TITLE OF THESIS: Texas Newborn Screening Program Research Study to Detect Severe Combined Immunodeficiency

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In partial fulfillment of the requirements for graduation with the
Dean's Scholars Honors Degree in (Department) Department of Chemistry and Biochemistry

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Texas Newborn Screening Program Research Study to Detect Severe Combined Immunodeficiency

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ACKNOWLEDGEMENTS

I would like to give my deepest thanks to Dr. Rachel Lee, my mentor, for her constant guidance and her willingness to always lend a helping hand. Dr. Lee has been such a tremendous help in helping me improve as a researcher and has shown me how meaningful it is to work in Public Health. She has taught me so much about how to ask questions and how to think critically, for which I am extremely grateful. Through her generosity, I continued working on the SCID project for two more semesters. I would also like to thank the other members of the newborn DNA analysis group, especially Ms. Nicole Kroutter, Ms. Leslie Covarrubias, Ms. Jenny Zhang, Mr. Brad Rolf, Ms. Lauren Kelley, and Mrs. D'Andra Morin for their supervision, tips, and work. I would like to also express my heartfelt gratitude to Dr. Leanne Field, who has sacrificed her weekends and so much time to meet with me and help me throughout this whole process. Her advice was essential in helping me through each step of writing the paper and preparing my presentation. I would also like to thank our generous sponsors, the Texas Department of State Health Services, The Association of Public Health Laboratories, and the Centers for Disease Control and Prevention, as well as the Office of Workforce and Career Development, who made the public health internship program possible.

I am so blessed to have been a part of this Public Health Internship program – it has undoubtedly been one of the most rewarding experiences of my undergraduate career. Without this program, I would never have seen how research so intimately saves so many lives on such a huge scale. This experience has shown me how to be a part of something that changes lives for the better.

Lauren Rego

May 4th, 2012

ABSTRACT

Severe Combined Immunodeficiency Disease (SCID) is a primary immunodeficiency disorder characterized by a lack of T-cell proliferation. It is important to screen newborns for SCID because without treatment, the disease is 100% fatal. If SCID is diagnosed early, gene therapy as well as stem-cell transplantation can restore T-cell function, saving the newborn's life. The purpose of this project was to demonstrate the validity of a proprietary assay for T-cell Receptor Excision Circles (TRECs) for diagnosis for SCID in Texas newborns developed by the University of Massachusetts Medical School. 848 newborns were screened at approximately two days and again at two weeks old, and their TREC and RNAse P concentrations were quantified. The results from the Texas screens were compared with those from the University of Massachusetts Medical School, which also screened the specimens. The results of the project will indicate whether or not the Newborn DNA Analysis Group at TDSHS should include SCID in its newborn screening panel.

INTRODUCTION

Newborn screening has been implemented in every state in the United States for early detection of life-threatening diseases (15). Texas currently screens roughly 400,000 newborns a year for a total of twenty-eight disorders, including metabolic, endocrine, and hemoglobinopathies, according to the Texas Department of State Health Services website (http://www.dshs.state.tx.us/lab/newbornscreening). The currently screened disorders all meet several criteria put out by the American College of Medical Genetics, or ACMG (19), that allowed them to be included in the panel: the disease must be treatable, easily testable, life-threatening, and difficult to diagnose without laboratory tests (19). The Texas newborn

screening mission statement emphasizes that one of its goals is "to decrease the morbidity and mortality of infants born in Texas." By allowing early detection of disorders that are not apparent at birth, Texas protects many infants from complications ranging from growth problems to seizures and even death, according to its website (http://www.dshs.state.tx.us/lab/newbornscreening). Most of these tests are performed at once using tandem mass spectrometry, or MS/MS, screening in the primary, or first-tier, test. An important advancement in newborn screening has come through simple DNA testing procedures—as of December 1, 2009, Texas has used DNA sequencing to screen for cystic fibrosis in second-tier specimen testing, according to the TDSHS website (http://www.dshs.state.tx.us/lab/newbornscreening).

In Texas, all newborns go through first-tier screening. If the assay returns a positive result from this level of screening, then the baby must be tested further, to decrease the chances of a false positive result and to even determine the specific mutation causing the disease. Texas uses DNA testing in the second-tier testing. These tests include DNA sequencing, PCR, and gel electrophoresis of DNA segments. Second-tier testing strives to pinpoint the genetic cause of the possible positive first-tier screen, allowing for a more precise diagnosis and to decrease the chances that false positive reports are mailed to parents. Some disorders that are currently have second-tier screens in place include hemoglobinopathies, cystic fibrosis, and glactosemia, according to the TDSHS website (http://www.dshs.state.tx.us/lab/newbornscreening).

"Severe Combined Immunodeficiency" (SCID) encompasses a group of disorders that result from a myriad of genetic mutations that result in a lack of T-cell proliferation (1, 10).

