



Newborn screening for severe combined immune deficiency (technical and political aspects)

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Purpose of review

Newborn screening for severe combined immune deficiency (SCID) has been implemented in more than half of the states in the United States. Despite the success of these programs, numerous challenges remain for implementing newborn screening. The present review will focus on technical, programmatic, and political aspects pertinent to newborn screening for SCID.

Recent findings

Recent data from newborn screening in 11 U.S. programs suggest that the birth prevalence of SCID is higher than previous estimates. In addition, a large number of other conditions causing T-cell lymphopenia have been detected. Several European countries have initiated pilot screening for SCID. Significant cost savings for treatment of infants with SCID detected at birth, compared with later in life, has been demonstrated. Published evidence of the favorable cost–benefit ratio for screening supports implementation of universal SCID newborn screening.

Summary

SCID fulfills criteria for a condition that should be included in routine newborn screening. Data presented from multiple newborn screening programs in the United States and Europe have shown that high throughput screening of all newborns is cost-effective. Screening improves early detection of this life-threatening condition and follow-up studies have shown a clear improvement in survival for early treatment.

Keywords

cost analysis, newborn screening, severe combined immune deficiency, T-cell receptor excision circles

INTRODUCTION

Severe combined immune deficiency (SCID) is a life-threatening primary immune deficiency caused by one of more than 15 different genetic defects resulting in an absence of functioning T and B lymphocytes. Infants with SCID are susceptible to frequent and severe viral, bacterial, fungal, and opportunistic infections. Nearly all infants die in the first 2 years of life without treatment by hematopoietic cell transplant (HCT) or gene therapy. Successful immune reconstitution restores T-cell function and sometimes B-cell function as well.

During T-cell development in the thymus, the T-cell receptor (TCR) gene undergoes recombination creating single-strand DNA excision circles or T-cell receptor excision circles (TREC). In 1998, Douek *et al.* [1] described the use of quantitative polymerase chain reaction (qPCR) to measure TREC in DNA from lymphocytes. TREC are considered a marker of newly formed T cells, recent thymic emigrants. TREC copy number per cell is highest in the first few months of life and drops off rapidly afterward.

Immunoglobulin light chain DNA undergoes similar recombination events in the bone marrow and produces kappa recombination excision circles (KREC). Newborn screening (NBS) for KREC, a surrogate marker for B cells, is abnormal in B⁺ SCID and identifies X-linked agammaglobulinemia and some cases of ataxia–telangiectasia, Wiskott–Aldrich syndrome, and Nijmegen breakage syndrome [2,3[–]]. Data on outcomes and cost-effectiveness are not yet available.

A real-time qPCR assay for TREC using dried blood spots (DBS) was adapted for use as a newborn screening test for SCID [4] and then optimized by the NBS program in Wisconsin [5]. TREC testing can

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KEY POINTS

- Publications of experience with SCID NBS from several large programs not only provide evidence that SCID fulfills the Wilson–Jungner criteria for a condition which is suitable for newborn screening, but also show that newborn screening for SCID is cost-effective with a favorable cost : benefit ratio.
- SCID newborn screening using qPCR assays to measure T-cell receptor excision circles, in DNA from newborn dried blood spots, can be integrated into existing newborn screening laboratories. There are currently two types of assays for SCID screening and several public health agencies and newborn screening programs in the United States and Europe are available to assist newborn screening laboratories in implementing SCID screening.
- Collaboration of newborn screening programs with public health agencies and consortium such as PIDTC, USIDNet, and organizations such as ESID is important for creating data collection systems to capture long-term follow-up information. This will facilitate the collection of evidence-based information on treatment outcomes, complications, quality improvement measures, and access to clinical research, which will improve the quality of life and provide opportunities for advancing the care and understanding of patients with SCID.

be performed using a laboratory developed real-time qPCR test, or commercial (Perkin-Elmer, Finland) end-point semiquantitative TREC assay.

Table 1 shows critical steps for developing a screening program. The present review will highlight these steps and some of the barriers to implementing SCID NBS, and measures and resources to address some of these issues.

