBS programs at that time. One important factor in the task force's decision was the lack of a sensitive, specific, and practical screening test to detect SCID.

This issue should be revisited in the broader context of NBS screening for profound T-cell lymphopenia and not just limited to SCID. The incidence of primary and secondary immunodeficiencies identified by the TREC assay (8:100,000) exceeds the required incidence of disease to institute screening. Even if one excludes the secondary immunodeficiencies that resolved with time, the TREC assay identified 5 cases of primary T-cell lymphopenia. Furthermore, the TREC assay is relatively inexpensive (approximately $5.50 per assay). Based on an analysis of potential cost-effectiveness of NBS for SCID, the relatively high incidence of T-cell lymphopenia and the low cost of the TREC assay suggest NBS may be cost-effective as well, although a formal cost-effectiveness analysis is needed.

One important limitation of our study is the inability to assess the total number of infants born in Wisconsin with clinically significant T-cell lymphopenia. Without such information, one cannot accurately calculate the true sensitivity and specificity of the TREC assay to detect severe T-cell lymphopenia. The TREC assay did not identify any infants with classic SCID during the first year of testing. However, there were no cases of SCID in Wisconsin during the screening program and the failure to identify an infant with SCID at this point is likely secondary to the relative rarity of the disorder (1-2:100,000) and not the lack of sensitivity of the TREC assay.

In conclusion, the Wisconsin screening program demonstrates the feasibility of the TREC assay performed on NBS cards to identify infants with primary and secondary forms of T-cell lymphopenia.

REFERENCES


14. Additional Information: The eFigure is available at http://www.jama.com.

15. Additional Contributions: We thank Dennis Schauer, BA (Medical College of Wisconsin), Thomas Litsheim, BS, MT (Wisconsin State Laboratory of Hygiene), Michael Cogley, BS (Wisconsin State Laboratory of Hygiene), and David Mickle, BS (Wisconsin State Laboratory of Hygiene), for their technical assistance. These individuals were compensated for their technical contributions in the form of salary from their respective institutions.

16. Author Contributions: Dr Routes had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.


20. Drafting of the manuscript: Routes, Grossman, Verbsky.

21. Critical revision of the manuscript for important intellectual content: Routes, Grossman, Verbsky, Hoffman, Brokopp, Baker.

22. Administrative, technical, or material support: Routes, Verbsky, Laessig, Hoffman, Brokopp, Baker.


24. Financial Disclosures: None reported.

25. Funding/Support: This work was supported by funding from the Children’s Hospital of Wisconsin, the Jeffrey Modell Foundation, the Wisconsin State Laboratory of Hygiene, and the Centers for Disease Control and Prevention (grant U01 EH000365).

26. Role of the Sponsors: The Children’s Hospital of Wisconsin, the Jeffrey Modell Foundation, and the Centers for Disease Control and Prevention did not have a role in the design and conduct of the study; in the collection, management, analysis, or interpretation of the data; or in the preparation, review, or approval of the manuscript.

27. Additional Information: The eFigure is available at http://www.jama.com.

28. Additional Contributions: We thank Dennis Schauer, BA (Medical College of Wisconsin), Thomas Litsheim, BS, MT (Wisconsin State Laboratory of Hygiene), Michael Cogley, BS (Wisconsin State Laboratory of Hygiene), and David Mickle, BS (Wisconsin State Laboratory of Hygiene), for their technical assistance. These individuals were compensated for their technical contributions in the form of salary from their respective institutions.
OCT 29 2009

Mr. and Mrs. Fred Modell
Jeffrey Modell Foundation
747 Third Avenue
New York, New York 10017

Dear Mr. and Mrs. Modell:

Thank you for your letter and for your kind words and good wishes as I undertake the directorship of the National Institutes of Health (NIH). I am enormously honored to have been appointed and feel privileged to be at the helm of this extraordinary agency at a remarkable time in its history.

I certainly agree with you that the early detection of Severe Combined Immune Deficiency (SCID) is very important and commend you and the Foundation for all of the work you have done and are doing to advance the diagnosis and treatment of SCID and other immune deficiency diseases. I am closely following the process underway at the Health Resources and Services Administration (HRSA) to establish the evidence needed to add SCID to the newborn screening uniform panel.