Since a wide array of genetic causes can lead to primary immunodeficiencies, SCID includes somatic mutations that result in Omenn, DiGeorge (also 22q11.2), Wiskott-Aldrich syndrome, as well as sex-linked SCID such as X-linked immunodeficiency (14). These diseases include mutations in JAK3, RAG1, RAG2, CD45, FOXN1, and IL2RG genes for X-linked SCID, which results in failure of T-cell activation by cytokines or antigen receptor development. The reported incidence of SCID is about 1 in 100,000 births, although this is probably an underestimate since newborns with SCID can die undiagnosed. The genetic mutations all result in failure of T-cell development in the body; therefore, not all lymphocytopenias can be classified under SCID. SCID only includes T-cell deficient primary immunodeficiencies, and may or may not be accompanied by B- or natural killer cell deficiencies (13, 12).

The phenotypes of various SCID-related diseases are summarized in Table 2. Although B- and Natural Killer cell function may or may not be impaired, all SCID patients lack the ability to produce T-cells.

It has been acknowledged that SCID meets the ACMG criteria for newborn screening (2, 19). The ACMG added SCID to its list of recommended screening panel in May of 2006 (19). Recently, some state public laboratories, including New York, California, Massachusetts, and Wisconsin, have begun state-wide screening of newborns for SCID (15).

A normal individual will have T-cell receptor genes that rearrange on the V, D, and L genes that code for T-cell receptors (2, 6, 10). When this rearrangement occurs, the body produces DNA

segments called T-cell receptor excision circles, or TRECs (2). These TRECs can be amplified and later quantified through RT-qPCR (8).

The compromise of the body's T-cells allows a myriad of opportunistic infections to invade unimpeded due to a lack of cellular and humoral immunity (5). A case study by Buckley and Adeli chronicles the symptoms of SCID on a 4-month old infant with no family history of primary immune disorders and who experienced oral ulcers, fevers, weight loss, and low blood oxygen levels. Two months after being referred to an ear, throat, and nose specialist, the patient finally was found to have no T- or B- cells after flow cytometry laboratory testing. However, by then an overwhelming adenovirus infection ruptured her liver, leading to her death. SCID patients like this girl do not display any symptoms during their first two months due to their still having the mother's immunoglobulin G--it is no surprise that the average diagnosis for SCID is at approximately 6 months, when it is often too late for treatment (1).

If SCID is caught early enough, gene therapy as well as stem-cell transplantation can help recover T-cell function (3, 4). 100% of infants with SCID die within their first year of life—however, if SCID is diagnosed within the first 3.5 months, about 95% will survive in the long term (2). This survival rate drops to 70% if diagnosis is delayed after 3.5 months (2). Patients who have been treated with gene therapy often succumb later to secondary cancers, such as leukemia (4). The most successful treatment for SCID lies in HLA-identical bone marrow transplants (4). If the baby has a type of SCID called "adenosine deaminase deficiency," which is caused by an accumulation of toxins that lead to SCID, then enzyme replacement therapy will be used to treat the baby (18). This therapy is effective for this type of SCID (18). Table 3 denotes

the survival of various SCID disorders along with their demographic information. SCID is a treatable disease if it is caught early enough, which is why including the SCID assay in newborn screening is essential.

Since SCID results from a variety of genetic mutations and neither B nor natural killer cell counts can be used to diagnose it, T-cell counts must therefore be quantified. In the laboratory setting, flow cytometry has been used to count cell types in the blood (10); however, the quantification of T-cell Receptor Excision Circles (TRECs) gives a direct measure of T-cell proliferation (2, 5). Since T-cell receptors rearrange when they mature, each T-cell produces a TREC. Thus, the number of TRECs directly relates to the number of T-cells being produced by the infant. Several other methods of SCID diagnosis in the laboratory have been developed as well—these methods include multiplexing assays, dideoxy sequencing, and heteroduplex analysis (9, 11,16). A resequencing microarray tested on somatic and X-linked SCID has been developed but is dependent on custom probes (1). Another immunoassay that uses actual Guthrie card specimens uses CD3 levels as a marker for SCID, with CD45 as a control (9). Problems of this methodology include a small sample size in its testing as well as the cutoff for CD3 SCID levels still undefined. The authors also note that the protocol would be difficult to expand for use for large sample sizes. The drawbacks for these techniques are too great for them to be used in newborn screening. The methodologies of dideoxy sequencing and heteroduplex analysis were tested using the IL2RG defect, with the conclusion that heteroduplex analysis had a specificity of only 47% (16). Dideoxy sequencing was the most specific and sensitive but also the most time-consuming.

However, the TREC assay is repeatedly reported as accurate, specific, and sensitive (12, 8, 2). A TREC level lower than 30 copies per µL is the lower limit for normal T-cell levels, and the TREC assay is reported to be 100% sensitive and 97% specific with only 1.5% of tested specimens as false positives (13). Recent advancements in the development of the TREC assay have further made it even more feasible to test large sample sizes—Wisconsin has already successfully implemented this assay using DNA extraction followed by RT-qPCR (2). The protocol used in Wisconsin has been streamlined and automated by the University of Massachusetts Medical School and is reported to have 100% efficiency with q-PCR and a lower false positive rate than reported in Systematic Evidence Review and by design avoids cross-contamination between samples by automating the DNA extraction process (8). In addition, this assay can be expanded to testing as many as 384 samples at once and allows quantification of TREC levels, which directly correlate to the quantity of T-cell production, thus not being mutation-dependent like the multiplexing assay. The TREC-assay has definitively been used to successfully diagnose infants with 22q11.2 (DiGeorge) syndrome (12). Indeed, the TREC assay can catch the vast majority of SCID disorders (it cannot successfully screen for adenosine deaminase deficiency). For these reasons, Texas has decided to use the TREC RT-qPCR assay that is optimized and developed by the University of Massachusetts (2). The assay has confirmed SCID diagnosis of patients who were known to have no T-cell counts.

