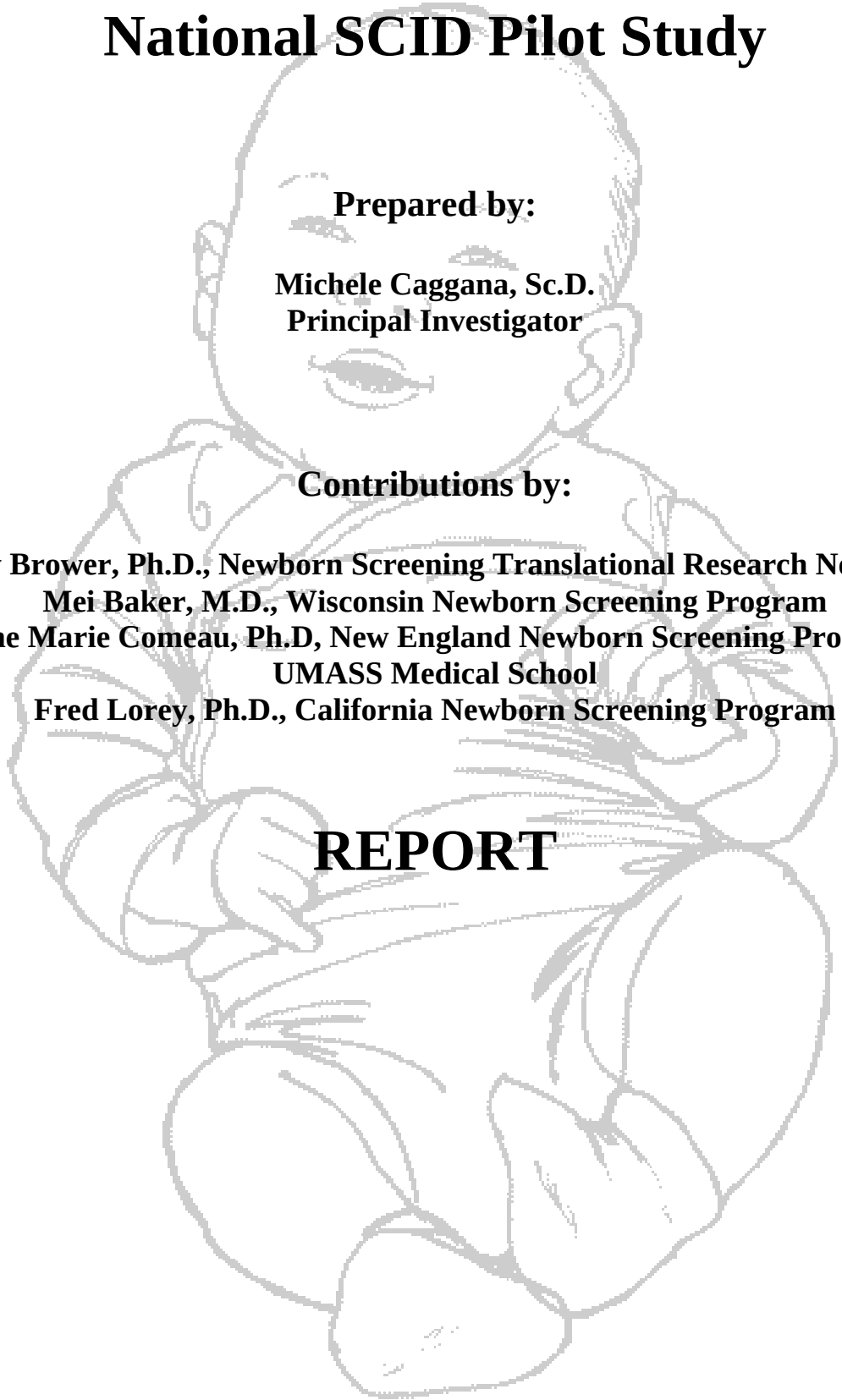
National SCID Pilot Study



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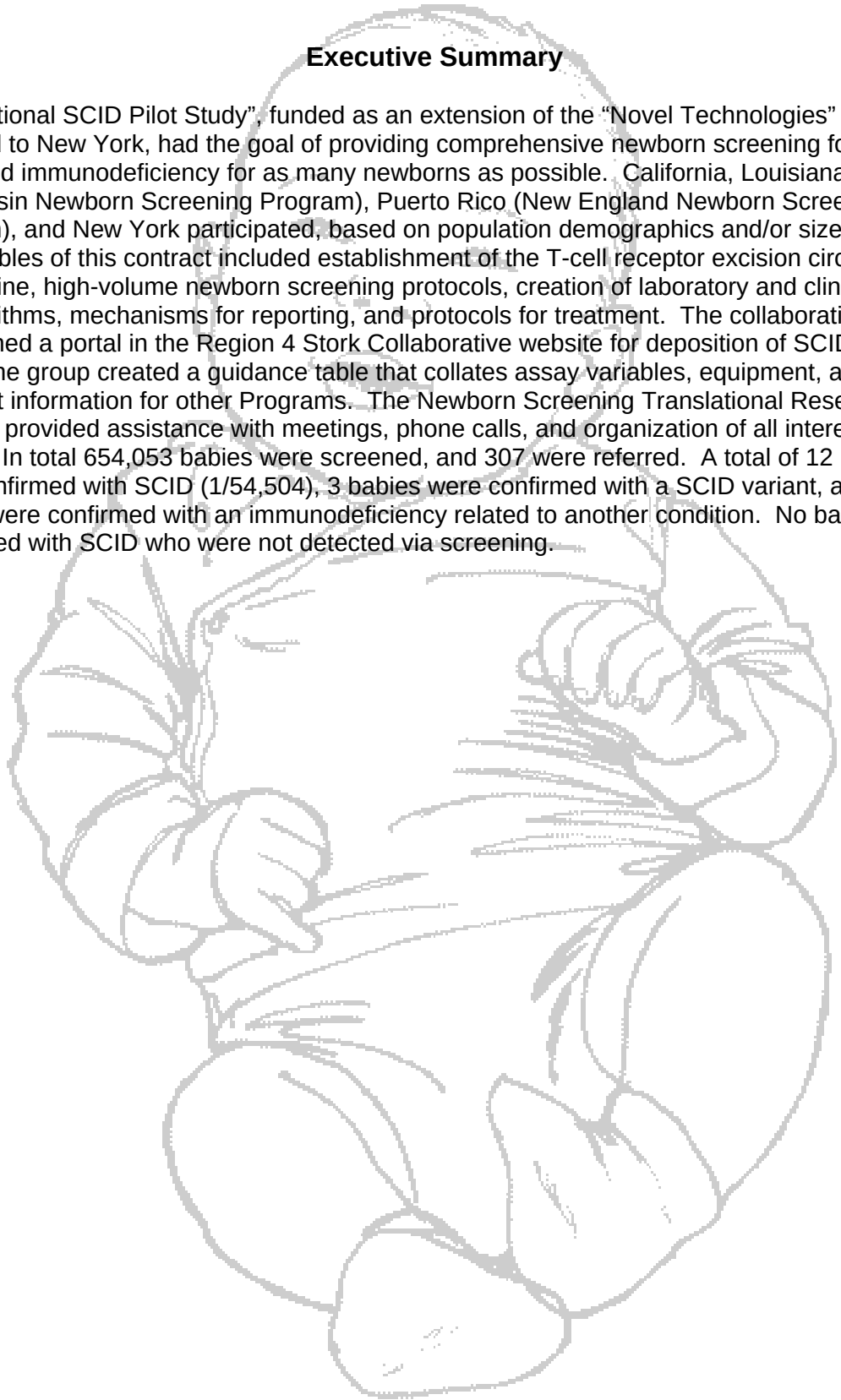
REPORT

Executive Summary

The “National SCID Pilot Study”, funded as an extension of the “Novel Technologies” contract awarded to New York, had the goal of providing comprehensive newborn screening for severe combined immunodeficiency for as many newborns as possible. California, Louisiana (Wisconsin Newborn Screening Program), Puerto Rico (New England Newborn Screening Program), and New York participated, based on population demographics and/or size. Deliverables of this contract included establishment of the T-cell receptor excision circle assay into routine, high-volume newborn screening protocols, creation of laboratory and clinical follow-up algorithms, mechanisms for reporting, and protocols for treatment. The collaborative established a portal in the Region 4 Stork Collaborative website for deposition of SCID cases. Lastly, the group created a guidance table that collates assay variables, equipment, and other pertinent information for other Programs. The Newborn Screening Translational Research Network provided assistance with meetings, phone calls, and organization of all interested parties. In total 654,053 babies were screened, and 307 were referred. A total of 12 babies were confirmed with SCID (1/54,504), 3 babies were confirmed with a SCID variant, and 38 babies were confirmed with an immunodeficiency related to another condition. No babies were diagnosed with SCID who were not detected via screening.