As you know, the NIH has an ex officio role on the advisory committee that makes recommendations in this area, the Secretary’s Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC). We have also been contributing to the development of additional evidence needed to fill current gaps in knowledge. Within the next six months, the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) will begin supporting an expanded pilot study to evaluate the feasibility and efficacy of technologies and protocols related to the newborn screening of SCID. This pilot study will also help to establish the related quality and performance control standards for SCID testing on a larger scale, while contributing to our understanding of the prevalence of SCID and related immunodeficiency disorders. I am aware of your commitment to co-fund the study, and I commend you for devoting Foundation resources to filling this critical knowledge gap and helping ensure that the policy decision will be based on a firm scientific foundation.

The SACHDNC is poised to respond rapidly and will consider additional data from the study just as soon as they emerge. I am hopeful that the evidence gaps will be closed forthwith and that a recommendation to add SCID to the uniform newborn screening panel will quickly follow.

Thank you again for your letter and for the great work you both do in memory of your son and on behalf of all children.

Sincerely yours,

Francis S. Collins, M.D., Ph.D.
Director
Background: Severe combined immunodeficiency (SCID) is a treatable, inherited lack of cellular and humoral immunity caused by diverse mutations in several different genes and leading to death in infancy unless immune reconstitution is provided. Currently no population screening exists for SCID, but early diagnosis would improve outcome.

Objective: Because all patients with SCID make few or no T cells, we asked whether the absence of T-cell receptor excision circles (TRECs), DNA episomes in newly formed T cells, could identify SCID regardless of genotype.

Methods: DNA isolated from dried blood spots was assayed by real-time PCR to quantitate TRECs. Control PCR was performed on a segment of the β-actin gene. After pilot studies with adult and cord blood control subjects, blood from SCID patients was spotted onto filters and tested, followed by screening of actual blood spots from the Maryland Newborn Screening Program. Finally, newborn blood spots were recovered and tested from 2 infants after their diagnosis of SCID.

Results: In contrast to filters from the newborn screening program, which had a mean of 1020 TRECs in two 3-mm punches, samples from 23 infants with SCID had <30 TRECs. The newborn screening filter was retrieved from a state laboratory for one of these infants plus another infant who had died of SCID previously; although both samples had detectable β-actin DNA, neither had TRECs.

Conclusion: TRECs are a stable analyte that can identify T-cell lymphopenia in newborn dried blood spots so that infants with SCID can receive early, life-saving treatment. (J Allergy Clin Immunol 2005;115:391-8.)

Key words: SCID, newborn screening, TREC, T-cell receptor excision circle, T-cell maturation, early intervention, primary immunodeficiency, cost-effectiveness, bone marrow transplant, dried blood spots

Severe combined immunodeficiency (SCID) represents a spectrum of genetic disorders characterized by profound defects in both cellular and humoral immunity.1-4 Infants with SCID die of infections in the first year of life unless immunity is reconstituted by bone marrow transplantation (BMT),5,6 enzyme replacement,7 or, in some recent cases, gene therapy.8-11 There is no current screening program for SCID, which is caused by diverse mutations in any of at least 11 known genes and others not presently known, as summarized in Table I.12-28

Screening newborns for treatable genetic disorders allows early intervention.29,30 After the introduction by Guthrie of filter paper spotted with heel-stick blood to screen for phenylketonuria (PKU),31 all states in the United States test newborns for PKU, hypothyroidism, and galactosemia.29,32 New tests use tandem mass spectrometry to detect many additional metabolic disorders30,33 and DNA analysis to diagnose specific mutations causing cystic fibrosis, hemoglobinopathies, and biotinidase deficiency.34-37 Screening programs are evolving to meet the growing number of conditions that can be identified and treated.29,30,38

A recent report on primary immunodeficiencies from the Centers for Disease Control and Prevention noted that SCID meets many of the accepted criteria for newborn screening.39 SCID is asymptomatic at birth, but it becomes fatal without treatment; effective treatment is available; and early intervention improves outcome. Infants receiving BMT before developing devastating infections have better survival at lower cost than those whose treatment is delayed.5,6,40 Indeed, Myers et al40 reported 95% survival among infants undergoing transplantation in the first month of life compared with only 70% for those treated after 3 months.