The purpose of this project is to demonstrate the validity of using the TREC assay developed by the University of Massachusetts Medical School to detect Severe Combined Immunodeficiency disorders in newborns in Texas. Although SCID does meet the criteria for newborn screening, pilot studies such as this show that Texas is correctly using the assay on a larger scale. The

success of the project will indicate whether or not Texas should include Severe Combined Immunodeficiency in its newborn screening program. SCID has been cited as a medical emergency (2), and the state of Texas can be at the forefront of averting this medical disaster. The residents of Texas will benefit from being able to receive an early diagnosis, which will greatly increase the chance of their child's survival.

Materials and Methods

Study Population

The specimens were collected from Austin-area infants whose blood was blotted on standard Guthrie cards. We screened both the initial Guthrie card, and the one sent two weeks later for the same specimen. The specimens were 848 newborns born at Austin hospitals whose parent or guardian had given informed consent for their participation in the pilot study. Each specimen was catalogued for anonymity using a coding technique so that neither the University of Massachusetts nor the person doing the SCID assay here in Texas would be biased in any way. Each specimen had an entire blood spot cut out and sent to the University of Massachusetts. Then each blood spot was coded, cut, and wrapped in glycine paper. The specimens were kept in a freezer unless there were being coded or tested. For each baby, we collected two specimens – one for when the baby is one to two days old, and the second for when they were one to two weeks old. In this experiment, 482 of the newborns had second screens completed.

The retrospective specimens were coded using codes TX-SCID-001 to TX-SCID-036, and were tested in the same way as the specimens from St. David's. One blood spot from each card was cut, catalogued using the code, wrapped in glycine paper, and frozen until it was mailed to the

University of Massachusetts for SCID testing, to provide a check on our results. These specimens were from a range of years and were from various parts of Texas.

The controls for the actual RT-qPCR step were known samples provided to us from the University of Massachusetts. The blank control was DI water, and two positive controls were DNA from two newborns who had been diagnosed with SCID. Another control was an adult DNA samples (low levels of TREC).

Laboratory Diagnosis of SCID with the Automated TREC Assay

All protocols are confidential and proprietary property of the University of Massachusetts Medical School, and thus the methodology cannot be described in detail.

The DNA extraction was performed using ordered reagents and following the protocol, including the automation process. The workflow for this experiment is shown in Figure 1.

First, each specimen was punched out and put into a MicroAmp Optical 96-Well Reaction plate from Applied BioSystems (2). Only one hole punch is needed for each specimen. The DNA extraction was performed with the Generation DNA Purification Solution and Generation DNA Elution Solution from Qiagen (2). Following the DNA extraction, the DNA was either frozen for later use, or primer/probe for TREC and RNAse P along with Applied BioSystem's TaqMan MasterMix added for immediate RT-qPCR amplification (2). A standard curve was prepared using various dilutions of TREC and RNAse P, and placed in three columns on the well-plate. Then the samples were subject to RT-PCR using the Applied BioSystems' ABi 7900HT Fast

Real-Time PCR System technology (2). The data were then standardized using the curve dilutions and a simple calculation performed, which allowed for the final quantification of TREC-levels (8).

Since the methodology uses RT-qPCR, we can use multiple probes at once (7). In this assay, we use probes for TREC and RNAse P. The TREC probes are specific for sequences common to all TRECS, which allows for the real time amplification of the TRECs. The second probe is for RNAse P. Each person has a relatively constant level of RNAse P in their blood. The RNAse P serves as an internal control, since all living humans have detectable levels of RNAse P in their blood. The expected outcomes for TREC and RNAse P levels are shown in Table 4. It is to be noted that low levels of RNAse P would indicate an error in DNA extraction or an invalid specimen (8).

This assay protocol allows testing of 96 specimens at once, using the automated procedure. All of the TaqMan Master Mix and the primer/probe was automatically dispensed to ensure accuracy and precision.

RESULTS

The data are all from the 848 newborns screened so far. Data from the analyses of the retrospective specimens as well as confirmatory testing from the University of Massachusetts has not been completed for all specimens.

Study Recruitment

Nurses were responsible for recruiting the study population and handing out the consent forms, which were both in English and Spanish. Nurses explained the purpose of the SCID pilot study to prospective parents.

Demographic Analyses

As seen in Figure 2, the gender distribution for the study population was 47% female and 53% male. The race/ethnic distribution is shown in Figure 3; 44% of the newborns were Hispanic, 41% were white, 7% were African American, 3% were Asian, and 2% were other. The birth weights distribution of the newborn in grams is shown in Figure 4. For comparison, the birth weight distribution for newborns born in the United States in 2002 to 2006 is also provided in Figure 4. The sample population seems similar to the national birth weight distribution except for extreme birth weights, and a normal distribution is clearly visible. Only one newborn in the study population qualified as Low-Birth Weight (LBW), or less than 2,500 grams.