SEVERE COMBINED IMMUNE DEFICIENCY A PUBLIC HEALTH PROBLEM

All 50 U.S. states perform newborn screening for 1 to 34 conditions. European countries screen routinely for 1 to more than 20 conditions [6]. SCID meets WHO criteria for inclusion in NBS panel : SCID is an important health problem; facilities for diagnosis and treatment are available; effective treatment exists with good agreement on which affected individuals should receive treatment; the natural history of the condition is understood; there is a suitable screening test with a reasonable cost–benefit ratio; and the benefits of screening outweigh the harm [7]. SCID was added to the U.S. Recommended Uniform Screening Panel (RUSP) in 2010 [8]. This does not mandate SCID screening. Each state independently determines which conditions will be included in their NBS panel.

In 2012, Petros [9] suggested expanding the Wilson–Jungner criteria [7] for NBS, including 10 additional items: reducing the patient’s ‘diagnostic odyssey’, providing adequate pretest information, demonstrating minimal harm from a false-positive test, showing positive effects or outcomes, testing may be declined by the parent(s), and adequate public health infrastructure to support and monitor testing, diagnosis, and treatment. NBS for SCID also meets all of these criteria.

Infants with SCID are typically asymptomatic at birth but become symptomatic by 2–6 months of age. Up to 35% of affected infants die at presentation [10]. Data from the Primary Immunodeficiency Treatment Consortium (PIDTC) registry supports the effectiveness of HCT for SCID, showing significant improvement in survival (from 50 to 94%) for infants transplanted before 3.5 months of age [11[■]]. Other therapies exist: enzyme replacement therapy for adenosine deaminase deficiency (ADA) and gene therapy, under investigation for ADA deficiency and interleukin 2 receptor gamma chain deficiency [12,13[■],14,15[■]]. Data from SCID NBS of over 2 million newborns in 11 states showed a birth prevalence of 1:58 000, which is considerably higher than previously reported estimates of 1:100 000 [16[■]]. The RUSP includes conditions that occur more frequently such as cystic fibrosis (incidence of 1:1450 in the Netherlands to 1:2500 in North American Caucasians) [17] and others that are less common such as maple syrup urine disease (incidence of 1:86 800 in Portugal [18] to 1:185 000 worldwide [19]).

As of June 2015, newborns in 32 states in the United States are screened for SCID (Newborn screening translational research network, NBSTRN). At this time, Taiwan and Israel perform SCID NBS on all newborns [20[■],21] and at least 11 countries are conducting pilot studies [2,22[■],23[■],24[■]]. Most other European countries have proposed consideration of SCID NBS to the Ministry of Health in their respective countries. Efforts to adopt universal screening have been hampered by the lack of support for NBS (11 countries currently do not mandate NBS). Economic considerations have proven to be a major barrier to initiating NBS for SCID [25].

Initial steps involve addressing general programmatic issues, including evidence-based review of clinical benefit, feasibility, and analytic validity of the screening test. A recent review [26] suggests that implementation of NBS for SCID necessitates a collaboration between clinical immunologists, experts in the diagnosis and treatment of primary immunodeficiencies, public health laboratories, patient advocacy groups, and policy makers. Given the relative rarity of SCID, clinical immunologists can

Table 1. Considerations for implementing SCID NBS

Process	Stakeholders	Resources
Initiation of plan for SCID NBS: Feasibility analysis Clinical utility Analysis of screening plan Resources Cost:benefit ratio of screening	Health authorities, newborn screening lab, newborn screening program, clinical immunologist, patient advocates/families	Literature review, expert opinion
Select testing methodology Determination of costs Feasibility: lab space, workflow, automated or manual	Health authorities, newborn screening lab Newborn screening lab, immunologist	APHL, EUNENBS, state and other established NBS labs, NBSB-CDC
Establishing TREC assay: Screening algorithm Validation of NBS test Establishment of cutoff values Protocol for confirmatory testing	Newborn screening team, immunologist Newborn screening lab Newborn screening lab Newborn screening team, immunologist, referral immunology lab	APHL, EUNENBS, NBSTRN, NBSB-CDC, various state and other established NBS labs
Quality assurance DBS collection Evaluation of cutoff values Evaluation of test performance (sensitivity, specificity, repeat specimens)	Hospitals, newborn screening program Newborn screening lab, immunologist Newborn screening lab	Proficiency testing samples from NBSB-CDC, sample exchange program with other NBS labs
Reporting Develop reporting formats Designation of recommendations Designation of responsibility	Newborn screening team, immunologist, referral facilities	APHL, expert opinion, NBSB-CDC, NBSTRN
Follow up plan Short-term follow-up Long-term follow-up	Newborn screening program, pediatrician Immunologist, patient advocates/families	Expert opinion, NBSB-CDC, NBSTRN NBSTRN, PIDTC/ESID/USIDNet registries