Arguments against newborn screening for SCID include its low incidence and the lack of a practical test. The true incidence of SCID is unknown; estimates of
TABLE I. Multiple genotypes of SCID

<table>
<thead>
<tr>
<th>Gene defect</th>
<th>Molecular pathogenesis</th>
<th>% of SCID cases*</th>
<th>Characteristic lymphocyte subpopulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2RG (X-linked)</td>
<td>Failure of IL-2 receptor (common) γ-chain signaling by cytokines IL-2, -4, -7, -9, -15, -21</td>
<td>45-50</td>
<td>T cells Low/absent B cells Present Natural killer cells Absent</td>
</tr>
<tr>
<td>ADA</td>
<td>Adenosine deaminase deficiency</td>
<td>16</td>
<td>T cells Low/absent B cells Absent Natural killer cells</td>
</tr>
<tr>
<td>IL7R</td>
<td>Failure of signaling through IL-7 receptor</td>
<td>9</td>
<td>T cells Low/absent B cells Present Natural killer cells Present</td>
</tr>
<tr>
<td>JAK3</td>
<td>Failure of Janus kinase 3 activation by common γ-chain</td>
<td>6</td>
<td>T cells Low/absent B cells Absent Natural killer cells Absent</td>
</tr>
<tr>
<td>DCLRE1C</td>
<td>Failure of antigen receptor rearrangement</td>
<td>&lt;5</td>
<td>B cells Absent Natural killer cells Present</td>
</tr>
<tr>
<td>RAG1</td>
<td>Failure of antigen receptor rearrangement</td>
<td>&lt;2</td>
<td>Natural killer cells Absent</td>
</tr>
<tr>
<td>RAG2</td>
<td>Failure of antigen receptor rearrangement</td>
<td>&lt;2</td>
<td>Natural killer cells Absent</td>
</tr>
<tr>
<td>CD45</td>
<td>Lack of a cell surface protein tyrosine phosphatase receptor (PTPRC), required for T- and B-cell activation by antigen</td>
<td>Rare</td>
<td>T cells Low/absent B cells Present Natural killer cells Low, maybe variable</td>
</tr>
<tr>
<td>TCRD</td>
<td>T-cell receptor δ chain deficiency</td>
<td>Very rare</td>
<td>T cells Low/absent B cells Present Natural killer cells Present</td>
</tr>
<tr>
<td>LCK</td>
<td>Lack of lymphocyte tyrosine kinase p56lck, required for T-cell development and activation</td>
<td>Very rare</td>
<td>T cells Low/absent B cells Present Natural killer cells Present</td>
</tr>
<tr>
<td>FOXN1</td>
<td>Lack of forkhead box N1transcription factor, required for thymus and hair follicle development (ortholog of nude mouse)</td>
<td>Very rare</td>
<td>T cells Low/absent B cells Present Natural killer cells Present</td>
</tr>
<tr>
<td>Deletion of Chr 22q11</td>
<td>Complete DiGeorge syndrome with thymus aplasia; part of DiGeorge-Velocardiofacial syndrome</td>
<td>Rare</td>
<td>B cells Absent Natural killer cells Variable</td>
</tr>
<tr>
<td>Currently unknown</td>
<td>Unknown, including reticular dysgenesis and congenital anomaly syndromes with SCID27,28</td>
<td>Each one rare</td>
<td>T cells Low/absent B cells Present Natural killer cells Variable</td>
</tr>
</tbody>
</table>

*Based on Buckley,15 Antoine et al,33 and unpublished estimates (J Puck).
†Some patients have substantial numbers of maternally derived T cells at time of diagnosis.
‡T- and B-cell antigen receptor genes include the DNA cross-link repair protein 1C (DCLRE1C, or ARTEMIS) and the recombinase activating genes (RAG).