Laboratory Analyses

Testing and data analysis of 848 first screen specimens were completed using the TREC assay and included data analysis techniques. Testing for additional newborns has not yet been completed. The analyses from the University of Massachusetts have not been received for all the specimens.

Figure 6 shows the number of TRECS per μL whole blood in the 848 newborn specimens for the first screen. As comparison, the results from the University of Massachusetts are also shown

here. Numbers of TRECS from the DSHS results ranged from 1000 – 11000 copies/ μL whole blood with an average of 2899 copies/ μL whole blood with a standard deviation of 1977 copies/ μL whole blood. As expected, the number of TRECS in the two positive controls was undetectable and the number of TRECS in the adult reference specimen was detectable, but lower than the threshold TREC concentration for all of the runs on these specimens. Since the TREC range for a normal newborn is greater than 252 copies/μL whole blood, all newborns in the study population were normal and did not have SCID. The few newborns shown in this paper whose TRECs fall below the cutoff were shown to be false positives with additional testing. All of the specimens had RNAse P levels that were higher than the cutoff, so the assays performed on these specimens were indeed valid. In comparison, the University of Massachusetts results had an average of 2355 copies/ μL whole blood, with a standard deviation of 1175 copies/ μL whole blood. The TDSHS results are higher than the Massachusetts results, indicating that the difference may have something to do with the standard curve.

482 newborns who had their first-screen test complete also had the second screen testing complete. Second screens were completed with an identical procedure.

Figure 7 shows the number of TRECS per μL whole blood for 482 two-week old newborns that were retested. The number of TRECS in these infants ranged from 1000 to 10,000 copies with an average of 3028 copies of TRECS per μL whole blood with a standard deviation of 2336 copies/ μL whole blood. All of the second screens were well above the cutoff for SCID. All of these newborns are confirmed to have normal TREC counts, since the counts are above the threshold. The controls for the second screens came out as expected, with the DI water with no TREC or

RNAse P, the two SCID controls with undetectable levels of TREC, and the adult with detectable, but lower than threshold values of TREC. The RNAse P levels for all of the specimens plus the SCID and adult controls were above the RNAse P concentration threshold – the assay was valid. However, the University of Massachusetts results had an average of 2903 copies/ μL whole blood with a standard deviation of 1328 copies/ μL whole blood. It is noted that the standard deviation from the University of Massachusetts was almost half the standard deviation from TDSHS.

The data appear to be showing the trend that the first and second screens are similar. However, to see if the first-screen and second-screen TREC levels were comparable, a paired two-tailed T-test was performed with the null hypothesis that there is no difference between the two screens in terms of TREC counts. Using an α of .05, the test yielded a T-critical value of 1.65 and a p-value of 9.29×10^{-5}. Since the null hypothesis is disproved, there is a 95% confidence that the two screens have a significant difference in the number of TRECs per μL whole blood. This may be due to the newborn having more TRECs produced as a result of their maturation. When the first screens for the from DSHS and the University of Massachusetts were compared using a paired two-tailed T-test, the p-value was 7.93×10^{-7}. Thus, the TREC counts from DSHS and the University of Massachusetts are statistically different with 95% confidence. It is interesting to note that that the TREC counts by DSHS and the University of Massachusetts are approaching a normal curve.

The standard curves that were used in these tests were curves prepared by the DNA analyses group in the Department of State Health Services using the methodology set forth in the

University of Massachusetts protocol. All curves met the University of Massachusetts guidelines to be used in the TREC assay.

DISCUSSION AND FUTURE STUDIES

Study Population Recruitment Conclusions

First, there needs to be a method in place to train the nurses on how to recruit and explain to parents the benefits of participation in the SCID study. In addition, creating another way to teach parents about how the study presents no personal risk and assuring them that all the DNA results will be confidential would increase the response rate. Programs could include a short video or small pamphlet that summarizes the goal of the study and explains to parents the importance of getting their child tested for SCID as soon as possible, especially since testing will require no additional blood work other than what is already required by state laws. Parents need to understand the SCID study poses no personal risk or risk of injury to their newborn. Also, if Texas can start collecting data on why parents denied consent, that would help pinpoint exactly what is going wrong in the recruitment process.

In the future, it would be ideal to expand the study population beyond Austin to allow for a more representative study of Texas newborns. Public health must reach out to everyone – in this case, it is evident that a failure to do so can sorely undermine a study. It is imperative that recruitment into the SCID study be fixed as soon as possible. This may even have to include personal recruitment directly to the patient from a Department of State Health Services representative.

Demographic Analyses Conclusions

The study population is skewed in terms of ethnic/race distribution. The study population had approximately the same number of males as females, as expected. The ethnic/race analyses show that the majority of the study participants were Hispanic, which is not representative of the Texas population. This could be due to a myriad of reasons. The Spanish form may be attracting more patients than the English form, or the patients may not have been told the whole scope of the project, and signed the form because a nurse handed it to them. The most probable reason is that the study population is reflective of the ethnic/racial distribution of the hospitals, although it is not representative of Texas. To evaluate this possibility, all the newborns born in the time period of interest need to be tabulated to see if the study population is similar to the ethnic distribution that St. David's normally encounters. This will be included in future studies of the patient demographic analyses.