Basis for this Report

The purpose of this report is to summarize work completed under the contract, "National SCID Pilot Study", which was funded by the National Institute of Child Health and Human Development beginning October 1, 2010. A history of the evolution of newborn screening for severe combined immunodeficiency (SCID) is described below.

In September 2007, SCID was nominated to the Secretary's Advisory Committee on Heritable Disorders in Newborns and Children [SACHDNC] for addition to the previously recommended uniform newborn screening panel. An evidence review was undertaken and the evidence report was discussed by SACHDNC in February 2009. At that time, the SACHDNC voted against recommending SCID screening. The Committee agreed the following should be addressed prior to inclusion of SCID in screening panels:

1. The prospective identification of at least one confirmed case of SCID in the United States through a population based newborn screening program;
2. A demonstrated willingness and capacity of additional states to implement newborn screening for SCID;
3. Studies to show reproducibility of the screening test and continuance of a false positive rate of <0.1%; and
4. The creation of a laboratory proficiency testing program through the Centers for Disease Control & Prevention's Newborn Screening Quality Assurance Program.

The following winter, in January 2010, the nomination of SCID was again brought to SACHDNC. At this time, the data obtained from the SCID NBS pilot studies that had been awarded to Massachusetts and Wisconsin by the CDC were reviewed. The high throughput SCID newborn screening already underway in these two states addressed the previously identified evidence gaps. SACHDNC voted to recommend to the Secretary of the Department of Health and Human Services [HHS] the addition of SCID and related T-cell lymphocyte deficiencies to the recommended panel and to the list of secondary targets, respectively (1), with the understanding that the following activities would take place in a timely manner:

1. The National Institutes of Health [NIH] shall fund surveillance activities to determine health outcomes of affected newborns with any T-cell lymphocyte deficiency receiving treatment as a result of prospective newborn screening;
2. The Health Resources and Services Administration [HRSA] shall fund the development of appropriate education and training materials for families and public health and health care professionals relevant to the screening and treatment of SCID and related T-cell lymphocyte deficiencies.
3. The CDC shall develop and distribute to performing laboratories suitable dried blood spot specimens for quality control and quality assurance purposes.

In May 2010, the Secretary adopted the recommendation to add SCID as a core condition to the recommended uniform screening panel, and related T-cell lymphocyte deficiencies to the list of secondary targets (2). The purpose of this report is to summarize work completed under the contract "National SCID Pilot Study", which was funded by the National Institute of Child Health and Human Development beginning October 1, 2010 in response to the SACHDNC's request to have the NIH fund surveillance activities and health outcomes.

Background

Immunodeficiency disorders are characterized by the lack of a functioning immune system. Babies born with SCID may appear healthy but are extremely vulnerable to infection, especially as maternal antibody protection wanes. Exposure to common infections and live vaccines is life-threatening. SCID leads to death in infancy unless treatment, usually stem cell transplantation, is provided (3, 4). Mutations in the DNA sequence of more than 13 different genes can cause SCID or a form of combined immunodeficiency, and in most cases these occur in a newborn with no family history of SCID. Since SCID is unlikely to be apparent at birth and early recognition is essential for lifesaving treatment, SCID has been recognized as a candidate for newborn bloodspot screening for many years (5). However, no laboratory test for detecting SCID on newborn bloodspots was available until the current testing platform for screening for SCID was developed and validated for population-based screening by NIH in 2005 (6). This screening test detects the presence of a by-product obtained during the development of an important part of a functioning immune system, the T cell. Patients with SCID have few or no T cells and the absence of this by-product, T-cell receptor excision circles (TRECs), identifies SCID regardless of the underlying genetic defect or DNA variation. The TREC test utilizes molecular methods to count the number of TRECs present in DNA obtained from dried blood spots.

SCID Newborn Screening Pilot Studies

In 2007 scientists in Wisconsin (State Laboratory of Hygiene and Medical College of Wisconsin) and the New England Newborn Screening Program of the University of Massachusetts Medical School both developed high throughput TREC assays to screen births in Wisconsin and Massachusetts on a trial basis (7-12). In 2008, a partnership among the Wisconsin State Laboratory of Hygiene, Children's Hospital of Wisconsin and the Jeffrey Modell Foundation led to the first pilot study screening all births in a state. Federal funding from CDC was then made available to continue the pilot study in Wisconsin and to initiate a second state-wide pilot in Massachusetts beginning in January, 2009. These two CDC-funded pilots are scheduled to conclude in October 2011. A third CDC-funded pilot study at the University of California at San Francisco study began in 2009 and is screening up to 2000 births at two Arizona hospitals on the Navajo reservation. The Navajo Nation has a high incidence of SCID, approximately 1 in 2,000.

The pilot studies in Wisconsin and Massachusetts generated screening and follow-up algorithms, created educational materials for families and health care providers, hosted multiple state programs for training in the assay and partnered with CDC in the development of proficiency materials that are now available to all state NBS programs (8, 10, 12). As described above investigators from these three pilots presented their findings to SACHDNC in January, 2010 and, at the time, had successfully screened over 200,000 newborns. Although no cases of classic SCID [total failure of immune system] were found, they did identify infants with immunodeficiency disorders [SCID variant, partial failure of immune system] that required medical intervention, documented the feasibility of screening for SCID, provided valuable information to SACHDNC, and paved the way for larger efforts (9, 11).

Purpose of this Report: Efforts to Increase the Number of Newborns Screened for SCID via a National Pilot Study

NIH initiated a pilot project in October 2010 through Health Research, Inc. (HRI), a not-for-profit corporation affiliated with the New York State Department of Health in order to maximize the number of infants screened for SCID. This project brought together collaborators in California and New York to accelerate their ability to screen for SCID, based on their large and diverse birth populations [CA ~510,000 births; NY ~250,000]. Inclusion of these two states established TREC screening to be compatible in a high-throughput, automated environment in addition to the high-throughput screening already underway in Massachusetts and Wisconsin funded by the CDC.

An additional key component of the study was establishment of a model for regionalization. The New England Screening Program presently screens for SCID in Massachusetts only, but these efforts were expanded under this contract. Wisconsin agreed to screen specimens for the state of Louisiana, and Massachusetts agreed to screen specimens from Puerto Rico. Thus an additional state and territory contributed to the number of newborns screened, regionalization was accomplished, and the diversity of newborns expanded with the inclusion of Puerto Rico.

The main NIH-funded research priorities for this project were to:

- Assess the TREC screening technology for SCID
- Establish immediate confirmatory tests and procedures for out of range results
- Ensure capacity and resources for tracking positive cases and arranging for appropriate follow-up care and referral in a timely manner
- Verify administrative structures necessary for a prospective pilot testing of SCID including the ability to obtain approval for human subjects research

The experience of Wisconsin and Massachusetts greatly enhanced these efforts as screening of their own populations has been active for 3+ years.

Statement of Work for the National SCID Pilot Study

The statement of work included the 12 deliverables. Below, each deliverable is shown *in italics* and the explanation follows. Appendices 1 to 12 have information that expands on information provided in this report.

1. Develop a conceptualized prototype technology that demonstrates a sound underlying technologic foundation. The Contractor must develop the entire technology platform or components of a platform.

The TREC assay is a lab-developed test that all states have either developed in house (MA, NY, WI) or with the help of Perkin Elmer Inc. (CA). In general, the TREC assay detects the δRec-ψJα signal joint (6, 13-15). This TREC is utilized because it is produced late in maturation, and 70% of the developing human T cells that have alpha/beta T-cell receptors have this TREC. The assays currently used depend on the quantitative detection of TRECs coupled with a control amplicon (actin or Rnase P), which is either subsequently or co-amplified in the TREC assay.