1/100,000 births39 exclude patients who die before their immunodeficiency is recognized. The incidences of galactosemia and biotinidase deficiency, included in many current screening programs, are comparable at 1/60,000 and 1/80,000, respectively.29,32 An inexpensive, high-throughput screening method for SCID is needed. Such a test should identify SCID caused by defects in known disease genes as well as SCID with unknown genotype. Characteristic lymphocyte phenotypes are associated with the different genotypes of SCID (Table I). Patients with IL2RG and JAK3 defects, for example, can make B cells and are designated T− B− SCID, whereas those with RAG1 defects are T− B+. Natural killer cells are typically absent in patients with IL2RG SCID but present in IL7R SCID. In some patients, regardless of genotype, maternal T cells are present at diagnosis in numbers substantial enough to cause spontaneous graft-versus-host disease. However, a very low number of patient T cells is a feature common to all SCID genotypes. Thus, attributes of infant T cells could provide the basis for a universal SCID screening test.

Normal thymic maturation of T cells requires cutting and splicing of the DNA encoding the T-cell antigen receptor (TCR) genes to produce T cells with diverse antigen specificities. As a byproduct of this recombination, excised DNA forms episomal T-cell receptor excision circles (TRECs). One species, the δRec-μJα TREC, is produced late in maturation by 70% of developing human T cells that express αβ TCRs.41,43 Quantitative PCR amplification across the joined ends of the δRec-μJα TREC reflects the number of recently formed T cells in peripheral blood. We have developed a newborn screening method for SCID based on measurement of TRECs from dried blood spots.

METHODS

Samples

Blood samples from healthy adults and from infants newly diagnosed with SCID were donated with consent under approved research protocols at the National Institutes of Health. SCID molecular diagnosis was performed by sequencing IL2RG in male subjects as described16 and obtaining JAK3 sequence (GeneDx, Rockville, Md) where indicated. Umbilical cord blood was obtained from routine deliveries via the National Disease Research Interchange (Philadelphia, Pa). Simulated blood spots were made by applying 50 μL of blood to the 1.3-cm circles on standard filter paper cards provided by the Maryland State Department of Health and Mental Hygiene (DHMH) (Baltimore, Md). The cards were air-dried and stored desiccated away from light. Actual, outdated newborn blood spots (stored >6 months desiccated at ~20°C), for which screening had been completed, were provided by Dr Susan Panny and colleagues at the Maryland State DHMH after approval by the DHMH Institutional Review Board. Samples had been obtained in routine care nurseries from babies at 1 to 2 days of age or in premature and special care nurseries at 2 to 3 days of age.42 Identifying numbers were removed from the filters.

T-Cell depleted simulated Guthrie cards

To model blood from SCID patients, adult blood was separated, depleted of T cells, and reconstituted as follows: after centrifugation,
the buffy coat was aspirated and spun over Ficoll (MP Biomedicals, Irvine, Calif). The granulocyte/erythrocyte pellet was retained. Mononuclear cells were treated with anti-CD3 coated microbeads and passed through a magnetic separation system (MACS; Miltenyi Biotech, Auburn, Calif). T-cell depleted mononuclear and granulocyte fractions were added back to the platelet-rich plasma and erythrocytes from the original centrifugation. Immunophenotyping showed 1% of the lymphocytes of this mixture were T cells. Aliquots were spotted onto filter circles as above.

**DNA extraction**

Disks 3 mm in diameter were punched out of the dried spots. DNA was extracted by using a Gentra Systems kit (Minneapolis, Minn). Glycogen 1 μL, 20 mg/mL (Invitrogen, Carlsbad, Calif), was added before precipitation. DNA was resuspended in 40 μL H₂O and heated at 65°C for 1 hour. Quality and yield were assessed by spectrophotometry and visualization in agarose gels. Preliminary DNA isolations showed optimal yields from 2 punches, corresponding to about 6 μL of blood.

**Quantitative real-time PCR**

Real-time PCR for δRec-αT TRECs used primer, probe, and control plasmid sequences of Douek et al. Each triplicate reaction of 20 μL contained 4 μL DNA, TaqMan 2× PCR Master Mix (Applied Biosystems, Foster City, Calif), 500 nmol/L forward and reverse primers (F, 5′-cacacctgactggactgct; R, 5′-gcaaggctgtagggtt), and 150 nmol/L probe (FAM-5′-acacctgattgtaaggtgccact-TAMRA). Samples were heated at 95°C for 5 minutes and then given 40 cycles of 95°C for 30 seconds, 60°C for 1 minute (7900HT Prism Sequence Detection System; Applied Biosystems). PCR for β-actin used identical cycles with 1 μL of DNA, 375 nmol/L primers (F, 5′-tcaccacgccgctcccttctac; R, 5′-cagcgaaccgtctactagg), and 250 nmol/L probe (FAM-5′-atggtccggctcctctactgt-TAMRA). TRECs and β-actin copy number were calculated relative to standard curves generated from serially diluted plasmids.

