Successful newborn screening for SCID in the Navajo Nation

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Navajo;
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SCID;
TREC

Abstract  Newborn screening (NBS) for severe combined immunodeficiency (SCID) identifies affected infants before the onset of life-threatening infections, permitting optimal treatment. Navajo Native Americans have a founder mutation in the DNA repair enzyme Artemis, resulting in frequent Artemis SCID (SCID-A). A pilot study at 2 Navajo hospitals assessed the feasibility of SCID NBS in this population. Dried blood spots from 1800 infants were assayed by PCR for T-cell receptor excision circles (TRECs), a biomarker for naïve T cells. Starting in February 2012, TRECs testing transitioned to standard care throughout the Navajo Area Indian Health Service, and a total of 7900 infants were screened through July 2014. One infant had low TRECs and was diagnosed with non-SCID T lymphopenia, while 4 had undetectable TRECs due to SCID-A, all of whom were referred for hematopoietic cell transplantation. This report establishes the incidence of SCID-A and demonstrates effectiveness of TREC NBS in the Navajo.

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Abbreviations: DBS, dried blood spots; dsDNA, double strand DNA; GVHD, graft vs. host disease; HCT, hematopoietic cell transplant; NBS, newborn screening; SCID, severe combined immunodeficiency; SCID-A, Artemis deficient SCID; TCL, T cell lymphopenia; TCR, T cell receptor; TREC, T-cell receptor excision circle.
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1. Introduction
Severe combined immunodeficiency (SCID) encompasses a group of genetic disorders characterized by a profound deficiency in both humoral and cell-mediated immunity [1]. SCID occurs in about 1/50,000 births, as assessed by unbiased, population based newborn screening [2–4]. Infants with SCID lack T cells and most have non-functional B cells, but 15–30% lack both T and B cells while retaining normal natural killer
(NK) cells (T-B-NK+ phenotype) [5, 6]. Most genetic mutations causing T-B-NK+ SCID disrupt antigen receptor recombination, required for development of a normal, diverse repertoire of T and B cells. In addition to lymphocyte restricted recombinase activating genes RAG1 and RAG2, DNA repair proteins expressed in all cells participate in antigen receptor rejoining; defects in these proteins can result in SCID as well as sensitivity to ionizing radiation and alkylating agents. The most common cause of radiosensitive SCID is mutation of DCLRE1C, the gene encoding the protein Artemis [7, 8].

Over 3 decades ago, a high incidence of TB-NK+ SCID, estimated at 1/2000 births, was reported in Navajo and Apache Native Americans from the southwestern U.S. [9], and in 2002 the cause was discovered to be a Y192X truncation mutation in DCLRE1C [10]. This autosomal recessive trait was presumably present in survivors of the Long Walk in 1864, from Fort Defiance, Arizona, to Fort Sumner, New Mexico, a forced relocation by the U.S. government [11]. The ensuing Navajo population reduction and subsequent recovery may have increased the SCID allele frequency [12]. Although awareness of SCID led to carrier testing and prenatal diagnosis for SCID in some families with previously affected infants [13], the nonspecific nature of presenting diarrhea and failure to thrive often delayed diagnosis. Early recognition of SCID is crucial for avoiding life-threatening infections that usually occur within the first 6 months of life. In contrast, definitive treatment by hematopoietic cell transplantation (HCT) leads to excellent survival in infants with SCID identified before the onset of infections [6].

The advent of a newborn screening (NBS) test for SCID presented an opportunity to implement population-based detection of affected Navajo infants before onset of infections. T-cell receptor excision circles (TRECs), circular byproducts of TCR V(D)J recombination, serve as a biomarker for newly formed naive T cells [14]. Low or absent TRECs in DNA isolated from dried blood spots (DBS) already universally collected indicate low numbers of circulating naive T cells regardless of the underlying genetic basis [15]. This is important because SCID from etiologies other than a DCLRE1C mutation has occurred among the Navajo [16].

Infants born at 2 hospitals in Chinle and Tuba City, Arizona, were invited to participate in a pilot study of TRECs test implementation, beginning in March 2009. After successful completion of this study, SCID NBS was transitioned to standard clinical care throughout the Navajo Nation in February 2012, and is currently ongoing. We present the introduction of SCID NBS and outcomes of screening 7900 infants through July 2014, including successful detection of 4 infants with SCID-A and one with T cell lymphopenia (TCL).