New York Validation

Validation of the assay in New York included method development, and submission to the Clinical Laboratory Evaluation Program. The Standard Operating Procedure and a summary of the validation is submitted as Appendices 1 and 2. In brief, >6,700 specimens were analyzed in an anonymized fashion as part of the daily specimen load. Another 4,000 were tested in smaller batches. The mean TREC value at that time was 3,911 TRECs/μL whole blood, and the

median was 2,975 TRECs/μL whole blood. A cut-off of 200 was established, and our Specialists wanted all of these to be referrals to flow cytometry in the beginning. Thus the number of babies New York sent to flow cytometry was higher than the other Programs. This practice typically occurs in newborn screening programs. After experience and data gathering, algorithms are further optimized. The NY algorithm was changed in January 2011 and our referral rate has dropped off substantially. During the validation, we also performed inter- and intra-assay reproducibility studies. In addition, we received 8 specimens from the Wisconsin newborn screening program, courtesy of Dr. Mei Baker, and 9 from Dr. Rebecca Buckley. Except for one sample, in which we highly suspected a sample labeling error, all of the immunodeficiencies were called correctly. In addition, we had a specimen from a child who died at 8 weeks of age of Omenn syndrome. The child's the mother allowed us to pull his sample and test it, and this was called correctly. We also pulled some specimens with 'immunodeficiency' or 'DiGeorge syndrome' noted in the electronic notebook and tested those. We correctly would have referred 3 of the DiGeorge syndrome infants. Based on our work since commencing population-wide screening, we have similar results for babies diagnosed prospectively at birth with DiGeorge syndrome.

California Validation

Summary

The TREC assay adapted by the California newborn screening program was validated by Perkin Elmer Inc. in Bridgeville, PA using 1,000 California newborn specimens. In addition to this validation, the assay was verified in Genetic Disease Laboratory, the site for screening SCID two weeks (births August 2 – 13, 2010) prior to the start date of the pilot study.

Briefly the verification process was as follows:

A) Replicates of the standards were run to verify reproducibility of standards. The mean and statistical limits were calculated.
B) Reference materials, which consisted of quality control (QC) materials supplied by CDC, were run. These reference materials are normal QC samples and negative (or SCID-like) QC samples. The mean TREC and beta-actin copy numbers and the statistical ranges were calculated.
C) Thirty blinded patients samples from the 1,000 samples tested in Bridgeville were re-tested for both TREC and beta-actin copy numbers. Absolute values of TREC and beta-actin were calculated against the standards that were run in duplicates. The run was judged acceptable based on the results of the negative (SCID-like), normal, water blank, and blank filter paper controls.
D) This study (C) was replicated.

This verification process successfully identified four confirmed SCID and T-cell lymphopenia cases. These four specimens were also identified as positive in Bridgeville, PA.

In December 2010, California and Wisconsin exchanged 6 coded specimens. While the actual numbers of TRECs vary between the two assays, the assessments matched. The 'calls', rather than the actual TREC numbers are the relevant data element for comparisons.

TABLE 1: Sample Exchange with Wisconsin for California's Validation Study

Tube #	ID_WI	Quantity TREC_WI (Ct)	Quantity TREC_CA (Cp)	Assessment
1	1	8 (37.73)	41 (37.16)	Abnormal-DGS
2	2	76 (33.96)	157 (34.86)	Normal
3	3	78 (33.91)	121 (35.3)	Normal
4	4	25 (35.57)	35 (37.48)	Abnormal-DGS
5	5	17 (36.57)	43 (37.11)	Abnormal-DGS
6	6	87 (33.76)	105 (35.57)	Normal

TABLE 2: Specimen Change Between Bridgeville Perkin Elmer Facility and California for the Analytical Validation in California for SCID/Lymphopenia Cases

Sample ID	Sample Received	NBS Run ID	ACTB	TREC	Ct	Plate ID
280-74-179/21-2010-11	10/22/2010	S28074179	56201	0	Undetermined	101022_Rpt
			49169	0	Undetermined	
280-53-001/31-2010-62	10/22/2010	S28053001	4536	15	36.22	101022_Rpt
			4779	12	36.66	
280-91-002/31-2010-62	10/22/2010	S28091002	15862	8	37.13	101022_Rpt
			15317	23	35.62	
280-30-003/31-2010-62	10/22/2010	S28030003	20352	14	36.41	101022_Rpt
			30933	5	37.91	

Louisiana and Wisconsin Validation

Several test exchanges were completed by Louisiana and Wisconsin prior to beginning live testing. The protocol is attached as Appendix 3. Briefly, samples are received at the Louisiana screening program each day and examined for quality. A barcode accession number is applied to each, and the laboratory punches samples into 96-well plates. A "no template" control is added at the end of the samples. The wells are covered with strip caps, and shipped via Federal Express to the Wisconsin newborn screening program. A data file is created by the Louisiana LIMS system and exported into an Excel plate map. A worksheet with accession numbers, gestational age and age at specimen collection is also created. The plate map and worksheet files are emailed to the Wisconsin program. The Wisconsin program then sends the worksheet back to Louisiana with the SCID screening results.

The Wisconsin program performs testing, and sends a re-punch list to Louisiana. In addition, all "inconclusive" results and "abnormal" results are given to staff at Louisiana State University

Children's Hospital electronically, and orally/electronically, respectively. All babies receiving a confirmatory diagnosis are also reported back to the Wisconsin Program.

Puerto Rico and Massachusetts Validation

In July 2010, Puerto Rico (PR) sent one batch of de-identified newborn blood spot specimens as a test run for the UPS shipping procedure. Notice that a test batch was being sent was given by email from PR newborn screening to the New England Newborn Screening Program (NENBSP) and a large UPS box containing the specimens was received the following day.

The specimens were viewed in preparation for the necessary requirements needed at NENSP (whether the assigned PR codes given the absence of a white slip could be read reliably, whether there was sufficient room on the card for the NENSP to assign its Guthrie number, etc.). The specimens were stored frozen until return shipping, and no testing was performed on these specimens. On September 23rd the test specimens were returned to Puerto Rico (UPS tracking number) to the following mailing address:

Puerto Rico Newborn Screening Program
UPR Pediatric Hospital
Building A 2nd floor - Lab 223
Attention: Sonia I. Ramirez
San Juan, PR 00935

The PR NBS confirmed return receipt of their specimens. The validation and standard operating procedure for screening of specimens from Puerto Rico is given as Appendix 4.

2. Develop a bioinformatics component for data collection and analysis, which will be a fundamental feature of the proposed technology platform.

Individual laboratories utilize their specific Laboratory Information Management Systems (LIMS). LIMS were adjusted in order to accommodate screening for SCID. In New York, the Neometrics system of Natus Inc. is utilized. The software was programmed and mnemonics developed to handle the referral and various repeat request categories. The system includes the ability to free text interpretations. It was decided, based on the validation data to incorporate a repeat specimen for premature infants (<37 weeks gestational age), due to their inherent low TREC levels (7, 9). SCID results are reported along with the rest of the screening panel.

California did not use their Program's LIMS system for the SCID pilot because samples are tested 2-3 weeks after birth at the central lab in Richmond, CA. Specimen Gate from Perkin Elmer Inc. sends the test results. The State Program has created an Access database for SCID and designed a mailer (report), which is sent out separately as an additional report. For this pilot, Perkin-Elmer Inc. is the lab of record, since it a lab-in-a-lab concept, and staff from Perkin Elmer Inc. signs the report. The Access database is designed to pull the demographic information from the State LIMS so the SCID report demographics match the regular NBS data/report, which is already complete and sent to providers.

As described above, Louisiana and Wisconsin exchanged data via an Excel plate map and worksheet exported from the LA LIMS without demographic data except for gestational age, age at specimen collection and accession number. After testing, Wisconsin transmits the results back to the Louisiana program using the same spreadsheet along with oral communication of abnormal results.

For Puerto Rico, all specimens are sent to Massachusetts coded and all PR specimens are included in the New England Newborn Screening Program LIMS. Within this LIMS, the recorded demographics for the PR specimens are the PR codes assigned to each specimen. For routine reports, the Massachusetts Program's custom LIMS at the University of Massachusetts Medical School is queried and a batch report is sent to Puerto Rico in an Excel format. All out of range reports are sent via mail, email and a phone call is made to the Puerto Rico newborn screening program at the time of result. The Puerto Rico Newborn Screening program in turn, reports the result, interpretation and recommended action to the healthcare provider, following algorithms set in Massachusetts. (Appendix 4)

While programs have begun to move forward with implementation of SCID screening in their states, and advisory committees, the National Library of Medicine Coding and Terminology Guide Data Standards for Electronic Reporting Project: (http://newbornscreeningcodes.nlm.nih.gov/nb/sc/measurement/TREC#footnote-condition) has begun to establish coding for electronic reporting for this condition. The disease name utilized in this coding system is: severe combined immunodeficiency, which was the agreed upon term by the Secretary's Advisory Committee on Heritable Disorders in Children and Newborns (SACHDNC). The analyte common name is "TREC" and the analyte long name is "T-cell receptor excision circle [#/volume] in Dried blood spot by Probe & target amplification". The Logical Observation Identifiers Names and Codes (LOINC) number assigned for this condition is 62320-7. The Systematized Nomenclature of Medicine—Clinical Terms (SNOMED-CT) code is 31323000. The International Classification of Disease, Ninth Revision, Clinical Modification (ICD-9-CM) code is 279.2 and the ICD-10-CM code is D81.9.

