Development of a routine newborn screening protocol for severe combined immunodeficiency

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

Background: Severe combined immunodeficiency (SCID) is characterized by the absence of functional T cells and B cells. Without early diagnosis and treatment, infants with SCID die from severe infections within the first year of life.
Objective: To determined the feasibility of detecting SCID in newborns by quantitating T-cell receptor excision circles (TRECs) from dried blood spots (DBSs) on newborn screening (NBS) cards.
Methods: DNA was extracted from DBSs on deidentified NBS cards, and real-time quantitative PCR (RT-qPCR) was used to determine the number of TRECs. Positive controls consisted of DBS from a 1-week-old $T^-B^-NK^+$ patient with SCID and whole blood specimens selectively depleted of naive T cells.
Results: The mean and median numbers of TRECs from 5766 deidentified DBSs were 827 and 708, respectively, per 3.2-mm punch (~3 μL whole blood). Ten samples failed to amplify TRECs on initial analysis; all but 1 demonstrated normal TRECs and β-actin amplification on retesting. No TRECs were detected in either the SCID or naive T-cell–depleted samples, despite the presence of normal levels of β-actin.
Conclusions: The use of RT-qPCR to quantitate TRECs from DNA extracted from newborn DBSs is a highly sensitive and specific screening test for SCID. This assay is currently being used in Wisconsin for routine screening infants for SCID.
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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.[1,2] Routine NBS began in the1960s with a single disorder, phenylketonuria, and grew to a core panel of 29 disorders as recommended by the American College of Medical Genetics.[3] 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."[4] 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.[5]

Severe combined immunodeficiency is a group of disorders caused by more than a dozen single-gene defects.[6,7] 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.[8] Consequently, the true incidence of SCID is unknown, but it is estimated at 1 in 66,000 live births.[9]

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.[6] 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, for 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.[6,8]

From [a]the Wisconsin State Laboratory of Hygiene and [b]the Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison; [c]the Division of Allergy/Clinical Immunology, Department of Pediatrics, Medical College of Wisconsin/Children's Research Institute, Milwaukee; and [d]the Wisconsin Division of Public Health, Madison.

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Reprint requests: Mei W. Baker, MD, University of Wisconsin School of Medicine and Public Health, 465 Henry Mall, Madison, WI 53706. E-mail: mwbaker@wisc.edu.

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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

Because most SCID cases are sporadic without a family history,[8] 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 antigenic markers, and quantitation of T-cell receptor excision circles (TRECs) on dried blood spots (DBSs).[8,10,11] 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.[12]

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 δRec-ψJα TREC, is produced from approximately 70% of all T cells that express the α/β T-cell receptor.[13,14] Seminal studies by Douek et al[13] established a method for the enumeration of the δRec-ψJα 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[12] 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 naive 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 $CD3^+CD45RA^+$ naive 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.

TABLE I. Sequences of the primers and probes

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

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 naive ($CD45RA^{bright}$) and memory ($CD45RO^{bright}$) T lymphocytes were obtained through the use of a 4-laser Aria high-speed cytometer sorter (BD Biosciences). Isolated naive 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).