2. Patients, materials and methods

2.1. Navajo pilot study

All research was approved by the Navajo Nation Human Research Review Board (NNHRRB) and the University of California San Francisco (UCSF) Committee on Human Research. An information pamphlet was approved for distribution in prenatal clinics and given to postpartum mothers (see Supp text). Navajo study workers provided information to mothers, obtained face-to-face written informed consent, logged and tracked samples, and encoded samples to maintain confidentiality. Following routine heel-stick for standard NBS, an extra DBS was obtained on a "SCID TEST" blotter (ID Biological Systems, Greenville, SC). SCID blotters were couriered weekly to UCSF, and tested within a week of arrival. Residual study DBS material was destroyed after analysis, with documentation provided to the NNHRRB.

DNA was isolated robotically (AutoGenPrep 965, AutoGen, Inc.) using 96-well deep plates (Corning, Sigma Aldrich) from 3.2 mm punches from control cord blood and study DBS [15]. After 16 h at 65 °C with protease K (0.5 mg/mL), samples underwent organic extraction, DNA precipitation, 2 washes with 70% ethanol, and suspension in TE buffer 50 μL. Quantitative PCR was performed using 5 μL DNA in 20 μL reactions containing 1X Taqman Gene Expression Master Mix (Applied Biosystems, Life Technologies), 0.5 μM TREC or 0.25 μM β-Actin primers, 150 μM FAM-TAMRA labeled probe (Supp Table 1) and 0.04% BSA (New England Biolabs) [14, 15]. PCR conditions were 2 min at 50 °C, 5 min at 95 °C, followed by 40 cycles (30 s at 95 °C, 60 s at 60 °C) (7900HT Real-Time PCR System, Applied Biosystems). Serial dilutions of plasmids encoding TREC and β-Actin gene sequences (1 × 10^13 - 12.5 and 1 × 10^7 - 100 copies/reaction, respectively) and a plasmid dilution (1000 copies of each ampolon) were included on each plate. In addition to local quality monitoring, proficiency testing was performed (through the Newborn Screening and Molecular Biology Branch, Center for Disease Control Prevention, Atlanta, GA).

Samples with ≥100 TREC/punch (equivalent to 33 TRECs/μL of blood) were considered normal based on analysis of anonymous DBS from the California Department of Public Health Genetic Disease Screening Program (kindly supplied by Fred Lorye, PhD), while those with fewer TRECs had a second punch analyzed for TRECs and β-Actin copies (Supp Fig. 1A). Samples with 2 poor PCR results or low TRECs with normal β-Actin were reported to the study workers at enrolling hospitals, enabling local physicians to contact infants as needed for clinical evaluation.

2.2. TREC screening as standard of care

In February 2012, TREC testing for SCID became part of routine clinical NBS for infants born in the Navajo Nation, reflecting the recommendations of the U.S. DHHS Secretary in 2010 [17] as well as the Navajo Area Indian Health Service Pediatric Advisory Board. Clinical TREC testing was performed at PerkinElmer Genetics, Inc. using a testing algorithm similar to the study procedure above (Supp Fig. 1B) [18].

2.3. Statistics

Confidence intervals (1-sided) were derived from inversion of the cumulative binomial distribution.

3. Results

3.1. Pilot study

TREC newborn screening was initiated at Tuba City Regional Health Care Corporation and Chinle Comprehensive Health
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Care Facility, serving a large area with a mostly rural Native American population. During the enrollment period, 2931 infants were born at the hospitals, of whom 61% had TREC screening completed; 2796 (95%) of the infants' mothers were visited by a Navajo study coordinator. Reasons that mothers were not interviewed included maternal or infant medical complications, infant out-of-hospital transfers for conditions requiring intensive care, and discharges prior to 24 h. Consent from 1837 mothers was obtained (66% of those interviewed), and 1800 "SCID TEST" samples were collected and tested. Mothers who did not enroll their infants for SCID NBS cited a range of reasons (Table 1). Despite incomplete enrollment, the feasibility of integrating the TREC test into regular infant care in this setting was demonstrated.