In addition, the Region 4 Stork (R4S) Collaborative originally funded by the Health Resources Services Administration was expanded to include a tab for SCID. To accomplish this, NIH provided a subcontract to the HRSA/Maternal and Child Health Bureau (MCHB)-funded Laboratory Performance Program to develop the SCID data portal as an expansion of the original Region 4 Regional Genetic and Newborn Screening Service Collaborative effort.(16). The subcontract was administered through the Eunice Kennedy Shriver National Institute of Child Health and Human Development's Newborn Screening Translational Research Network (NBSTRN). Drs. Fred Lorey of California and Roshini Abraham from the Mayo Clinic were given administrative access. In addition, Drs. Mei Baker, Michele Caggana, and Anne Marie Comeau were given access to enter Louisiana, New York, and Puerto Rico data. Drs. Lorey and Caggana entered data for California and New York, respectively, and information from Massachusetts and Wisconsin was welcomed. Furthermore, any other state program, clinician or researcher who has interest can ask for access to view the information in the de-identified database.

The NBSTRN aided in the identification of key players to make this pilot and its associated data collection a reality. This was accomplished in several ways: the NBSTRN helped facilitate phone calls between the states, the curators of the site, and others with the clinical expertise (Drs. Rebecca Buckley, and Jennifer Puck) to ensure the portal was user-friendly, and had the appropriate categorizations. The portal is still undergoing some minor modifications as experience collects. Classification of conditions related to immunodeficiency, and others that may be ascertained via TREC screening are listed below:

Classic SCID

1. *IL2RG* (common g chain SCID); X-linked
2. *JAK3*
3. *IL7 Ra*
4. *RAG1*
5. *RAG2*
6. *DCLRE1C (*Artemis SCID*)*

7. *PRKDC (*DNA-PKcs SCID)
8. *LIG4 (*DNA Ligase IV SCID*)*
9. *CD3D (*CD3δ SCID*)*
10. *CD3E (*CD3ε SCID*)*
11. *CD3Z (*CD3ζ SCID*)*
12. *PTPRC (*CD45 SCID*)*
13. *AK2 (*Reticular dysgenesis*)*
14. *ADA (*Adenosine deaminase SCID*)*
15. *PNP (*Purine nucleoside phosphorylase SCID*)*
16. *WHN* (Winged-helix-nude SCID)
17. Unknown

Variant SCID/ Leaky SCID

1. *NHEJ1* (Cernunnos/ XLF)
2. *AK2*
3. *PNP*
4. *WHN*
5. *RAG1*
6. *RAG2*
7. *ADA*
8. *IL2RG*
9. *IL7Ra*
10. *LIG4*
11. *DCLRE1C*
12. *CHD7* (CHARGE syndrome)
13. *RMRP* (Cartilage Hair Hypoplasia)
14. *CORO1A* (Coronin-1A)
15. Unknown

Non-SCID

1. DiGeorge syndrome (del22q11.2; ~ >35 genes)
2. *CHD7*
3. *ORA1*
4. *STIM1*
5. *CD3G (*CD3γ SCID*)*
6. *ZAP70 (*primarily affecting CD8 T cell development*)*
7. *LCK* (p56lck*)*
8. MHC class II deficiency (*CIITA, RFXANK, RFX5, RFXAP*)
9. *RMRP*
10. Hoyeraal-Hreidarsson syndrome, HHS (*DKC1, TERT, TINF2, DCLRE1B*)
11. *SLC46A1* (Hereditary folate metabolism, HFM); reversible SCID
12. *ATM* (Ataxia telangiectasia)
13. *RAC2*
14. Down Syndrome (Trisomy 21)
15. Jacobsen syndrome (del 11q)
16. Lymphopenia of prematurity
17. Idiopathic T cell lymphopenia
18. Unknown

In addition a summary of assay elements, conditions, equipment, cut-off values, and quality control measures was established by Drs. Caggana, Baker, Comeau, and Lorey. This document, “Assay Elements”, was made into an Excel spreadsheet and is attached as Appendix 5. We believe this summary is an important outcome of this pilot. It allows Investigators to view assays currently in practice and make decisions about screening in their own Programs.

3. Develop appropriate protocols to use in conjunction with the technology. The finalized protocols shall include a technical research plan, a measurement plan, performance metrics, and a quality assurance plan. The Contractor shall develop the final protocols with the input of the Project Officer and the Contracting Officer. Coordinate protocols across multiple sites.

Two programs (Massachusetts and Wisconsin) had been screening, prior to the award of this contract as described above, so the additional testing for Louisiana and Puerto Rico increased throughput and added an additional state and territory. Two more states, California and New York significantly increased the number of babies screened. Both NY and CA had to develop new protocols for testing. California chose to use the lab-in-a-lab program offered by Perkin Elmer Inc. In this way, the company installed equipment and sent personnel to perform testing, which they had developed and offered previously to about 11,000 newborns from their

Bridgeville, PA facility as part of their StepOne screening assay. This pilot study generated data to be used for submission of the Perkin Elmer assay to the Food and Drug Administration.

New York developed a fully automated assay in-house. Briefly, a lab-developed method prepares DNA in a 96-well format using the Biomek NX. Next the DNA eluate from four 96-well plates is transferred to a single 384-well plate for amplification and detection. The multiplex PCR (TREC and RnaseP) assay is set up in a 10μL total volume with 2μL of input DNA using the Biomek NX. The plasmid calibrators were graciously supplied by Dr. Anne Comeau and Jacalyn Gerstel-Thompson of the Massachusetts program (7, 15). A serial dilution is made in water from the working stock plasmid and 3 sets of standards are run on each 384-well plate. A total of 2 μL is added per reaction giving final concentrations 1,000,000, 200,000, 40,000, 8,000, 1,600, 64 and 12.8 TREC copies/rxn.

The coordination of protocols across multiple sites was accomplished early in the process by the creation of the "Assay Elements" summary spreadsheet discussed above (Appendix 5). In addition, the assays were implemented into the normal newborn screening specimen testing schemes in New York, and all testing in California was centralized after specimens were received from the contract laboratories 2-3 weeks after the rest of the newborn screening testing was completed. Each program determined their laboratory (Appendices 6 and 7) and clinical follow-up algorithms (Appendices 8 and 9), and these were discussed on monthly phone calls supported and arranged by the NBSTRN. The Contract Officer and NBSTRN staff were available for these monthly calls and provided input during the pilot study. Quality assurance was carried out in the individual programs, and by contributions to and participation in the Centers for Disease Control and Prevention Model Performance Evaluation Survey sponsored by the Molecular Biology Branch as described below.

Data from programs will be added to the new data portal as described, and via this pilot 654,053 infants were screened for SCID. So the goal of screening as many babies as possible was brought to fruition via this project.

4. Ensure that the technology is amenable for use with dried bloodspots on a filter paper matrix.

Because all the participants in this project are newborn screening laboratories, the specimens used in method development, validation, and the screening are dried blood spots. In addition, the Centers for Disease Control and Prevention Molecular Biology Branch, developed the previously described Model Performance Evaluation Survey using specimens created on the same filter paper matrix for the quality assessment. Thus this matrix is the sole specimen source for all of work completed under this contract and going forward.

5. Develop a technology that includes analytic performance of the test under conditions that permit its adoption into a high throughput situation. As part of the development of the technology, the Contractor must validate the performance of the test in appropriate populations, using population-based newborn samples across multiple sites.

In the development phase of the assays in California and New York, there were several sample exchanges across multiple sites. Wisconsin shared a panel of specimens with California and New York. New York has also committed to sharing control specimens with Illinois and Michigan.

As described above, New York and California were chosen because of the high volume of births. In New York the DNA is isolated in an automated 96-well format. To facilitate completion of the assay, aliquots of the DNA samples are collapsed into 384-well plates prior to PCR and quantitation. The assay is carried out on 2 Biomek NXs with 4 Peltier units each, and all steps are automated on these instruments. Thus high throughput is expanded to these two laboratories. Since testing was done at four sites as part of routine newborn screening, all race/ethnic categories are included in this pilot study.