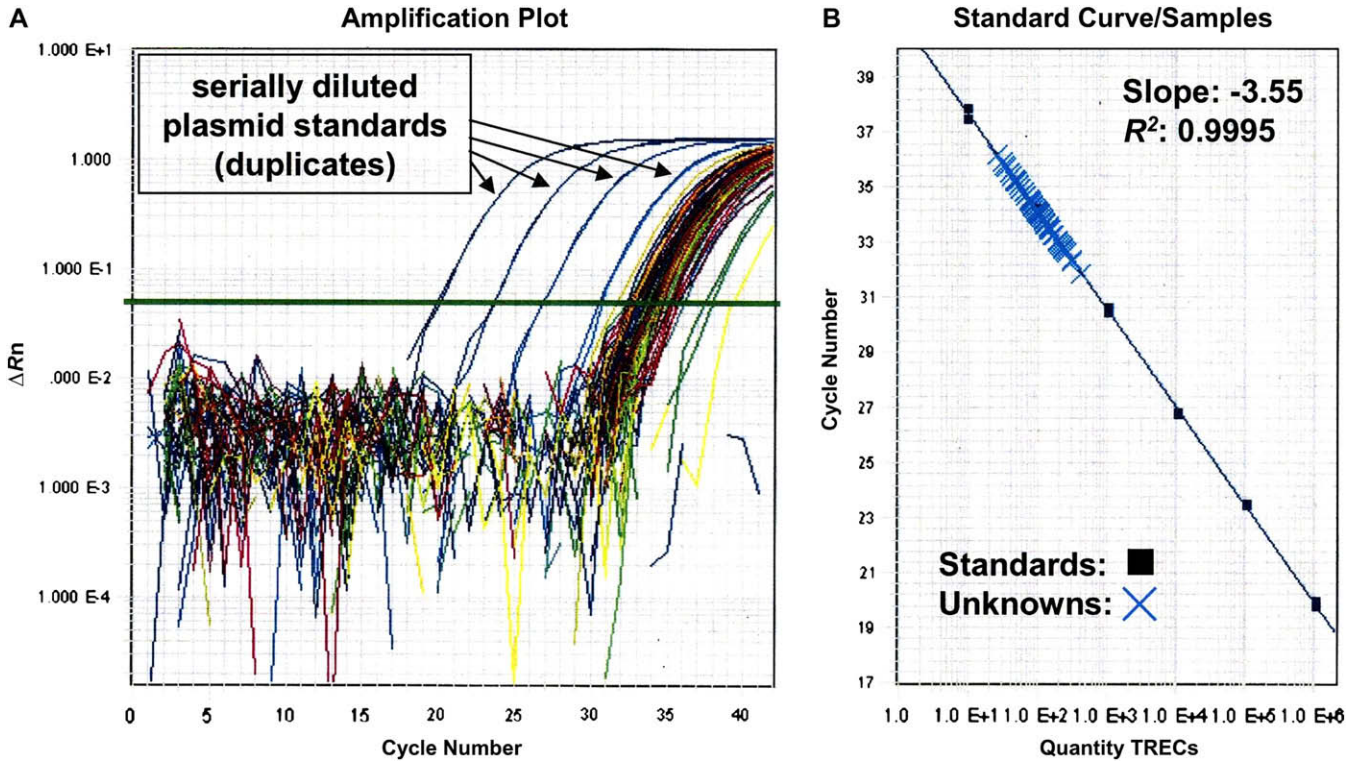

FIG 1. Representative RT-qPCR TREC assay on DNA samples from a 3.2-mm DBS. **A,** *Arrows* indicate some of 10-fold diluted plasmid standards ranging 1,000,000 to 10 copies of TRECs. **B,** A standard curve of serially diluted plasmids containing a known copy number of TRECs *(solid squares)* and 82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

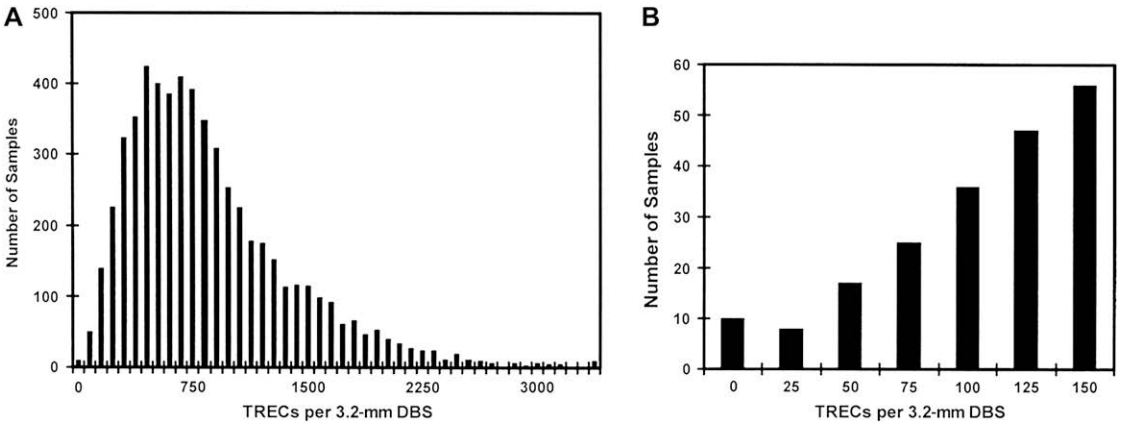

FIG 2. Results of initial RT-qPCR TREC assay on 5766 NBS cards. **A,** TREC copy number distribution in 5766 deidentified 3.2-mm DBSs. The mean is 827, and the median is 708. **B,** The number of samples with a TREC copy number ≤150 per 3.2-mm DBS. Sixty-one samples (1% of the total) have less than 75 TRECs per 3.2-mm DBS.