In the first summer of the study a decline in mean TREC number occurred in samples from both sites between May (398 and 422 TRECs/μL for Tuba City and Chinle, respectively) and June (185 and 203, respectively) (Supp Fig. 2). Although no samples during June had TRECs below the normal cutoff, a procedural review was conducted. PCR artifacts were excluded based on standard curves and controls that accompanied every assay. Further investigation revealed that in the Arizona desert summer, evaporative coolers were used where the DBS samples were being stored. To reduce effects of humidity, likely to degrade DBS DNA quality based on observations from other NBS tests (Fred Lorey, personal communication), the study procedure was modified to require sealing each sample in a Mylar bag containing desiccant discs. With this change the TREC copy numbers returned to baseline by July (Supp Fig. 2), exhibiting no further variation.

Of the 1800 initial DBS samples, 1787 (99.3%) had normal TRECs (Supp Fig. 1A). At the study hospitals, a second DBS was obtained routinely at each infant's 2-week pediatric visit, allowing for collection of repeat TREC samples where required. Eleven infants with initial inconclusive DBS had a second DBS showing normal TRECs. Two infants were initial positive cases with low TRECs and normal β-Actin; one infant was withdrawn from the study because the parents did not want repeat TREC testing. The other initial positive case (Patient 1, Table 2) had TCL by flow cytometry (discussed in Section 3.3).

Table 1 Reasons giving for declining to enroll infant in SCID screening study (959 of 2796 mothers interviewed).

<table>
<thead>
<tr>
<th>Reason</th>
<th>Proportion of mothers endorsing</th>
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<tbody>
<tr>
<td>Mother is not interested.</td>
<td>25%</td>
</tr>
<tr>
<td>Baby looks healthy, so test not required.</td>
<td>23%</td>
</tr>
<tr>
<td>Mother needs more time to consider it.</td>
<td>10%</td>
</tr>
<tr>
<td>If test is voluntary, mother does not want it.</td>
<td>9%</td>
</tr>
<tr>
<td>Enrolling would be against traditional beliefs.</td>
<td>7%</td>
</tr>
<tr>
<td>Baby has had enough testing.</td>
<td>4%</td>
</tr>
<tr>
<td>Father or other family members are against it.</td>
<td>3%</td>
</tr>
<tr>
<td>Mother is not Navajo.</td>
<td>3%</td>
</tr>
<tr>
<td>(Uncommon reasons, each)*</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>(No reason recorded)</td>
<td>11%</td>
</tr>
</tbody>
</table>

* Examples: prior baby already tested, privacy concerns, does not think SCID is a serious problem.

3.2. Expansion to standard care

In February 2012, SCID NBS was rolled out as part of the routine NBS at all Navajo Nation maternity hospitals in Arizona and New Mexico. DBS samples for TREC testing were collected and stored according to established study protocol and sent weekly to PerkinElmer Genetics, Inc. for SCID screening. Results were returned to designated local pediatricians and also reported to the senior pediatric consultant in Tuba City (Supp Fig. 1B). By June 2012, all infants born at participating hospitals and present at 24 h of age were screened, as were infants born elsewhere but enrolling for care by 6 weeks of age at Navajo Reservation clinics; none of 6100 DBS samples screened were inconclusive, but 4 infants had undetectable TRECs and proved to have SCID-A. A summary of the first 17 months of standard care screening with a single SCID-A case in 3498 infants (Pt 2 here) was included in a publication on SCID NBS in 11 programs [4]. This current, comprehensive report reaches back to include findings from the pilot (1800 infants) as well as 13 more recent months of screening 2602 previously unpublished infants, including 3 more with SCID-A.

3.3. Infants with low TRECs

Five infants, 1/1580 births (95% CI 1/870–3300) were identified as abnormal, one with low TRECs and TCL associated with congenital anomalies during the pilot phase and 4 with undetectable TRECs and mutation-proven SCID-A during standard care screening (Table 2). All 5 infants presented with low absolute lymphocyte counts (ALC) and no visible thymus tissue on initial chest radiographs. SCID-A patients received treatment at UCSF.

Pt 1: this term female had 11 TRECs/μL blood, with normal β-Actin on NBS (Table 2). Family history was negative. In addition to lack of a thymic shadow, chest radiogram showed thoracic scoliosis with incomplete fusion of T7 and a T10 hemi-vertebra. She developed neonatal tetany due to primary hypoparathyroidism, responding to calcium supplementation. Flow cytometry showed only 632 CD3 T cells/μL (normal >2500/μL), but proliferation to PHA was normal, as was the diverse T cell repertoire demonstrated by spectrotyping. A chromosome copy number array, fluorescent in-situ hybridization for DiGeorge syndrome and TBX1 gene sequence were normal. Live rotavirus vaccination was given without sequelae, and routine killed vaccines elicited robust antibody responses at 7 months. Despite persistent TCL she has experienced no severe infections.