**Statistical analysis**

Relationships between DNA concentrations, TRECs, and β-actin copies were determined by linear regression. Analysis of the variance was used to compare samples from routine versus special care nurseries. A test based on simulating the Kolmogorov-Smirnov statistic, adjusting for having to fit mean and variance, was used to establish whether values were normally distributed.

**RESULTS**

Although simple to collect, dried blood spots vary widely in cellular content and extractable material, as illustrated by the 10-fold variation in DNA yields in our samples from 0.12 to 1.35 μg (mean, 0.46 μg) per 2 punches. Nonetheless, preliminary experiments showed significant positive correlations between TRECs and DNA content; β-actin copies and DNA content; and TRECs and β-actin copies (not shown). Multiple independent DNA preparations and PCR reactions were performed on a series of spots made from cord and adult blood, demonstrating that TREC number was reproducible for a given individual’s sample (Fig 1, A). Mean TREC values for cord and adult blood were 740 and 164, respectively, consistent with published observations that cord blood has more newly formed T cells and TRECs. To assure that adequate DNA for PCR had been extracted, TREC values were normalized to copies of β-actin genomic DNA (Fig 1, B). The mean cord blood TREC/β-actin was 0.0109 (median, 0.0125; range, 0.0028-0.0158; SEM, 0.0014), 4-fold higher than the adult mean of 0.0024 (median, 0.0022; range, 0.0013-0.0042; SEM, 0.0003).

To model SCID blood, an aliquot of adult blood was depleted of T cells, spotted onto filters, and used for 5 DNA extractions and PCR quantitations of TREC and β-actin (Fig 1, C, circles). Two of the T-cell–depleted DNA preparations had no amplification, whereas 3 had minimal amplification, corresponding to <30 copies, the lower limit of detection in standard dilutions.
### Table II. Clinical and molecular data and TREC analysis at time of diagnosis in 23 patients