APHL, American Public Health Laboratory association; ESID, European Society for Immunodeficiencies; EUNENBS, European Network of Experts on Newborn Screening; NBSB-CDC, Newborn screening branch, Centers for Disease Control and Prevention; NBSTRN, Newborn screening translational research network; PIDTC, primary immune deficiency treatment consortium; USIDNet, US immunodeficiency network.

provide essential context regarding the suitability of SCID for screening, the importance of early diagnosis and treatment, and the economic impact of NBS.

TREC ASSAY METHODOLOGY

Several NBS programs have published methods for the TREC assay [2,27–29,30^{***}] and the Clinical Laboratory Standards Institute published guidelines in 2013 [31]. Briefly, DNA is extracted from a 1.5 or 3.2 mm DBS punch. The laboratory developed assay uses DNA eluted into solution, or from a punch left in the PCR well (in-situ or direct PCR), a polymerase, TREC primers, and a probe for the TREC signal joint. A single-plex assay (TREC alone) requires retesting for both TREC and reference gene if the initial test is abnormal. A multiplexed assay measures TREC and the reference gene, either β -actin or RNaseP, as the initial screening test [31]. Reference genes, β -actin or RNaseP are controls for DNA amplification

failure. Specimens without amplification of TREC and reference DNA are considered ‘unsatisfactory’ or ‘inconclusive’.

The C_T or C_q is the number of amplification cycles in which the fluorescence signal reaches the threshold for detection. The TREC copy number is calculated from a calibration curve using serial dilutions of plasmids, or cell lines [32] transfected with a known number of TREC copies. Alternately, normal cutoff values may be established based solely on the C_q of the sample. TREC copy number is considered zero when there is no reportable C_q . Categorical results (screen negative or positive) appear comparable for both reporting methods.

The analytic validity of the TREC test should be evaluated using reference materials (e.g. SCID cases, normals), blank samples, and quality control samples with varying concentrations of TREC. These samples can be used to determine the limits of detection, type 1 error (detection of TREC in a blank), and type 2 errors (failure to detect TREC in a sample

containing TREC). Quality control samples and specimens from sample exchange or proficiency testing are also used to validate an assay. Values for absolute TREC copy number vary between laboratories; therefore, each lab must establish their own cutoff values. Cutoffs are often adjusted based on the rate of positive samples. Excessive false-positive tests adversely affect the cost-effectiveness of the test. Most laboratories refer 0.02–0.04% of samples for confirmatory testing and repeat 0.08–0.13% inconclusive specimens [28,29,30[•]].

The commercially available test kit for TREC qPCR (Perkin Elmer, Waltham, MA, USA) uses a 1.5 mm DBS punch, with similar primers and a fluorescent probe. An elution buffer releases DNA from the DBS. After DNA amplification, the amount of fluorescence from probe hybridization to TREC is measured using time-resolved fluorescence resonance energy transfer (TR-FRET). This assay received approval for marketing by the USA Food and Drug Administration (FDA) and the European CE in 2014. To date, there have been no published double-blind comparisons of results between these two assays.

Utilization of equipment such as automated card punchers, plate washers, and liquid handling devices improves the performance efficiency of this assay, increasing the number of specimens that can be run while reducing the number of hours of labor and the risk of repetitive motion injuries.

The sensitivity of this assay is estimated to be 99.5% [29] with a specificity of 99.98% [33]. TREC qPCR is sensitive, specific, and suitable for use in a high throughput laboratory.