Pt 2 (listed in [4]): this term female had undetectable TRECs with normal β-Actin on NBS; immune studies were consistent with T-B-NK+ SCID (Table 2). Although no family history of SCID was recorded before the NBS result, upon review 2 distant cousins had succumbed to SCID, one with complications following attempted HCT and the other with an infection before HCT could be performed. Physical examination at 4 weeks was normal except for an oral ulcer, a previously described non-infectious characteristic of SCID-A [19]. At 2 months she received a haploidentical, CD34 selected, T cell depleted peripheral HCT from her mother without pre-conditioning, followed by a stem cell boost at 7 months. She developed mild graft vs. host disease (GVHD), autoimmune
hemolytic anemia and thrombocytopenia, all resolving with steroids. Her CD3 T cell count was 304/μL at 20 months of age, with normal proliferation to PHA. She is healthy, but remains B-lymphopenic, and requires gammaglobulin support.

Pt 3: this male infant born at 36 weeks' gestation had two NBS (repeated for preterm birth) with undetectable TREC, but normal β-Actin, and absent T and B cells (Table 2). He was healthy at 4 weeks of age except for an oral ulcer that interfered with feeding. Review of the family history revealed a deceased older sibling who had experienced recurrent pneumonias with fatality at age 6 months, but for whom no immunologic studies were done. At 5 weeks he received a haploidentical, CD34 selected, T cell depleted peripheral HCT from his mother without pre-conditioning. His course was complicated by infection with HHV6 and refractory GVHD treated with sirolimus, prednisolone, basiliximab and methylprednisolone. Because of persistent T-lymphopenia, he received a second maternal HCT at 13 months with reduced-intensity conditioning chemotherapy. He continued to have further complications including veno-occlusive disease, ongoing GVHD, HHV6, pulmonary infiltration causing respiratory distress and eventual demise at 15 months.

Pt 4: this term female was discharged after birth without SCID NBS collected and failed to return for this test until 2 months of age. At this visit, a TREC test was obtained, but 2-month vaccinations were also given, including live attenuated rotavirus oral vaccine. Within two days, her NBS showed undetectable TREC. At her local hospital, she had loose stools that tested positive for rotavirus antigen, and further studies confirmed SCID-A (Table 2). At 3 months she received a haploidentical, CD34 selected, T cell depleted peripheral HCT from her mother without pre-conditioning, with a stem cell boost at 5 months. She has developed donor CD3 T cell engraftment at 8 months, but remains on immunoglobulin infusions.

Pt 5: this healthy term female had undetectable TREC with normal β-Actin on NBS, undetectable T or B cells, and normal NK cells (Table 2). At 8 weeks, following serotherapy with rabbit anti-thymocyte globulin, she received a haploidentical, CD34 selected, T cell depleted peripheral HCT from her mother. Due to failure to engraff a second HCT with a matched unrelated donor was performed at age 5 months.

### 4. Discussion

This report describes the successful implementation of newborn screening for SCID in the Navajo Nation, and emphasizes the importance of mandatory SCID NBS in this population with increased frequency of SCID due to the founder mutation in Artemis. The initial pilot study with informed consent was undertaken to establish whether SCID NBS could be accomplished in this rural population with its
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Distinctive traditions and values. A face-to-face written consent process was mandated during the study phase to assure that participants were fully informed and knew that participation in research was voluntary [20,21]. Only two-thirds of new mothers agreed to enroll their infant, with refusals reflecting attitudes shared across cultures [22] as well as deriving from traditional values (Table 1); nonetheless sufficient enrollment occurred to demonstrate DBS sample collection, TREC testing and follow-up could be integrated into pediatric care, provided DBS samples were protected from humidity. Based on the pilot study, together with the known high risk for SCID among the Navajo, the health benefits of screening were judged sufficiently important that this test was adopted as standard care. The high specificity of the TREC test throughout both phases reported here, with 0.2% indeterminate results, is comparable to other SCID NBS programs [2–4]; repeat DBS testing where needed was facilitated by the existing practice of obtaining a routine second DBS sample for all infants during the first month of life.