The birth weight distribution of the study population does seem representation of the US population as a whole. The smaller sample size of the study population explains why there is not a more identical distribution. The fact that the study did not encompass newborns on the extremes of the birth weight scale, namely Low Birth Weight newborns, does skew the study. Texas has yet to determine whether or not an extremely low birth weight can affect the specificity of the TREC assay.

In the future, the best way to get an accurate sample that is representative of the demographics of Texas, is to set up state-wide sampling. Increasing the scope of the sampling will hopefully diminish any gender, birth weight, or ethnic/race skewing seen here in this study. The best way

to prove that the TREC assay can be successfully implemented and help all Texans is to test as many as possible and with as representative of a study population as possible.

Laboratory Analyses Conclusions

The 848 newborns all had TREC levels that were well above the cutoff – thus, none of them have SCID since they display evidence of T-cell maturation. Texas has shown that the TREC assay can successfully be used to screen for SCID using the University of Massachusetts protocol as far as absolute indication of SCID is concerned. This test so far has indeed been valid to use for SCID screening. Texas can quantify the number of TRECs a baby is producing both at one-day and two-weeks old, but not in a way that is reliable. The Texas public health laboratory can tell if a baby has SCID, a primary immune deficiency disorder, using this assay.

Since the paired T-test from THSDS and the University of Massachusetts showed that the TREC levels for the two screens had a statistically significant difference, the results of this experiment may not be repeatable with great precision, since it was expected that the TREC counts for both screens would indeed be similar. There are several possibilities why the counts would differ. One reason could be that TREC counts are significantly different between the blots on the Guthrie card, or that TREC counts are significantly different between even hole-punches on the same blood spot. The other possibility is that the standard curve needs to be reanalyzed. If the concentrations in the standard curve are too high, that would give an artificially high result for TREC counts, and it was seen that the TDSHS TREC counts were higher than the University of Massachusetts results.

The paired t-test between the TDSHS first screens and second screens revealed interesting results. There is indeed a significant difference between the first and second screens in terms of TREC counts. This indicates a couple of findings. One finding could be that newborns have various levels of T-cell maturation in their first two weeks—it fluctuates and does not stay the same as their first few days of life. Another possibility is that the TDSHS results are not consistent. This must be further investigated. The same newborn should be tested several times using the TREC assay from the same blood spot to see how much the TREC counts vary. In this experiment, the standard curve used must be identical in all these experiments.

The retrospective specimens all had TREC counts that were too low for the rt-qPCR to detect. Since there are no TREC counts associated with these specimens, they are not reported in any figure. The RNAse P values for these specimens were all above the threshold, so the tests were valid. This shows that Texas can correctly use the assay to diagnose patients with SCID, since all the retrospective specimens were SCID patients.

It is not clear yet whether identical TREC counts are important, or whether the absolute SCID indication is more important. As far as absolute SCID indication, TDSHS has demonstrated 100% accuracy in determination of whether or not the newborns have SCID, since the University of Massachusetts has determined that none of the screened newborns have SCID, and the TDSHS results agree.

The results from Massachusetts and Wisconsin also confirmed the TREC assay, and Texas has followed suit in terms of absolute SCID indication (8). It remains to be determined whether absolute TREC counts should be matching between different tests. All of these public health agencies have successfully completed pilot studies for the TREC assay, and the Texas results are comparable. The results from this study further verify that the assay can be used in the realm of public health. However, as the largest public health agency in the United States, Texas will be able to see how the TREC assay can be used on a much larger scale.

However, the study will still be expanded upon. Not only is the patient population so far too small, but the Texas results have not yet been compared with the University of Massachusetts results for the second screens. The results must be checked so that the results of the TREC counts can be compared for their accuracy. Also, the standard curves used in this experiment need to be verified by the University of Massachusetts. Texas also needs to finish the second-screens for the remaining specimens. In addition, various confounding variable must be excluded, including how TREC levels can change in an infant over time, and how TREC levels vary across blood spots and even hole punches of those blood spots. It could also be of interest to see if there are any trends in TREC counts with respect to gender or race/ethnic categories.

The next step in the SCID study is to continue testing specimens, and to move to even more automation, and increase the number of specimens that can be tested at one time. As the response rate increases, larger machines will be used to cope with the increased number of specimens being submitted. The public health department at Texas is equipped to test 384

specimens at once, all automated. Future steps in the pilot study will be to test this equipment for its validity for use in the SCID assay.

There are materials – SCID specimens – sent to the Texas Department of State Health Services from the Centers of Disease Control. These materials will further show that Texas can test materials even outside of Texas newborns, further expanding the scope of the assay. This will also test how accurate Texas is in SCID diagnosis on various types of SCID.

Since the true incidence of SCID is unknown, Texas will be able to contribute to data to find out its true incidence (8). Public health laboratories will know around how many SCID patients they can catch a year, and the public will benefit from knowing the dangers of SCID, and will be protected by having a much greater chance of an early diagnosis. When the data from all the public health laboratories is pooled, population parameters for TREC levels can be definitively quantified.

Future Goals

Since Texas can successfully screen for SCID, the future is open to what the public health can do next. The ultimate goal is to add SCID as the 29th newborn screening test and to start saving lives as soon as possible. This study's success shows that adding SCID to the newborn screening panel will allow the public health laboratory to save even more lives.