BARRIERS TO TEST PERFORMANCE

The establishment of a molecular laboratory within an existing newborn screening laboratory may be a significant barrier to implementing SCID screening. Assay reagents cost \$3–5 per test, but the cost of equipment (thermocyclers, automated plate washers/liquid handling devices) can be considerable. Implementation of SCID screening has been delayed in some programs pending availability of FDA approved tests. The TREC assay should fit into the established work flow of the NBS laboratory. Modification of the laboratory space may be needed; however, a separate facility or room is not needed; creation of a workspace for unidirectional workflow (from clean to dirty) avoiding DNA contamination is sufficient [31]. Another barrier to implementation may be the need for additional personnel and special training in molecular techniques. Consultation regarding development of the laboratory test is available without cost from the Newborn Screening Branch at the Centers for Disease Control and

Prevention (NBSB-CDC) in Atlanta, Georgia. In addition, grants to assist states in implementing SCID NBS have been available through the CDC and APHL (USA only).

Creation of calibration materials requires laboratory expertise to maintain plasmid preparations or cell lines. Calibrators must be continuously evaluated to ensure that TREC copy number remains constant and contamination by PCR products does not occur. Monitoring of the NBS test method using SCID-like spots may be difficult for a program that has not yet initiated NBS for SCID. DBS reference materials from pools of cord blood with normal TREC copy number, TREC copy number below expected, and samples that fail to amplify genomic DNA can be developed in individual laboratories or proficiency testing samples can be obtained from the NBSB-CDC or the Newborn Screening Quality Assurance Program (NSQAP) once screening has started. In addition, sample exchange programs between states can be used to assess analytic validity.

SCREENING RESULTS AND CONFIRMATION

After confirmation of abnormal TREC screen (repeat assay on initial DBS), the NBS team (coordinators, genetic counselors or nurses, and immunologist) is notified electronically or by fax/phone. The primary care practitioner, or birth hospital, is notified of the result and the need for additional testing. Usually, primary care practitioners are responsible for notifying the parents. They are key partners in teaching parents about the test, diagnosis, and treatment.

Three types of reports are issued: negative (e.g. TREC values are within normal range), positive (e.g. TREC values are below normal, confirmatory testing is recommended), or unsatisfactory/inconclusive (e.g. the TREC result is not valid and repeat NBS specimen is requested). The report of a positive TREC NBS should include a basic description of SCID, the TREC test, the interpretation of the test, and the recommended next steps. These should include measures protecting the infant from infection, avoidance of live vaccines, and the need to obtain blood sample for confirmatory testing as soon as possible [34]. Some programs refer the infant directly to a diagnostic center experienced in the diagnosis and care of patients with SCID for evaluation. This often occurs when no TREC is detected because the likelihood of a SCID diagnosis is very high [29].

Clinicians have an important role in the development of educational materials, including written materials, educational lectures, and other

presentations for primary care providers, birth hospitals, and parents. Several patient advocacy groups (Immune Deficiency Foundation, Jeffrey Modell Foundation, International Patient Organisation Primary Immunodeficiencies) have produced written materials and videos on SCID NBS. In addition, patient advocates have testified before newborn screening advisory boards and state legislatures and been instrumental in adopting NBS for SCID.

Confirmatory testing consists of CBC with differential and lymphocyte subset enumeration of T, B, and Natural Killer cells by flow cytometry. Enumeration of naïve and memory T cells is highly recommended [31,35]. It is critical that the laboratory requires a minimum sample size and reports results directly to the NBS program or immunologist for interpretation. If confirmatory testing is consistent with SCID or another immune defect, diagnostic testing will be performed in a specialty referral center with physical exam and specific immune tests. Evaluation in a specialty referral center is also necessary to diagnose other conditions causing T-cell lymphopenia at birth. A list of these conditions is included in Table 2.

BARRIERS FOR CONFIRMATORY TESTING

Excessive sample repeats will increase program costs and adversely affect the cost : benefit ratio. Test performance should be evaluated after a pilot test and at regular intervals thereafter. Premature infants may have low TREC, but there are no specific recommendations regarding separate cutoffs based on gestational age. If TREC are undetectable, many programs proceed to confirmatory testing [28,30[■]]. Most repeat screening when the infant is at term. Programs should consult with laboratories to determine their expertise in interpreting flow cytometry results in newborns, test cost, and accessibility of testing prior to implementing screening. Many programs utilize a central laboratory, shipping blood samples overnight if necessary. Educational materials are frequently available at no cost to programs either through patient advocacy groups or through the NBSTRN or American Public Health Laboratory association (APHL).