The benefit of this public health measure to the Navajo people has been realized, with detection of absent TREC in 4 infants leading to otherwise unsuspected diagnoses of SCID-A. TREC screening in one infant was delayed, resulting in exposure to live rotavirus vaccine that caused diarrheal illness [23], emphasizing the importance of complete implementation and physician awareness to avoid infectious exposures in infants whose screening tests have not been reported as normal [23]. The remaining 3 SCID infants were diagnosed early and protected from infectious exposures with isolation, prophylactic antibiotics, and prompt referral for HCT. Given the known DNA repair defect of SCID-A, DNA damaging chemotherapy and radiation were minimized. Unfortunately persistent HHV6 in Pt 3 was associated with prolonged lymphopenia and refractory GVHD, ultimately leading to his demise.

One case of non-SCID TCL and associated anomalies was also found by NBS; Pt 1 received prompt follow up, avoided serious infections, and remained healthy at 2.5 years of age.

NBS has confirmed that the incidence of SCID-A in the Navajo Nation is very close to the original estimate of 1 per 2000 births, nearly 30-fold higher than in the general population [4]. In contrast to screening programs in many states [2–4], most abnormal TREC screens in Navajo infants, 1/1580 births, are due to SCID because the incidence of SCID-A is high and that preterm and ill infants, a major source of abnormal TREC tests and TCL in other screening programs, are transported to high level care beyond the Navajo Nation before newborn screening tests are sent. Continuation and expansion of SCID NBS is particularly important in areas where Navajo and Apache infants at high risk for SCID are born, but universal implementation will afford early detection for all forms of SCID and other TCL conditions to all infants.

5. Conclusion

In the Navajo population, where a founder mutation causes frequent SCID, benefit from SCID NBS has been demonstrated, with affected infants receiving prompt diagnosis and early referral for definitive treatment. The benefit of SCID NBS in this high-risk population demonstrates the importance of early detection and supports extending SCID NBS to newborns everywhere.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was made possible through collaboration with the Navajo Nation Institutional Review Board, Beverly Becenti-Pigman, LL.D., Chair. Drs. Robert Vogt and Harry Hannon provided encouragement and helpful suggestions and initiated the Center for Disease Control and Prevention TREC Proficiency Testing Program. Dr. Fred Lorey provided helpful suggestions and access to materials for standardization. The authors thank all of the study staff and physicians who provided invaluable assistance at the Navajo Area maternity centers, and Yanning Wang for expert technical assistance with TREC samples. Bill Slimak and colleagues at PerkinElmer donated clinical TREC testing to introduce SCID newborn screening in the Navajo Nation. Preliminary versions of the pilot results were presented at the Navajo Nation Research Conference, Window Rock, AZ, 2011.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcim.2015.02.015.

References

Appendix A.

Supplementary text: Approved information pamphlet distributed in medical facilities participating in the pilot SCID newborn screening study:

**SCID TESTING STUDY**
Approved by Navajo Nation Tribal IRB and UCSF CHR, DATE 2009.

**Voluntary Newborn Screening for Severe Combined Immunodeficiency (SCID).**

Babies can look very healthy at birth and still have a serious disease. That is why all babies are tested for certain conditions that cannot be seen, but need to be treated. The tests are done by a heel-stick, with drops of blood being collected on a paper blotter.

The **SCID Testing Study** is trying out a new blood-drop test that is not yet part of routine newborn screening everywhere, but is used in Wisconsin and Massachusetts. The study is being done by Dr. Puck and Dr. Cowan of the University of California in San Francisco, Dr. Hu of the Tuba City Regional Health Care Corporation. The new test is to find babies who may not be able to fight infections. The problem these babies have is called Severe Combined Immune Deficiency, or SCID. These babies need treatment right away.

You and your new baby are invited to join the **SCID Testing Study**. This study is only for babies whose parents give permission. A study worker will tell you about the study and ask if you wish to join. Before you decide you may talk it over with your family and your doctor.

*Why is this study for Navajos?* SCID is a rare problem that can happen in babies all over the world, but Navajo and Apache Indians have a rate of SCID that is 20 times higher than the general population. About one in every 2,000 Navajo infants may be born with SCID. SCID is treated by giving the baby a transplant of blood-forming cells from a healthy person. The healthy cells enable the baby to fight infections. This treatment has to be done as early as possible. If not treated, infants with SCID do not survive.