An attractive feature of the testing in NY And CA is that both states opted for different types of testing models. The former using a lab-developed assay, and the latter using a lab-in-a-lab concept. Either model may be attractive and applicable to other states because of the individual state requirements or preferences.

In March of 2010, the CDC's Newborn Screening Quality Assurance Program began a Model Performance Evaluation Survey (developed in collaboration with Massachusetts and Wisconsin) to all who were interested in screening for SCID. Laboratories report TREC values and make a clinical assessment (No Follow-up/Screen Negative or Follow-up Required/Screen Positive). As of 6/16/11, there were 10 participants, including Wisconsin, Massachusetts, California, New York, Taiwan, the Centers for Disease Control and Prevention, the University of California at San Francisco, Perkin-Elmer Genetics (Bridgeville, PA), Perkin-Elmer (Turku, Finland), and one additional state who is working towards implementation. New York and California began participating in this evaluation scheme in October 2010, shortly after statewide TREC screening efforts began. Massachusetts and Wisconsin were using this quality assurance tool since its inception, and they contributed to its development.

TABLE 3: Summary of NY Results v. Other Participants on CDC Model Performance Evaluation Survey Reference Materials in Blinded Send Outs

	---------- Categorical Screening Result ----------			
	------ NY State Lab---------		-- All Laboratories --	
Sendout	No Follow-Up Required	Follow-up Required	No Follow-Up Required	Follow-up Required
Mar 2011	3/3	2/2	30/30	20/20
Feb 2011	2/2	3/3	18/18	27/27
Dec 2010	3/3	2/2	24/24	16/16
Nov 2010	3/3	2/2	24/24	16/16
Oct 2010	3/3	2/2	24/24	16/16

NOTE: "Follow-up Required" category includes SCID-like (low TREC with normal reference gene) and unsatisfactory/Indeterminate (low TREC and reference gene).

The 'call' for this prototype program is the overall post-screening action, and not the individual TREC values. This allows for flexibility and variability in the assays performed by the various institutions. Many screening schemes/assays may be utilized, but the outcome of the screening test is the basis for proficiency.

6. Provide data management and quality assurance methodology, which are essential parts of population testing. Including the quality assurance, the Contractors shall consult with the Centers for Disease Control and Prevention's Newborn Screening Quality Assurance Program (NSQAP) for advice on appropriate quality control assurance protocols. Coordinate data acquisition from a variety of sources.

From the CDC website : http://www.cdc.gov/labstandards (17):

"Laboratory Quality Assurance (QA) encompasses a range of activities that enable laboratories to achieve and maintain high levels of accuracy and proficiency despite changes in test methods and the volume of specimens tested. A good QA system does these four things:

- establishes standard operating procedures (SOPs) for each step of the laboratory testing process, ranging from specimen handling to instrument performance validation;
- defines administrative requirements, such as mandatory recordkeeping, data evaluation, and internal audits to monitor adherence to SOPs;
- specifies corrective actions, documentation, and the persons responsible for carrying out corrective actions when problems are identified; and
- sustains high-quality employee performance"

In order to implement screening in the pilot states/territories, standard operating procedures have been developed that describe the testing process. As part of general laboratory standards, and newborn screening practices, this was essential for implementation. As described above, there were several specimen exchanges that were completed between states to determine assay performance, and these are described elsewhere in this document. SCID screening, being a newborn screening test was incorporated into the system in each state as required by good laboratory practice.

The CDC offers help in several areas to maintain quality lab standards. These include proficiency testing, reference materials, consultation, training, and guideline development. The Newborn Screening Translational Research Institute at the CDC (NBSTRI) has helped pilot states by providing a Model Performance Evaluation Survey as described above. In addition, they are beginning to prepare proficiency testing for SCID via the Newborn Screening Quality Assurance Program.

Each site has its own methodology for data acquisition, which depends on the laboratory and follow-up algorithms utilized. This is similar to all aspects of newborn screening, in that the protocols are tailored to population size, population make-up, and laboratory capabilities. By design, data management systems must be altered to enable data acquisition and reporting in each Program. As described above, while states have different methodologies, and different information systems, all effectively were used to screen over 650,000 infants, in the short time frame of this pilot study.

In addition, the expansion of the R4S system enables data analysis within states, and comparisons can be made to the national cohort being screened by the other participants in this pilot.

7. Provide definitive answers concerning the utility of the new technology for newborn screening. In particular the Contractor must determine the technology's:

--utility to screen newborns for conditions currently screened within the United States (including its predictive value, sensitivity, and specificity);

Table 4 below shows the total number of newborns screened, and the actions taken by the 4 newborn screening programs. Pre-term infants are defined as those in the neonatal intensive care unit for California and Massachusetts. In New York and Wisconsin, pre-term infants are those that are <37 weeks gestational age. Programs request repeat samples for these infants when their TREC values are low, a common occurrence in this cohort. Action is taken when TRECs are zero in this group. The number of positive screens resulting from repeat testing, and the number of babies whose results warrant flow cytometry are also shown in this table.

TABLE 4: Actionable Measures Taken by Programs for the Pilot Study

State/Territory	Number of Infants Screened for Pilot Study *(10/01/2010-06/16/2011*	Number of Pre-term Infants (# repeated; # to flow cytometry)	Total Number of Infants Re-sampled	Referral to Flow Cytometry
LA (by WI)	32,000	3,871 (21; 0)	44	8
PR (by MA)	34,544	296 (11; 5)	32	9
NY	167,509	16,085 (207; 5)	433	247
CA	420,000	37,604 (301; 13)	355	43
Totals	**654,053**	**57,856 (540; 23)**	**864**	**307**

New York had a high referral rate because the Immunologists in the state that head the 8 Specialty Care Centers involved in the follow-up of screen-positive infants wanted to start very conservatively. The laboratory algorithm is included as Appendix 6. Since the beginning of testing, the cut-off has been re-evaluated, and changed to create a presumptive positive range where a repeat specimen is requested for a newborn with 150 TRECs or less. We are in the process of implementing a second cut-off revision. The rate of infants being referred to flow cytometry is 0.047% overall. With the adjustments to the screening algorithm in New York, we anticipate this to drop off significantly. Our current referral rate is 15 per 21,000 newborns (0.07%) and with the impending change, this figure will drop by another 30-40%.

Interesting information has already evolved regarding the distribution of SCID in babies born in the different geographic locations. Most notable is an apparently higher frequency of SCID than estimated in other studies (12 cases in 654,053 births yields a frequency of 1 in 54,504). In California, there was 1 case of SCID due to mutation in the IL2RG gene, 2 cases due to mutations in the RAG1 gene, and 2 cases due to mutations in the IL7Ra gene, the molecular defect for the 6th infant has not been determined. In New York, there were 2 cases of SCID due to mutations in the adenosine deaminase (ADA) gene, 2 cases due to mutation in the IL2RG gene, one of these babies has a sibling with the same condition. The fifth case in New York died in April, while molecular analysis was pending. This baby had multiple congenital anomalies and complications due to prematurity. This infant had a T-B+NK+ phenotype. Population-based screening has been in existence for some time due to the screening programs already established in Massachusetts and Wisconsin (7-12). In addition, SCID seems to be more prevalent in persons of Hispanic descent as 2 of 5 babies in New York were of Hispanic

descent (1 X-linked, and 1 ADA deficiency) and 5 of 6 babies confirmed with SCID in California were of Hispanic descent (all but the IL2RG) . In California, if just the Hispanic population is considered, SCID and SCID variant has an incidence of 1 in 25,000 births, and this frequency rises to 1 in 18,000 births if all T-cell lymphopenia is considered. It is important to note that the pilot was established in October 2010, and ended mid-June 2011. In this 8.5 month period, 654,053 newborns were screened, and continued testing will determine if this higher than expected frequency, and over-representation of Hispanics with SCID will continue.