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 when 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

TABLE II. Comparison of published data.

	Chan and Puck[12]	Wisconsin data
Routine NBS samples	140	5766
Average TRECs/DBS (3 μL whole blood)	510	827
Median TRECs/DBS (3 μL whole blood)	Not provided	708
Samples without TREC amplification	2 in 140	1 in 5766

numbers of TRECs and β-actin were automatically determined based on this curve. PCR amplification of the β-actin gene was used as a reference gene to assess successful DNA extraction. The β-actin gene primers were designed to generate amplicons flanking an intron/exon junction. BSA was an addition in the RT-qPCR reaction mixture to reduce the inhibitory effects of hemoglobin and lactoferrin present in DBS on RT-qPCR.[15]

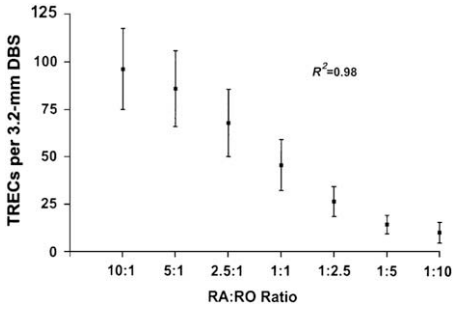

FIG 3. TREC determination from DBS with varying CD45RA:CD45RO ratios. Pure populations (>90%) of naive ($CD45RA^{bright}$) and memory ($CD45RO^{bright}$) T cells were obtained from peripheral blood by cell sorting, mixed at varying ratios, and spotted on newborn screening cards for TREC analysis (see Methods). The data presented are the average TREC ± SEM (each ratio cohort represents 4-8 individual TREC analyses).

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, spotted on NBS cards (see Methods), and analyzed at the Wisconsin State Laboratory of Hygiene for TRECs in a blind manner. TRECs are not replicated during cell division and therefore should be present only in recently emigrated naive T cells. We found that the number of TRECs was directly proportional to the CD45RA:CD45RO ratio, with virtually no TRECs detected at low CD45RA:CD45RO ratios (Fig 3).

For an assay to be used for NBS, the variability of the assay over time must be low. The robustness of the assay is demonstrated by calibration curve data from 75 consecutive TREC assay runs. We found that the correlation coefficient of the standard curves was 0.996 ± 0.004 (2 SD), and the slope of the standard curves was –3.52 ± 0.14 (2 SD; Fig 4). The small SD attests to the reproducibility of the assay. The traditional linearity of the assay is valid, but moot, because the objective of this assay is to identify specimens with low or 0 TRECs.