Sometimes a brother, sister or other relative has been diagnosed with SCID, making families and doctors know to look for SCID in a new baby by doing special tests. In many cases, however, babies are not diagnosed quickly
because they seem normal until they get infections or fail to gain weight. Unfortunately, by this time treatment is more difficult and chances for a full cure are not as good. The **SCID Testing Study** is to learn whether our new test will be a good way to check all newborns for SCID.

**How does the SCID test work?** In healthy blood, cells called “T-cells” handle the infections all people are exposed to. When T-cells are forming, “T-cell Receptor Excision Circles” also called “TRECs” are produced. Babies with SCID can't make T-cells, so they do not have TRECs. The new test looks for TRECs. If a baby has low or absent TRECs, further testing for SCID is needed.

**What will happen in the SCID Testing Study?** At the same time as routine newborn screening, approximately 2 extra drops of blood will be collected on a paper blotter for the **SCID Testing Study**. No additional heel-sticks are needed. The filter will be sent to Dr. Puck’s laboratory at the University of California, San Francisco, for TREC testing. To protect your privacy, no names will be on any samples. Samples will have code numbers, and the key to the code will be kept in a locked office on the Navajo Reservation.

If the test shows a normal number of TRECs (as expected for 98% of babies or more), you will not hear anything more about it.

If the test shows low TRECs, or the test does not come out well, Dr. Hu in Tuba City will notify the study team and the care provider for that baby. If this happens, a further test will find out whether there was just a problem with the TREC test or if the baby’s T-cells are low.

Any baby with abnormal follow-up tests will be referred immediately to an immunology specialist. If SCID is confirmed, early treatment will be arranged.

**Does this study have risks?** Getting a drop of blood from a baby’s heel briefly causes pain like getting stuck with a pin. However, the research blood sample is taken at the same time as the routine test, with no additional heel-stick.

Being in a study may involve loss of privacy. We will protect your family’s privacy by keeping information about your baby as confidential as possible. All samples have code numbers, not names. Files are kept on the Reservation in locked drawers or secure computers. No names will be used in any publications.

**Does the study offer benefits?** This is a study to learn about a new SCID test. There may be no direct benefit to you or your baby. It is possible that if a baby
has SCID, being in the study will provide an earlier diagnosis, which in turn could lead to a better outcome.

The information gained from this study may benefit other Navajo and Apache Indians, and babies across the country if an effective newborn screening test for SCID can be developed. We hope that information gained from this study will help identify every child, of every nationality, who has SCID.

*What if I do not want my baby in the study?* Taking part in this study is voluntary. You may not want to take part. If you do not join, neither you nor your baby will lose any rights to medical care or services. Also, after joining, you may change your mind and leave the study by contacting Dr. Hu or the study team.

*Other Questions?* If you have more questions, please ask the study team to help you contact a study leader.

<table>
<thead>
<tr>
<th><strong>Table A.1</strong>: Sequences of TREC and β-Actin primers and FAM-TAMRA probes used for real-time PCR.</th>
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<tbody>
<tr>
<td><strong>TREC Forward</strong></td>
</tr>
<tr>
<td><strong>TREC Reverse</strong></td>
</tr>
<tr>
<td><strong>TREC Probe</strong></td>
</tr>
<tr>
<td><strong>β-Actin Forward</strong></td>
</tr>
<tr>
<td><strong>β-Actin Reverse</strong></td>
</tr>
<tr>
<td><strong>β-Actin Probe</strong></td>
</tr>
</tbody>
</table>
A. Research

TREC Screening

Fig. A.2: Algorithms for testing and reporting results from dried blood spots (DBS), showing full blood in the DBS.

Kwan et al., Supp Figure A.1A.

Total numbers of samples: B, standard of care testing performed by PerkinElmer Genetics, Inc (numbers of total samples; B, standards of care); C, SCID Research Pilot Phase with 1800 numbers and % of samples in parentheses at each stage. A, SCID Research Pilot Phase with 1800 numbers and % of samples in parentheses at each stage.
Kwon et al. Suppl. Figure A2

Reversed in July, when the protocol was modified to keep samples in desiccated, sealed bags.

Mean TREC number in samples from both Chimal (diamonds) and Turba City (circles) in June, 2009, was

**Figure A.2:** Seasonal variation in pilot study TREC copy numbers due to humidity artifact. A significant dip in

**Month 2009**

August | July | June | May | April

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![Graph showing TREC numbers across months with significance levels denoted.](image-url)