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Figures and Tables

***Table 1*. "Abnormal genes known to cause SCID." Buckley, R.H. 2004. Molecular Defects in Human Severe Combined Immunodeficiency and Approaches to Immune Reconstitution. Annu Rev Immunol. 22:625-655.**

Chromosome	Disease
1q31–32	SCID caused by CD45 deficiency*
5p13	SCID due to IL-7 receptor alpha chain deficiency*
10p13	SCID (radiation sensitive; Athabascan) due to mutations in the Artemis gene*
11p13	SCID caused by RAG1 or RAG2 deficiencies*
11q23	SCID caused by CD3 delta chain deficiency
19p13.1	SCID caused by Jak3 deficiency*
20q13.11	SCID caused by adenosine deaminase (ADA) deficiency*
Xq13.1	X-linked SCID caused by common gamma-chain (γ_c) deficiency*

Table 2. "Lymphotype phenotypes of the different molecular types of SCID." Buckley, R.H. 2004. Molecular Defects in Human Severe Combined Immunodeficiency and Approaches to Immune Reconstitution. Annu Rev Immunol. 22:625-655.

Lymphocyte phenotype	Type of SCID
$T^-B^+NK^-$	X-linked (γ_c deficiency) Jak 3 deficiency CD45 deficiency
$T^-B^+NK^+$	IL-7R alpha chain deficiency CD3 delta chain deficiency
$T^-B^-NK^-$	Adenosine deaminase deficiency
$T^-B^-NK^+$	RAG1 or RAG2 deficiency Artemis deficiency

Table 3. "Survival of 89 Patients with Severe Combined Immunodeficiency Who Received Transplants Between May 1982 and August 1998." Buckley, R.H., S.E. Schiff, R.I. Schiff, M.L. Markert, L.W. Williams, J.L. Roberts, L.A. Myers, F.E Ward. February 1999.

VARIABLE	NO. OF PATIENTS	NO. SURVIVING	PERCENT SURVIVING	P VALUE*	
				WILCOXON	LOG RANK
Type of severe combined immunodeficiency				NS	NS
γ_c-Chain deficiency	43	34	79		
JAK3 deficiency	6	6	100		
Interleukin-7 receptor α deficiency	2	2	100		
Adenosine deaminase deficiency	13	11	85		
Autosomal recessive, unknown cause	20	17	85		
Cartilage–hair hypoplasia	1	1	100		
Male, unknown cause	4	1	25		
Total	89	72	81		
Race or ethnic group				<0.001	<0.001
White	69	61	88		
Black	10	5	50		
Hispanic	10	6	60		
Total	89	72	81		
Sex				0.047	0.06
Male	75	58	77		
Female	14	14	100		
Total	89	72	81		
Age at time of transplantation				0.088	NS
<3.5 mo	22	21	95		
≥3.5 mo	67	51	76		
Total	89	72	81		

*NS denotes not significant.

Table 4. Cutoffs of TREC and RNAse P Levels for Different Specimen Types.

	TREC Levels	RNAse P Levels
SCID Newborn	< 252 copies/µL whole blood	> 4,032 copies/µL whole blood
Non-SCID Newborn	> 252 copies/µL whole blood	>4,032 copies/µL whole blood
Unsatisfactory	Not applicable	<4,032 copies/µL whole blood

***Figure 1*. "The T cell excision circle (TREC) assay on dried blood spots." Puck, J.M. 2007. Neonatal Screening for Severe Combined Immune Deficiency. Curr Opin Allergy Clin Immunol. 7:522-527**