NEWBORN SCREENING FOLLOW-UP

Short-term follow-up includes reporting the number of screen positive or inconclusive results,

Table 2. TREC values at birth for immunodeficiencies and other disorders

Disorder	TREC values	Affected genes
Typical SCID	0 to very low	ADA, CD3D, CD3E, CD3Z, DCLRE1C (Artemis), IL2RG, IL7RA, JAK3, PTPRC, PRKDC, RAG1, RAG2, others
Reticular dysgenesis	0 to very low	AK2
Coronin-1A deficiency	0 to very low	CORO1A
Thymic aplasia (complete DiGeorge syndrome)	0 to very low	22q11.2 deletion, others
Leaky SCID/Omenn syndrome	Below cutoff	ADA, DCLRE1C, IL2RG, IL7RA, JAK3, LIG4, RAG1, RAG2, others
Cartilage hair hypoplasia	Below cutoff (variable)	RMRP
Cobalamin/folate metabolism defects	Below cutoff (variable)	MTHFD1, MTR, SLC46A1
Variant SCID	Below cutoff (variable)	Other defects
Syndromes with defective T cells DiGeorge syndrome [1], Cernunnos-XLF [2], CHARGE [3 [■]] syndrome, Jacobsen [4] syndrome, RAC2 defect [5], dedicator of cytokinesis 8 deficiency [6], Ataxia telangiectasia [7], VACTERL association [8], Barth syndrome [9], TAR syndrome [10], Trisomy 21 [11 [■]], EEC syndrome [12]	Below cutoff (variable)	22q11.2 deletion, 10p- [1], NHEJ1 [2], CHD7 [3 [■]], 11q deletion [4], RAC2 [5], DOCK8 [6], ATM [7], multiple [8], TAZ [9], RBM8A- [10], Chr 21 trisomy [11 [■]], TP63 [12]
Prematurity	Variable TREC	
Secondary T-cell lymphopenia (other) Chyllothorax PIK3CA [13 [■]], CLOVES syndrome [13 [■]], gastrointestinal atresia, gastroschisis, intestinal lymphangiectasia, cardiac surgery, hypoplastic left heart syndrome, neonatal leukemia, other congenital anomalies, some metabolic disorders, idiopathic	Variable TREC	PIK3CA [13 [■]]

Data from [27].

documentation of follow-up actions and results (e.g. confirmatory testing), and disposition (diagnosis, treatment, survival). In the United States, NBS programs use internal data collection systems to allow sharing of data with state public health agencies. Several programs have published short-term outcome data on their SCID NBS experience [28,29,30^{aa},35]. Prior to NBS for SCID, the median age at diagnosis was 4.6 months and at transplant was 6 months, with only 28% having been transplanted before 3.5 months [11^{aa}]. After initiation of NBS in California, median age at diagnosis was 2 weeks with 12 of 14 cases transplanted before 3.5 months of age [29].

Long-term follow-up is not well defined for NBS. A workshop convened by the U.S. Secretary of Health and Human Services Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) proposed data collection for care coordination, including access to care, evidence-based treatment outcomes, continuous quality improvement, and engagement in new knowledge discovery including access to clinical research [36]. At a minimum, data collection on treatment efficacy, complications from treatment, treatment outcomes, complications from underlying disorders, quality of life, and cost-effectiveness should occur. Long-term follow-up is critical for demonstrating program effectiveness and that the benefits of screening outweigh the harm.

BARRIERS TO FOLLOW-UP

Programs need to establish data collection protocols. Some consider that reporting follow-up measures or collecting data in a prospective manner constitutes human subject research and should require informed consent. Many programs consider this to be outside the scope of NBS. Available resources include: national registries through PIDTC, U.S. Immunodeficiency Network (USIDNet), and

European Society for Immunodeficiencies (ESID). PIDTC is limited to patients with SCID undergoing HCT. The USIDNet registry is being amended to collect data on infants with T-cell lymphopenia identified through SCID NBS. The NBSTRN holds monthly meetings of NBS programs to facilitate exchange of information and data collection.