<table>
<thead>
<tr>
<th>Case code</th>
<th>Age</th>
<th>Sex</th>
<th>Family history</th>
<th>Clinical history</th>
<th>Total lymphocytes</th>
<th>T cells</th>
<th>B cells</th>
<th>NK cells</th>
<th>SCID genotype</th>
<th>TREC genotype</th>
<th>PCR from blood spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>340</td>
<td>2 d</td>
<td>M</td>
<td>Healthy</td>
<td></td>
<td>801</td>
<td>15</td>
<td>136</td>
<td>68</td>
<td>IL2RG</td>
<td></td>
<td>** +</td>
</tr>
<tr>
<td>126</td>
<td>1 wk</td>
<td>M</td>
<td>Healthy</td>
<td></td>
<td>Not available</td>
<td>0</td>
<td>444</td>
<td>3</td>
<td>JAK3</td>
<td></td>
<td>- +</td>
</tr>
<tr>
<td>220</td>
<td>2 wk</td>
<td>M</td>
<td>27-wk gestation, respiratory distress</td>
<td>2820</td>
<td>24</td>
<td>448</td>
<td>NA</td>
<td>IL2RG</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>140</td>
<td>1 mo</td>
<td>M</td>
<td>Healthy</td>
<td></td>
<td>441</td>
<td>9</td>
<td>361</td>
<td>13</td>
<td>IL2RG</td>
<td></td>
<td>- +</td>
</tr>
<tr>
<td>339</td>
<td>3 mo</td>
<td>F</td>
<td>Thrush, pneumonia, adenovirus, FTT† (died in post-BMT period)</td>
<td>1740</td>
<td>0</td>
<td>1672</td>
<td>0</td>
<td>IL2RG</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>344</td>
<td>3 mo</td>
<td>M</td>
<td>Diarrhea, thrush, Parainfluenza 3, sepsis</td>
<td>2780</td>
<td>0</td>
<td>2724</td>
<td>0</td>
<td>IL2RG</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>345A</td>
<td>4 mo</td>
<td>M</td>
<td>FTT, chronic upper respiratory infections, rash</td>
<td>446</td>
<td>101</td>
<td>96</td>
<td>167</td>
<td>Not IL2RG or JAK3</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>345B</td>
<td>4 mo</td>
<td>M</td>
<td>FTT, chronic upper respiratory infections, rash</td>
<td>623</td>
<td>204</td>
<td>146</td>
<td>158</td>
<td>Not IL2RG or JAK3</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>346</td>
<td>4 mo</td>
<td>M</td>
<td>Diarrhea, PIP††</td>
<td>987</td>
<td>0</td>
<td>809</td>
<td>138</td>
<td>Not IL2RG</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>318-2</td>
<td>4 mo</td>
<td>M</td>
<td>Thrush (brother of 318-1)</td>
<td>2160</td>
<td>22</td>
<td>1805</td>
<td>NA</td>
<td>IL2RG</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>336</td>
<td>4 mo</td>
<td>M</td>
<td>Cystitis, pneumonia</td>
<td>395</td>
<td>16</td>
<td>371</td>
<td>0</td>
<td>IL2RG</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>349</td>
<td>4 mo</td>
<td>M</td>
<td>PIP, rash eczema</td>
<td>1210</td>
<td>35</td>
<td>236</td>
<td>0</td>
<td>IL2RG</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>317</td>
<td>6 mo</td>
<td>M</td>
<td>FTT, Rotavirus, PIP</td>
<td>1000</td>
<td>160</td>
<td>800</td>
<td>0</td>
<td>IL2RG</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>348</td>
<td>6 mo</td>
<td>M</td>
<td>FTT, Rotavirus, PIP</td>
<td>1020</td>
<td>10</td>
<td>950</td>
<td>70</td>
<td>AR, not JAK3 Trace§ §</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>352</td>
<td>6 mo</td>
<td>M</td>
<td>PIP</td>
<td>2390</td>
<td>93</td>
<td>2008</td>
<td>NA</td>
<td>AR</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>7 mo</td>
<td>M</td>
<td>PIP</td>
<td>3348</td>
<td>10</td>
<td>3367</td>
<td>34</td>
<td>IL2RG</td>
<td>Trace +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>321</td>
<td>7 mo</td>
<td>M</td>
<td>PIP, absent</td>
<td>396</td>
<td>40</td>
<td>350</td>
<td>0</td>
<td>SCID with radii and thumbs</td>
<td>Trace +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>353</td>
<td>7 mo</td>
<td>F</td>
<td>PIP (died before BMT)</td>
<td>1300</td>
<td>28</td>
<td>1057</td>
<td>285</td>
<td>AR</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>354</td>
<td>7 mo</td>
<td>M</td>
<td>FTT, diarrhea, PIP</td>
<td>1080</td>
<td>0</td>
<td>921</td>
<td>38</td>
<td>Not IL2RG</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>326</td>
<td>8 mo</td>
<td>M</td>
<td>FTT, diarrhea, PIP</td>
<td>710</td>
<td>24</td>
<td>670</td>
<td>14</td>
<td>JAK3</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>342</td>
<td>9 mo</td>
<td>M</td>
<td>FTT, diarrhea, dehydration</td>
<td>2750</td>
<td>82</td>
<td>2336</td>
<td>385</td>
<td>IL2RG</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>318-1</td>
<td>12 mo</td>
<td>M</td>
<td>FTT, diarrhea, CMV, bronchitis</td>
<td>800</td>
<td>260</td>
<td>420</td>
<td>80</td>
<td>IL2RG</td>
<td>Trace +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>355</td>
<td>13 mo</td>
<td>M</td>
<td>Eczema, elevated IgE, HSV, Rotavirus, Adenovirus, pneumonia, sepsis</td>
<td>1000-2448</td>
<td>268 (DR+, RO+)</td>
<td>558</td>
<td>285</td>
<td>Not IL2RG or JAK3</td>
<td></td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>350</td>
<td>3 mo</td>
<td>F</td>
<td>Rash, PIP, mother with incontinentia pigmenti</td>
<td>4550</td>
<td>4573</td>
<td>939</td>
<td>NA</td>
<td>Not SCID; NEMO mutation</td>
<td>596 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>329</td>
<td>5 mo</td>
<td>M</td>
<td>PIP</td>
<td>1400</td>
<td>506</td>
<td>458</td>
<td>60</td>
<td>Not SCID; T cells normal at 1 y</td>
<td>624 +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>343</td>
<td>7 mo</td>
<td>M</td>
<td>FTT, 3 episodes of pneumonia, died after 2 BMT attempts</td>
<td>2226</td>
<td>140</td>
<td>4</td>
<td>171</td>
<td>Not IL2RG</td>
<td></td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Abnormal values are in bold type. Values approaching normal range are underlined.