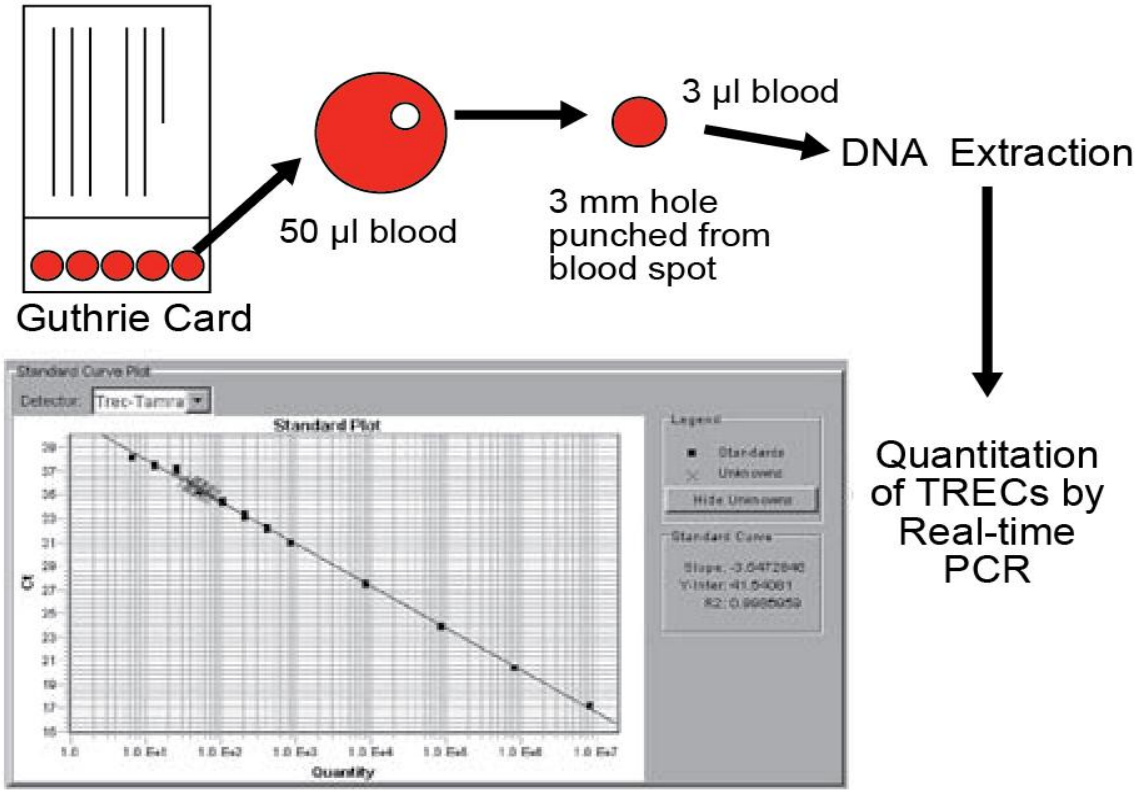


Figure 2. Gender Distribution of Study Population.

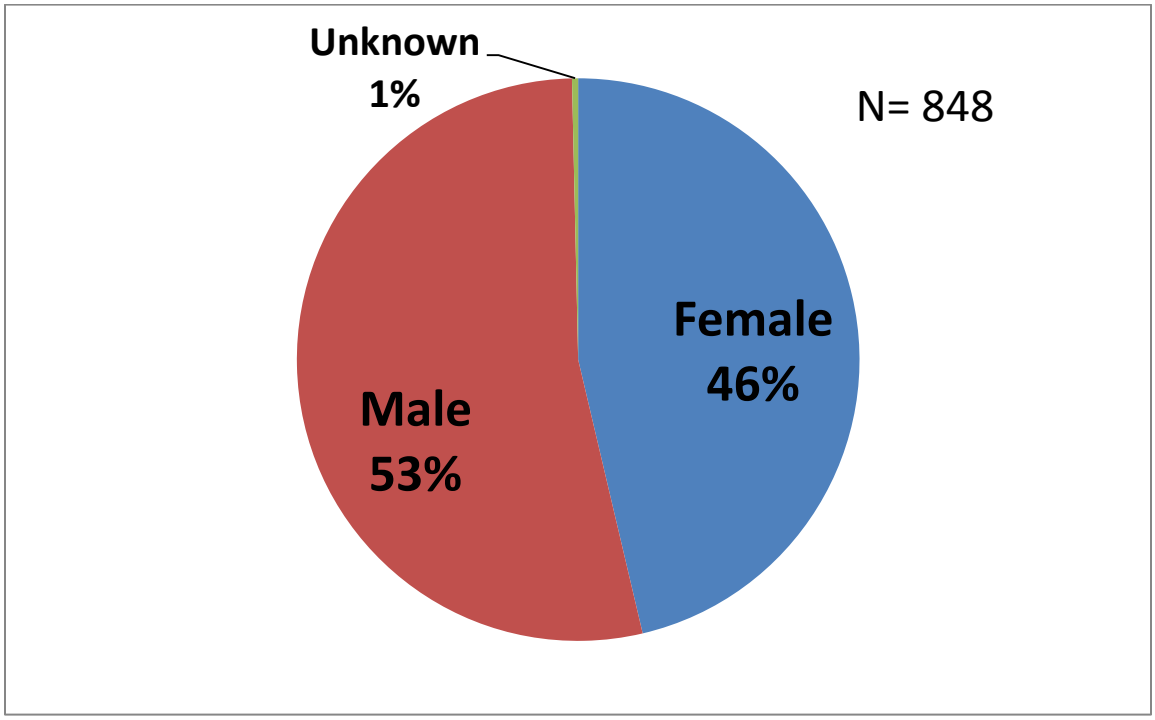


Figure 3. Ethnic/Race Distribution of Study Population.