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

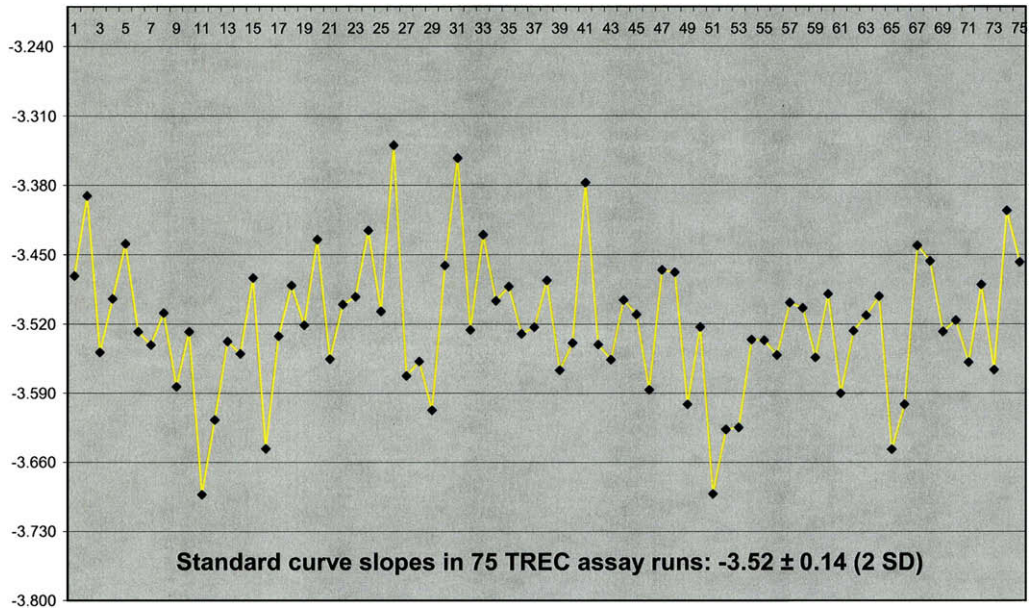

FIG 4. Reproducibility of the TREC assay. The slopes of the standard curves observed from 75 consecutive TREC assay runs. The range of the slopes at –3.52 ± 0.14 indicates consistent amplification efficiency.

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.[16] 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 ($CD45RO^+$). 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 $CD45RO^+$ T cells. In these situations, because of a paucity of $CD45RA^+$ 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 $CD45RO^+$ 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

1. McCabe LL, McCabe ER. Expanded newborn screening: implications for genomic medicine. Annu Rev Med 2008;59:163-75.
2. Wilcken B, Wiley V. Newborn screening. Pathology 2008;40:104-15.
3. Newborn screening: toward a uniform screening panel and system. Genet Med 2006;8(Suppl 1):1S-252S.

4. Lindegren ML, Kobrynski L, Rasmussen SA, Moore CA, Grosse SD, Vanderford ML, et al. Applying public health strategies to primary immunodeficiency diseases: a potential approach to genetic disorders. MMWR Recomm Rep 2004;53:1-29.
5. Lipstein E, Knapp A, Perrin J. Evidence review: severe combined immunodeficiency (SCID), version: 11/12/08. Prepared for: Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children. Available at: http://www.hrsa.gov heritabledisorderscommittee/reports/evidencereviewSCID.htm. Accessed January 28, 2009.
6. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. Annu Rev Immunol 2004;22:625-55.
7. Geha RS, Notarangelo LD, Casanova JL, Chapel H, Conley ME, Fischer A, et al. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. J Allergy Clin Immunol 2007;120:776-94.
8. Puck JM. Population-based newborn screening for severe combined immunodeficiency: steps toward implementation. J Allergy Clin Immunol 2007;120:760-8.
9. Quie PG, Mills EL, Roberts RL, Noya FJD, Stiehm ER. Immunologic disorders in infants and children. WB Saunders; 1996.
10. McGhee SA, Stiehm ER, Cowan M, Krogstad P, McCabe ER. Two-tiered universal newborn screening strategy for severe combined immunodeficiency. Mol Genet Metab 2005;86:427-30.
11. McGhee SA, Stiehm ER, McCabe ER. Potential costs and benefits of newborn screening for severe combined immunodeficiency. J Pediatr 2005;147:603-8.
12. Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. J Allergy Clin Immunol 2005;115:391-8.
13. Douek DC, Vescio RA, Betts MR, Brenchley JM, Hill BJ, Zhang L, et al. Assessment of thymic output in adults after haematopoietic stem-cell transplantation and prediction of T-cell reconstitution. Lancet 2000;355:1875-81.
14. Verschuren MC, Wolvers-Tettero IL, Breit TM, Noordzij J, van Wering ER, van Dongen JJ. Preferential rearrangements of the T cell receptor-delta-deleting elements in human T cells. J Immunol 1997;158:1208-16.
15. Al-Soud WA, Radstrom P. Purification and characterization of PCR-inhibitory components in blood cells. J Clin Microbiol 2001;39:485-93.
16. Villa A, Notarangelo LD, Roifman CM. Omenn syndrome: inflammation in leaky severe combined immunodeficiency. J Allergy Clin Immunol 2008;122:1082-6.