*Known sibling(s) and/or maternal male uncle(s) with SCID or severe immune compromise in infancy.

†Total white blood counts for the patients ranged from 5,110 to 15,300/μL; total lymphocytes/μL, 10th to 90th percentiles⁴⁷: 0-3 mo, 300-7600/μL; 3-6 mo, 3900-9000/μL; 6-12 mo, 3400-9000/μL; 1-2 y, 3600-8900/μL. Below 2000/μL considered abnormal in infants.⁵,⁶⁴

††0-30th to 90th percentiles for T cells⁴⁷: 0-3 mo, 2500-5500/μL; 3-6 mo, 2500-5600/μL; 6-12 mo, 1900-5900/μL; 1-2 y, 2100-6200/μL.

§§0-30th to 90th percentiles for B cells⁴⁷: 0-3 mo, 300-2000/μL; 3-6 mo, 430-3000/μL; 6-12 mo, 610-2600/μL; 1-2 y, 720-2600/μL.

∥0-30th to 90th percentiles for NK cells⁴⁷: 0-3 mo, 170-1100/μL; 3-6 mo, 170-830/μL; 6-12 mo, 160-950/μL; 1-2 y, 180-920/μL.

*No PCR product detectable.

††Failure to thrive (weight <5% and falling behind on growth curve).

‡‡Pneumonia due to Pneumocystis jiroveci (formerly P carinii).

§§Trace PCR detected below threshold level of 30 TREC/c2 punches.\\n
||DNA from newborn screening filter spot; no other sample available.
Our laboratory receives samples for mutation testing for SCID. In 2002, 34 newly diagnosed infants with confirmed SCID who were born in the US had blood, cell lines, or DNA submitted for mutation diagnosis; 19 (56%), had disease-causing mutations in \textit{IL2RG}. Beginning in 2003, blood submitted in excess of the amount needed for diagnostic studies was spotted onto a filter and dried for TREC analysis. Twenty-three spots were analyzed from newly diagnosed SCID patients, whose characteristics are summarized in Table II. The 3 female and 20 male patients included 2 affected brothers in family 318 and twins in family 345. Four patients were diagnosed in the first month of life, all recognized to be at risk for SCID because of a positive family history. Although one of these infants had respiratory difficulties associated with prematurity, none had infections or failure to thrive as a result of SCID. Three other infants, 339, 318-2, and 336, had known immunodeficiency in siblings or maternal uncles, but they were not investigated by medical providers before onset of infectious complications of SCID. All patients were referred to BMT centers for treatment. Clinical features (including 2 deaths caused by infections with onset before BMT) and lymphocyte phenotypes are indicated. Twelve of the 23 cases (52%) had defects in \textit{IL2RG}.

Duplicate DNA isolations from blood spots of all 23 infants had detectable \(\beta\)-actin. In contrast, 19 had no detectable TREC\s, and 4 had \(<30\) TRECs (Table II and Fig 1, C, triangles). The patients with trace amounts of TREC amplification were 6 months of age or older at diagnosis. Of the latter, patient 348, diagnosed at 6 months, had autosomal recessive T\(^+\)B\(^+\)SCID with some natural killer cells; patients 320 and 321, diagnosed at 7 months, had X-linked SCID and SCID with congenital anomalies, respectively; and patient 318-1, diagnosed at 12 months with X-linked SCID, had a younger affected brother with no detectable TREC\s when diagnosed at 4 months of age.