TABLE 5: Outcomes of Newborn Screening by SCID Category

State/Territory	Start of Screening	Total Number of Births in State	Number of Infants Screened for Pilot Study *(10/01/2010-06/16/2011)*	SCID[a]	SCID Variant[b]	Non SCID[c]
LA (by WI)	10/1/2010	69,232 (WI) 65,268 (LA)	32,000 (LA)	0	0	4
PR (by MA)	10/1/2010	77,022 (MA) 45,620 (PR)	34,544 (PR)	1[#]	0	7
NY[^]	10/1/2010	245,282	167,509	5	0	19
CA	10/1/2010	510,000	420,000	6	3	8*
	Total	**1,012,424**	**654,053**	**12**	**3**	**38**

[a] SCID: Deleterious mutation in the DNA of one of the following genes, resulting in total failure of normal function of the protein encoded by that gene, whether IL2RG, JAK3, IL-7Ra, RAG-1, RAG-2, ADA, CD45, Artemis/DCLRE1C, CD3δ, CD3ε, CD3ζ, DNA PKc, or DNA Ligase IV. These proteins are crucial to the normal development of lymphocytes; therefore, any defect in one of these genes will result in a significant problem with immune function and associated susceptibility to infection. AKT2 defects, which cause severe lymphopenia and granulocytopenia, may have low TRECs but also poor amplification of peripheral blood DNA due to low numbers of nucleated blood cells. Patients with SCID have fewer than 300 autologous T cells per mL of blood, and their proliferative responses to the mitogen PHA are less than 10 percent of normal control responses. Some SCID patients do not have defects in any of the above genes, suggesting that additional disease genes for SCID remain to be discovered.

[b] SCID variant: Variation in the DNA of one of the following genes resulting in impairment of functioning of the protein encoded by that gene. Also known as “leaky SCID”; Combined Immunodeficiency (CID); or Omenn syndrome, a particular clinical entity with skin rash, eosinophilia, and T cells that represent expansion of a restricted thymic output. CID and Omenn syndrome may be due to hypomorphic variations in the above SCID genes or may be caused by defects in genes such as PNP, AK2, Cernunnos, Coronin-1A, RMRP, or WHN/FOXN1. In addition, there are SCID variant patients s for whom defects in known genes are not found.

[c] Non-SCID: Other defects either related directly to a component of the immune system with an associated malfunction or related to the loss of a section of DNA (e.g., DiGeorge syndrome [full and some partial], Jacobsen syndrome) or, in some cases, abnormal gain of DNA (e.g., Down syndrome/trisomy 21). Multisystem syndromes may be associated with variable severity of defects in immune function along with other serious health problems, including heart defects and developmental delay. The non-SCID category is a mixed group and includes individuals with a variety of genetic defects as well as infants who have poorly developed immune systems due to premature birth. Lymphopenia of prematurity, idiopathic T cell lymphopenia, DiGeorge syndrome/del(22)(q11.2), CHARGE syndrome, Jacobsen syndrome/del(11)(q24.1-11qter), Down

syndrome/trisomy21, thymectomy, and RAC2 deficiency may be associated with low or undetectable TRECs in some cases. There are additional defects of cellular immunity, including CD25 and ataxia telangiectasia, in which TRECs may or may not be abnormal. There are insufficient data at this time to predict whether these conditions may be detected by TREC newborn screening. In addition, there are many non-SCID immunodeficient patients for whom a genetic cause is not found. *Note:* not all DiGeorge syndrome infants will have immune deficiency, and thus this screening lacks the ability to detect all cases of this condition.

[#] two children died prior to flow cytometry, one was highly suspected to have SCID and is included

^ one child under treatment for leukemia and one child has JAK3 deficiency but was born prior to the start of screening; neither is included

* three additional infants are pending a diagnosis in California

TABLE 6: Forms of Non-SCID Detected via TREC Newborn Screening

Forms of Non-SCID Detected During Screening	California	New York	Louisiana	Puerto Rico	**Totals**
DiGeorge syndrome	5 (includes1 possible)	3	0	2 (includes 1 possible)	**5**
Trisomy 21	1	1	0	0	**2**
Charge syndrome	0	1	0	0	**1**
T-cell lymphopenia	2	7	0	5	**14**
Jacobsen syndrome	0	0	1	0	**1**
Surgery/Other	0	0	3	0	**3**
Trisomy 18	0	1	0	0	**1**
6p deletion	0	1	0	0	**1**
B-cell deficiency^	0	4	0	0	**4**
Bare lymphocyte syndrome (pending)	0	1	0	0	**1**

^These diagnoses are based on flow cytometry data and are under further investigation by New York State immunologists.

The duration of this pilot was relatively short. It is difficult at this time to estimate the PPV and NPV, as well as the other parameters, so these should be taken in context of the numbers of infants screened, and the time-framing for screening. There are several cases that appear to be SCID, and are undergoing molecular diagnosis. This impacts the data significantly, depending on the ultimate outcomes of these ongoing studies. Clearly, a timely molecular diagnosis is key to our understanding of the true benefit of SCID screening. It is likely that additional genes will be discovered causing SCID in the United States due to population-wide newborn screening. We are already aware of the study by Routes (2009) that described the rare condition of RAC2 deficiency, a non-SCID variant, which affects neutrophil migration (9).

--utility to screen conditions for which investigators could, potentially, develop therapeutic interventions in the near future;

SCID is one of the few conditions for which there is a veritable cure. First, knowledge of the condition allows parents and their physicians to prevent opportunistic infection by keeping the child out of circumstances where it is likely they can become infected such as crowds, daycare situations, and away from individuals who are ill. Also prophylactic antibiotics can be adminstered to prevent opportunistic infections. Children with SCID will not receive live virus vaccination for rotavirus to prevent infection. Administration of intravenous immunoglobulins (IVIG) to give a level of antibody protection is also provided.

As can be seen from the data gathered in this pilot study, there are several children identified with adenosine deaminase deficiency. Treatment for this condition is primarily via enzyme replacement therapy, called pegylated- or PEG-ADA. Sometimes children continue to receive IVIG infusions even with PEG-ADA. Hematopoietic stem cell (bone marrow or cord blood) transplantation is considered curative for other forms of immunodeficiency.

The study of McGhee et al. 2005 showed an 86% likelihood of SCID screening being cost-effective. This study used estimates for variables considered because at the time, there was limited information on the effectiveness or costs of screening. The most experience regarding costs of transplantation were from oncology practices. A cost to charge ratio of 0.373 was used in the calculation. Screening costs are still difficult to enumerate due to personnel, equipment, supplies, reagents, and throughput. Estimates range from 3-9$ per infant, depending on what aspects of the screening test are used to calculate the true cost. Flow cytometry costs range from $100-$200 depending on the institution performing the test. The study used Medicare reimbursement rates for visits, lymphocyte studies (flow cytometry and proliferation). Nonetheless, even by use of conservative estimages, the authors concluded the costs of delayed diagnosis were likely to exceed screening costs (18).

Anecdotally, specialists and parents have stated that costs up to $1 million dollars can accrue with emergency room visits, prolonged hospitalizations in intensive care units, isolation, treatment of infection etc. These children are either transplanted very late with poorer outcomes, or not at all. Early transplantation has a high success rate, and ablation is avoided using a parental donor, particularly if the treatment is initiated within the first 3.5 months of life (3, 4). Costs for this treatment, per Dr. Rebecca Buckley, are estimated at $50-100,000.

--efficacy to screen newborns for conditions currently screened, comparing this new technology with traditional and alternative technique;

This is a new condition for inclusion into newborn screening panels. The TREC assay has proven itself to be efficiacious because of the large-scale screening effort funded by this pilot with 654,053 babies screened, and 12 cases of SCID detected, in addition to 41 other infants with actionable immunodeficiencies. While there is no traditional technique currently in practice, a comparison with a newly developed alternative antibody test was carried out in New York.

An assay was developed in New York using Luminex technology prior to the initiation of this pilot and preliminary data from the Massachusetts program showed it to be promising (7, 19, 20). The antibody used in this study only detected the CD3 marker. The CD45 marker was not used. For the comparison with an alternative method, we tested >700 specimens using the CD3 immunoassay for which newborn screening and clinical outcomes were determined previously using the TREC assay; results are reported here.

For the study, specimens for all of the referrals from New York up to that point, including cases of T-cell immunodeficiency were included in a blinded panel. A total of 731 specimens were tested in duplicate. The cut-off values used for the CD3 assay were:

X ≥1.08 CD3 cells/mL blood	"within acceptable limits" (WAL)
X ≥0.495 and X <1.08 CD3 cells/mL blood	"borderline; request a repeat sample"
X <0.495 CD3 cells/mL blood	"referral"

The panel included 158 samples with TRECs ≥1000 TREC/μL whole blood (high); 158 samples with TRECs 500-1000 TREC/μL whole blood (medium); 158 samples with TRECs 200-499 TREC/μL whole blood (low); 213 referrals; 29 presumptive positive samples with TRECs 150-200 TREC/μL whole blood; and 15 repeat specimens from premature infants. All of the high, medium, and low TREC specimens had a WAL result on the CD3 assay. All presumptive positive and premature repeat infants had a WAL result on the CD3 assay.