COST CONSIDERATIONS

The justification for SCID NBS has been based on the demonstration of superior medical outcomes for early detection [10,11^{aa},37]. Key variables for estimating the economic impact of screening must include the prevalence of the condition, the sensitivity and specificity of the test, treatment availability and alternatives, the cost of the test (and the confirmatory test), downstream healthcare costs, societal costs, and mortality and morbidity [38]. In the United States, the threshold for cost-effectiveness has been \$50 000 per quality-adjusted life-year [39]. Few NBS programs have published articles detailing their analysis of the incremental cost-effectiveness ratio (ICER) [40^{aa}]. However, several publications demonstrate the cost-effectiveness of adding SCID to the newborn screening panel [33,40^{aa},41^a,42^{aa},43]. The ranges for ICER vary widely depending on the test cost and the treatment cost for infants with SCID identified late (without NBS) compared with those identified by screening (Table 3). For an average test cost of \$4 to \$6, ICER ranges from a negative value, indicating cost savings to a value slightly more than \$50 000.

The clinician understands the natural history of SCID, the appropriate diagnostic tests, and therapy, and differences in outcome for infants with and without screening [42^{aa}]. In this capacity, the clinician can provide valuable information to the newborn screening committee, who will make recommendations to governmental policy makers.

Table 3. Incremental cost-effectiveness ratios for SCID NBS in the USA and Europe

Source: country/year	Test cost	Treatment cost – early	Treatment cost – late	ICER
France – 2015 [38]	€4.69–6.79 ^a	€86 179 (59 014–272 577)	€195 778 (165 884–257 160)	\$22 960/QALY ^c
USA – 2014 [36]	\$16.67 ^b vs. \$4.50	\$365 785 ^c (\$126 000)	\$1.43 million ^c (\$494 000)	~\$38 000 vs. ~\$16 900
UK – 2014 [18]	\$7.08 ^d	\$100 000 ^d	\$250 000 ^d	~\$22 270 ^d
USA – 2014 [37]	\$4.25 (mean)	\$120 000	\$ 2 million (approx.)	Not reported
USA – 2011 [29]	\$ 4.22	\$120 000	\$360 000	\$27 907/QALY

ICER, incremental cost-effectiveness ratio; QALY quality-adjusted life-year.
^aTest cost per sample and cost of secondary screen for 0.02% positive results.
^bIncludes staff, equipment, supplies, referral contracts, test cost alone = \$4.50.
^cCharges (costs).
^dEstimated based on published costs.

Patient and family organizations can be instrumental in supporting implementation of SCID NBS. Disease-specific advocacy groups have advocated NBS for SCID and in many cases provided funds to initiate screening or create patient and family educational material. Representatives from SCID family support groups have related their stories to local NBS advisory boards, the lay press, and national groups.

BARRIERS TO COST CONSIDERATIONS

The budget of many NBS programs encompasses testing for all conditions on their panel. Adding a new condition may require approval of the state (United States) or federal (Europe) government. Approval may not mean allocation of additional funds, so programs may need to apply for grant funding for screening. Resources for proficiency testing materials, educational materials, and consultation regarding technical considerations are available without charge.

CONCLUSION

The cost-effectiveness of SCID NBS is well documented. Federal, public health, and other professional societies have recommended adding SCID to routine NBS. Although some barriers have delayed implementation of SCID NBS, there are considerable resources available to assist newborn screening programs in their efforts to initiate SCID NBS. It is hoped that the evidence in this review will assist programs in securing support and addressing impediments to national implementation.

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Conflicts of interest

There are no conflicts of interest.

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- of outstanding interest

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A total of 106 391 newborns were screened between May 1, 2010, and December 31, 2011, in Taiwan. Five newborns were referred for confirmatory testing. Two had SCID, two had persistent T-cell lymphopenia and a third patient was found to have SCID after the study period. All three patients received stem cell transplantation. This is the first report of SCID NBS in Taiwan.

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Over 5000 anonymous dried blood spots and 18 SCID DBS were tested with the commercially available EnLite assay to measure TREC and β -actin. All 18 SCID samples were identified by the assay. The presumptive positive rate ranged from 0.04 to 1.0% of samples tested depending on the TREC cutoff values. This is the first published study demonstrating the performance of the EnLite assay on newborn DBS.

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