Blood spots were also made from 2 additional immuno- deficient patients (Table II, 329 and 350) for whom SCID was considered but later ruled out. TREC\s were positive in samples from both patients. Patient 329 had intermittent lymphocytopenia and pneumocystis pneumonia at 5 months, but by 1 year his lymphocytopenia had resolved without a definite diagnosis. Patient 350 and her mother, who had incontinentia pigmenti, were found to have a previously reported, disease-associated missense mutation of the nuclear factor kappa B essential modulator (NEMO), a condition in which naïve T cells are produced normally, but memory T cells are not\(^{49,50}\).

To measure performance of the TREC assay on actual newborn screening spots, we collected an arbitrary number of sequential, outdated, anonymous Guthrie filters being discarded after testing. Enough saturated blood area for our assay was present on 242 filters. Repeated DNA isolation and PCR were done on samples that had either \(<1000\) \(\beta\)-actin copies or \(<30\) TREC\s. Three were rejected for failure to yield DNA that could be amplified for \(\beta\)-actin, leaving 140 cards from routine and 99 from special care nurseries. Mean TREC number for all 239 samples was 1020; the mean (median) ratio of TREC\s to \(\beta\)-actin copies was 0.0168 (0.0120) for samples from routine nurseries and 0.0140 (0.0086) from special care nurseries, comparable to filters spotted in the laboratory from cord blood (Fig 2, A). Two spots from routine and 5 from special care nurseries had no detectable TREC\s, but they had \(\beta\)-actin amplification. Although blood from a SCID patient would have this profile, these results were almost certainly false positives, given our small sample size. The function \(\log[TREC/(\beta\text{-actin})]\) evaluated for all samples with non-zero values of TREC and \(\beta\)-actin was normally distributed (Fig 2, B). Means and ranges for this function did not differ between routine versus special care nurseries, and a 1-way
Basic and clinical immunology

SCID is most often sporadic. Moreover, positive family presence of high numbers of B cells or maternal lymphocyte counts approaching normal values as a result of the treatment of SCID, around 10% of SCID samples had lymphocyte nurseries. Although a pilot series identified most newborns for SCID that could be performed on cord blood locally in nurseries. Double the national average, suggesting that recognition of the time of diagnosis SCID patients lack TRECs. Samples from all of 23 patients with various forms of SCID had <30 TRECs in two 3-mm punches (equivalent to <5 TRECs per µL of circulating blood). One patient with NEMO deficiency and one with an undefined, transient T-cell lymphopenia had normal TRECs. Further testing will establish the range of cellular immunodeficiency phenotypes that can be found on the basis of low numbers of TRECs.

Importantly, the TREC test was abnormal in 2 actual Guthrie cards obtained from newborns with SCID. The clinical course of these affected infants illustrates the benefit of early diagnosis. Patient 126 has never been ill; a previously diagnosed affected older brother triggered presymptomatic diagnosis and led to his successful treatment by early haploidentical T-cell–depleted BMT. However, patient 343 was not diagnosed for 7 months, during which he experienced chronic diarrhea, failure to thrive, and 3 episodes of pneumonia. Despite 2 attempted haploidentical BMTs during long hospitalizations his immune function remained poor, and he died at 2 years of a sudden, overwhelming infection.

As with other screening tests, failure to amplify TRECs would trigger further screening and/or follow-up for definitive diagnostic tests. Our rates of failure to amplify TRECs were 1.5% in routine and 5% in special care nurseries. Failures in samples from infants in special care (9% of infants) may reflect prematurity, stress, infection, or medications. Prospective screening with larger numbers of samples and follow-up of infants with low TRECs.
will reveal both clinical and technical factors that give false positive results as well as identify infants in need of treatment for immunodeficiency.

We thank Rebecca Buckley and Robert Vogt, Jr for helpful discussions; Susan Panny and staff at the Maryland DHMH for providing newborn screening cards; Stacie Anderson, Jolie Carter, Javier Chinen, Joie Davis, Dan Douek, Roxanne Fischer, Amy Hsu, and Martha Kirby for advice and assistance; Nealoden of The EMMES Corporation for help with statistics; and the physicians and genetic counselors who referred patients to our study.

REFERENCES