Results of Comparison for TREC Assay to CD3 Immunoassay

138 of 213 TREC Assay Referrals were called Borderline "PP" by the Luminex CD3 Assay

122	diagnosis of no disease, no immune dysfunction
4	diagnosis of idiopathic T-cell lymphopenia
2	diagnosis of B-cell deficiency
9	cases open to follow-up
1	case lost to follow-up

51 of 213 TREC Assay Referrals were called Borderline "PP" by the Luminex CD3 Assay

35	diagnosis of no disease or no evidence of immune dysfunction
1	diagnosis of idiopathic T-cell lymphopenia
2	diagnosis of DiGeorge syndrome or possible DiGeorge syndrome
1	diagnosis of trisomy 21 w/ mild lymphopenia
1	diagnosis of neutropenia
2	infant expired
7	cases open to follow-up
2	cases lost to follow up

24 of 213 TREC Assay Referrals were called Referrals by the Luminex CD3 Assay

9	diagnosis of no disease or no evidence of immune dysfunction
2	diagnosis of idiopathic T-cell lymphopenia
1	diagnosis of neutropenia
1	diagnosis of leukemia
1	diagnosis of DiGeorge syndrome or possible DiGeorge syndrome
1	diagnosis of SCID type currently unknown, baby expired
1	diagnosis of Charge syndrome/SCID
2	diagnosis of ADA deficiency/ SCID
1	diagnosis of X-linked SCID
2	infant expired
3	cases open to follow-up

Thus 75 cases would have been actionable by the CD3 assay, and 6 additional cases detected by the TREC assay might have been not picked up by the CD3 assay (4 cases of idiopathic T-cell immunodeficiency and 2 cases of B-cell deficiency). None of the SCID cases would have been missed using these cut-off values for the CD3 assay.

An estimate of cost for this assay is ~$1.50 (exclusive of personnel and instrument costs; reagents and service contract are included). In our laboratory currently the assay is labor intensive, and not automated. It requires an overnight incubation, followed by 3-4 hours of work the following day, and would nicely fit into the workflow of a laboratory performing first tier TREC analysis. However, further development of this assay as a standalone is dependent on the availability of the detector antibody, and a written agreement from a supplier to ensure adequate supplies for the assay if used in a high-throughput laboratory. In addition use of this assay would require calibrator and control builds to withstand stability and cross-validation studies. An attractive feature of this technology, is the ability to multiplex T- and B-cell markers into the same assay, for example having CD3, CD19, and CD20 all detected in the same assay. Further studies are warranted to determine if CD3 can be used as a second-tier test to minimize the number of referrals, at least in New York.

--ability to exhibit sufficient and inherent flexibility to faciliatate expansion of its multiplexing capabilities to include new conditions in the future;

SCID screening using TREC analysis is a “DNA-first” test. Previously, molecular capability in newborn screening laboratories has been very limited as “second-tier” testing. Most often after a positive immunoreactive trypsin test result for cystic fibrosis screening, and in this instance, most states use an FDA-approved kit. Few laboratories had capability and staff to implement lab-developed molecular tests. This project has outlined for states the basic steps/requirements to perform high-throughput, automated DNA isolation, amplification, and detection. In the attached “Assay Elements” Excel file (Appendix 5), it is important to note that 3 of the 4 states/territories funded by this pilot, and the 2 additional state programs (MA and WI) that are funded currently by the CDC are obtaining DNA for testing in lieu of *in situ* amplification. .

Use of this technology opens up the important possiblity for other molecular applications for newborn screening. Experience and information gained by this pilot will have a significant impact on newborn screening programs for many years because the DNA obtained for SCID screening is useable for other molecular assays in the future. In addition, as has been described in SCID screening (7), and in Krabbe disease screening development (NY Newborn Screening Program), it is possible to obtain amplifiable DNA from a newborn screening specimen even after biochemical testing has been performed (immunoassays and ms/ms assays).

--ability to streamline and fully automate, so that it is suitable for population screening;

In developing SCID screening, laboratories must be able to incorporate the punching, DNA isolation, amplification, detection and resulting into their daily workflow. This requires, for larger states, the ability to automate the entire process. During the development of this assay in New York, a Biomek NX with Peltier units with heating/cooling functions was used to obtain DNA with automation. The assay requires beginning with the 96-well format so the dried blood spot can be punched accurately into the well and allow for liquids to be added and withdrawn while isolating DNA. The same instrument is used next to collapse four 96-well plates to one 384-well plate for amplification and detection. The instrumentation used by the pilot states is given in the “Assay Elements” spreadsheet (Appendix 5). The utility of screening was performed

throughout the pilot providing proof of principle for all aspects of this technology platform; the end result of this pilot was successful screening using TREC analysis for over 650,000 babies.

--bioinformatics capabilities for data collection and analysis for use in follow-up care and public health research;

Utilization of the R4S portal insures access by public health departments and researchers. In New York, we are leveraging funding for effective long-term follow-up to include SCID screening. Our clinicians meet quarterly, three times quarterly by conference call and once annually in person to address long-term follow-up. In California, all babies who screen positive are subjected to flow cytometry studies at a single clinical laboratory. The results of the testing are reviewed by one of two clinicians; these clinicians meet with the family, and provide treatment and follow-up. The National Library of Medicine efforts to systematically define SCID screening and follow-up are described in detail above, thus ensuring there are bioinformatic capabilities for data collection and analysis for use in follow-up care and public health research. The accumulation of the data from this pilot and ongoing efforts will also help better define referral and follow-up care patterns.

--appropriateness based on the framework of established screening procedures;

A requirement of this pilot was to establish SCID newborn screening into the daily work flow for newborn screening. This was accomplished in California and New York where SCID was added to the state screening program. The current standard of care in newborn screening, including accessioning, laboratory testing, referral, diagnosis and treatment, was achieved. This approach allowed for all newborns born after October 2010 to be tested in both states in a systematic way. The pilot expanded the pool of screened newborns through the collaboration between the New England Program and Puerto Rico, and the Wisconsin Program and Louisiana.

New York implemented an emergency regulation to include SCID in its newborn screening program. As mentioned above Natus and the IT staff in newborn screening established reporting for the condition. For Louisiana, reporting is done by exchange with the follow-up staff in Shreveport. There was no IRB review in Wisconsin. In California, a waiver of consent was obtained, and the reporting system is described above. For Puerto Rico, reporting is via electronic exchange with Puerto Rico, and the project underwent Institutional Review Board approval in Puerto Rico and Massachusetts.

--potential risks and benefits (e.g., its ability to detect genetic variance, clinical heterogeneity, carrier status), and;

The only risks associated with this pilot hold for any conditions on newborn screening panels. They are: 1) the risk of missing an affected infant, the false negative result, and 2) the increased anxiety in families who have a screen positive result. None of the participants in this pilot reported infants who were affected with SCID, and missed in the screening program. The sharing of information amongst programs, and outcome data in the R4S system allows for algorithm changes to minimize the number of infants who are referred, come to medical attention, and require flow cytometry. One laboratory concern is cross-contamination. Again, this is a normal concern on the part of newborn screening in general for galactosemia, biotinidase deficiency, and lysosomal storage disorders, where the laboratory detects the condition by testing for absence of an activity or analyte. This is mitigated by the re-testing, from the same specimen in duplicate, any samples with a ‘low’ activity/concentration.

Screening for SCID and related T cell lymphopenias has resulted in reduced morbidity and mortality in the 12 children diagnosed with SCID and the 41 additional babies diagnosed with SCID variants and other conditions resulting in T-cell deficiency. Increased molecular analysis during the diagnostic phase has the potential to add to our knowledge of genotype-phenotype correlations, and will likely identify new molecular causes of SCID. The identification of carriers is not within scope of this effort, since the follow-up tests, flow cytometry, complete blood counts, and mitogen activation studies as appropriate are diagnostic for these conditions, and point to a molecular target to study.

The availability of the test at a commercial laboratory will also allow states contracted with them to institute SCID screening. Furthermore, the lab-in-a-lab model adapted by California for this pilot study will lead to FDA approval of a test kit to measure TREC number in newborns. FDA approval of such a test kit will satisify requirements in some states for the use of an FDA-approved assay in newborn screening.

--detailed quality control and quality assurance standards.

Because the contract agreements were established with states who were interested in full implementation, or regionalization of an existing assay, they already adhere to quality assurance standards that follow CLIA guidelines for laboratory operation, training and competency of staff (21). In New York, the quality assessment standards are described as:

“Ongoing processes to assess conformance of practices with specifications established by the laboratory, and protocols for resolution of non-conformance, including monitors for the effectiveness of interventions for problem resolution”.

Laboratory processes are observed as part of routine surveys of the newborn screening program by the Clinical Laboratory Evaluation Program (22) in New York, which is a CLIA-exempt state, and thereby is obligated to adhere to standards that are equal to or more stringent than those set forth by CLIA. New York, like all other states are also surveyed by CLIA. One way quality assurance is accomplished by the participating laboratories is uptake of the CDC Model Performance Evaluation Survey, which is also available for any comers. This program is scheduled to become part of the routine proficiency testing materials administered by the Newborn Screening Quality Assurance Program in October, 2011. Thus there will be a successful transfer from its current location in the laboratory of Dr. Robert Vogt to the NBSQAP.

8. Characterize the following elements of the proposed technology relating to analytical validity, quality control, and assay limitations across multiple sites by the end of the Period of Performance

The results provided here have demonstrated that the TREC assay is robust, and laboratory algorithms including duplicate repeat runs for those with low TRECs and incorporation of multiple controls in each assay run help ensure reporting is accurate and that specimens with low/no TRECs are reported accurately and not missed from contamination or other inconsistencies in the blood specimens collected on dried filter papers (Appendices 6 and 7).

While it is early in screening to assume the numbers gathered in this pilot study will hold with continued large scale population screening, it is possible to get early estimates on the positive predictive value (PPV), the negative predictive value, and the analytical sensitivity and specificity.

For the PPV, two calculations can be made. First, using the number of referrals in the denominator (n=307) and 12 cases of true SCID detected, the PPV is 3.9%. If this number is recalculated using the current referral rate for New York, a revised expected number of referrals is 15 per 21,000 newborns. During the course of this study, with 167,509 newborns screened, the estimated referral rate using the newer cut-off (established in January 2011) is 119, and the denominator for the entire pilot comes becomes 179. Thus the PPV in this instance would be 6.7%. When one considers the PPV for all T-cell immunodeficiencies, the figure using the denominator of 307 for the entire study is 17.3% with 53 cases of SCID and other T-cell immunodeficiencies. Using the revised referral to flow cytometry calcuation for New York, the PPV becomes 29.6% for all detected forms of T-cell immunodeficiency. The NPV is 100% since we are not aware of any infants tested during the course of this study who have come to medical attention with T-cell immune dysfunction.

The sensitivity of this test is 100% at present, because the true positive cases were all detected by screening, and no cases that were positive for SCID or T-cell immunodeficiencies were detected clinically, i.e., there were no false negative results. The specificity of this test is 99.95% since there were 654,041 infants with a screen negative result. The denominator is 654,348 for all true negative infants 654,041 + 307 referrals. Similarly, for all cases of T-cell immunodeficiency, the specificity does not change.

9. Ensure that the technology developed through this contract satisfies the requirement of applicable FDA statuts and regulations. Additionally, the Contractor shall review and consider recommendations provided in applicable FDA guidance documents. The Contractor shall also review appropriate Clinical and Laboratory Standards Institute's (CLSI) voluntary standard controls and guidelines related to the proposed technologies and consider recommendations provided in these guidance guidelines when developing their proposals.

In order to get screening approved in the participating states, the methods had to be developed and validated according to the Clinical Laboratory Evaluation Program in New York (22) and the Clinical Improvement Amendments '88 (21). This was accomplished as it would have been for any new test addition to the newborn screening programs involved. California and Perkin-Elmer are working on a collaboration which will enable their test to be submitted to the Food and Drug Administration as an *in vitro diagnostic* product (23). This is an important aspect of this work because some state programs interested in beginning SCID screening must have an FDA approved test available in order to move forward with the addition of SCID to their panel. The laboratories have incorporated appropriate calibrators and controls. Calibrators are plasmids with TREC inserts. In addition, blank filter paper punches, and SCID-like controls are used on plates. Options can include a true SCID patient, adult blood spotted onto filter paper cards, lymphocyte-depleted blood specimens, and dried blood spots made from red blood cells only. Internal controls are normal dried blood spots, or adult blood spiked with a known number of TRECs and spotted onto filter paper. In addition, if multiplexing the control amplicon, controls for this are also included. The Clinical and Laboratory Standards Institute is in the process of formulating a guideline for SCID screening. Many of the investigators in this pilot project have been asked to participate in the preparation of this guideline, which will assist other State Programs in their efforts.

10. Establish a collaborative partnership with more than one State Newborn Screening Program throughout the development and conduct of these projects so as to ensure

appropriate perspectives during the design of the technology and incorporate certain unique components that are specific to newborn screening.

Prior to October 2010, the Massachusetts and Wisconsin were actively screening and preparing to begin testing for Puerto Rico and Louisiana, respectively. California had begun screening in August . By virtue of this pilot, these 3 states began a collaboration by actively participating in monthly calls. These were helpful in the start-up of this project, as Drs. Caggana and Lorey were able to learn from the experience of Drs. Baker and Comeau. In addition, clinical experts were recruited to help advise the group as the Programs joined on. The NBSTRN coordinated 2 meetings (September 2010 and March 2011) of the key participants. Most importantly, the NBSTRN also coordinated the monthly phone calls with our growing group. NBSTRN extended invitations to other states who were interested in or beginning the process of moving forward with SCID screening. By the last conference call in March, 8 states had joined including: New Jersey, Nebraska, Colorado, Delaware, Minnesota, Virginia, Illinois, and Michigan. In addition to States, the Immune Deficiency Foundation also participated and updated us on their efforts in different states, made us aware of their educational materials, and learned from our screening experiences. Representatives from SCID Angels for Life also participated in our last call. These calls have continued, with its last one conducted in July, 2011. They are held each month on the 4th Friday, and anyone interested is invited to join the call and ask questions. Based on this newly formed network, we are aware that following states are moving towards implementation of screening for SCID: Colorado, Minnesota, Delaware, New Jersey, North Carolina, Illinois, Connecticut, Florida, Iowa, Michigan, Rhode Island, Maryland, Oklahoma, and the District of Columbia.

11. Provide a system of referral for follow-up and treatment for children who have screened positive.

Reporting for Louisiana and Puerto Rico followed their usual protocols incorporating the specific SCID follow up algorithms developed and already in use in Wisconsin and Massachusetts, respectively. The state programs relayed results for follow-up in Louisiana and Puerto Rico. A provider fact sheet was created for physicians in the state that describes screening (Appendix 10).

In California, educational materials were developed in English and Spanish (Appendix 11). In addition, all infants for which flow cytometry and complete blood counts were required were seen and followed by Dr. Jennifer Puck from the University of California at San Francisco, Dr. Sean McGhee of Stanford University or Dr. Joe Church of Children's Hospital, Los Angeles. The follow-up system in California was set up so that all flow studies were conducted by Quest Diagnostics in San Juan Capistrano, California. Follow-up molecular testing, if necessary was conducted by laboratories such as Correlegan or GeneDx, which offer a full panel of immunodeficiency sequencing assays.

In New York, the follow-up system includes a network of Specialty Care Centers across the state and via several conference calls, their expertise was used to create a clinical evaluation protocol for follow-up (Appendix 8). Parent and provider fact sheets were also developed (Appendix 12). The Immunodeficiency Centers are in the same locations as our Inherited Metabolic Disease Care Centers, which facilitate any necessary genetic counseling. A follow-up coordinator at the Wadsworth Center is responsible for reporting results to the physicians at these Centers. Flow cytometry and complete blood counts are done at either commercial laboratories (Quest/Mayo) or sometimes in their own institutions. The physicians report back the results via a diagnostic form, which is included as Appendix 8 as well.

The American College of Medical Genetics developed an ACT sheet for use by Programs to help educate primary care physicians. The Primary Immunodeficiency Network also developed educational materials for newborn screening for parents whose babies screen positive. These fact sheets for parents and physicians all serve to better educate them about immunodeficiencies and newborn screening and are freely available.

12. Work collaboratively with the Newborn Screening Translational Research Network Coordinating Center, with the input of the Project Officer and the Contracting Officer, to identify the most appropriate states to participate in the study.

The states participating in this contract were chosen early on in the process with input from the NBSTRN, the Program Officer, and the Principal Investigator. California and New York were chosen to participate because of their large size and diverse population. Massachusetts and Wisconsin were chosen because they were already operational, and could apply their expertise to the inclusion of an additional state, Louisiana and a territory, Puerto Rico. Puerto Rico was chosen because of its unique population. Inclusion of the latter two also enabled the feasibility of regionalization to be explored.

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