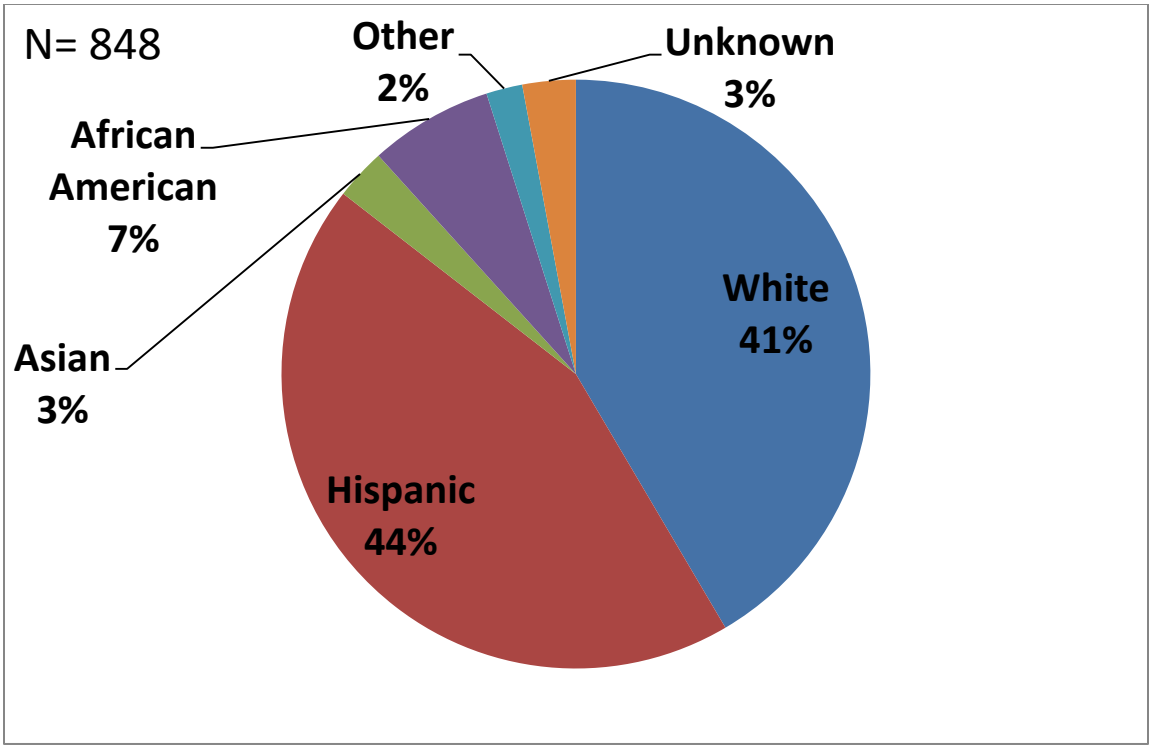


Figure 4. Birth Weight Distribution for Study Population.

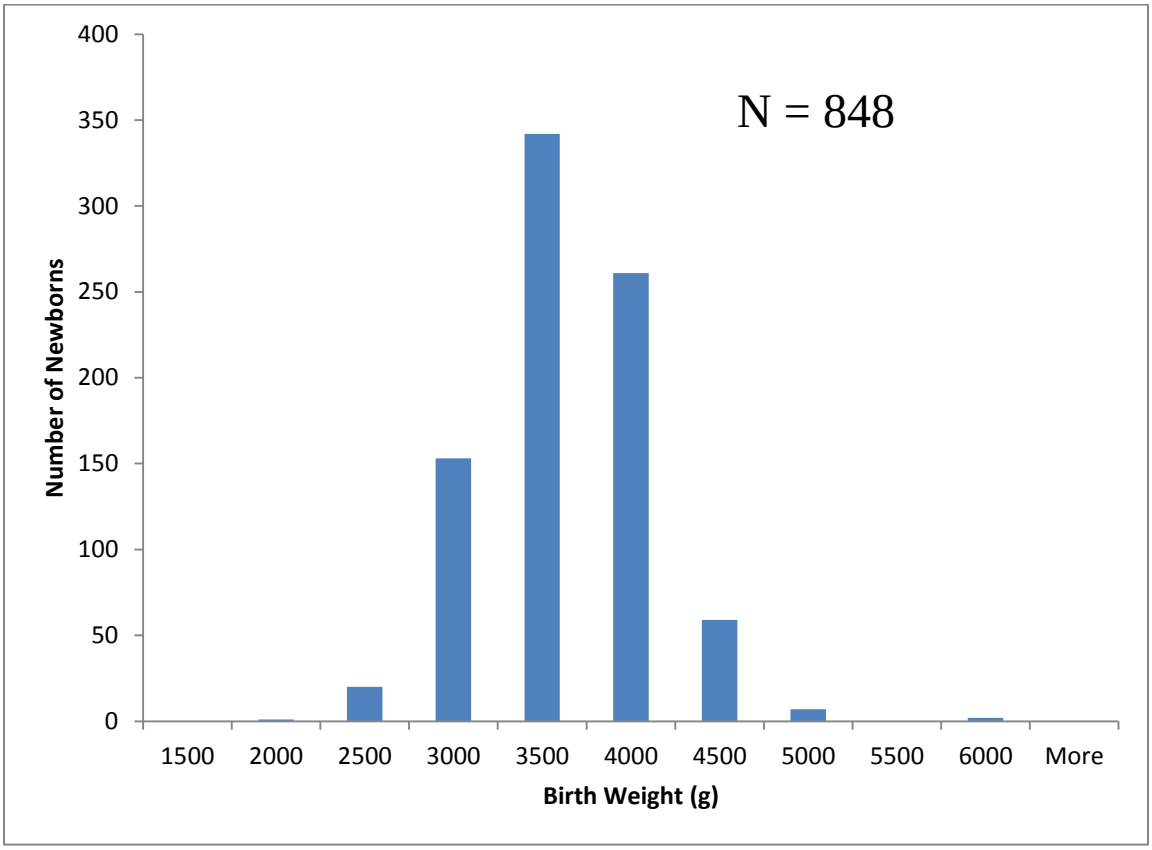


Figure 5. Birth Weight Distribution of US Newborns in 2002-2006 (CDC).

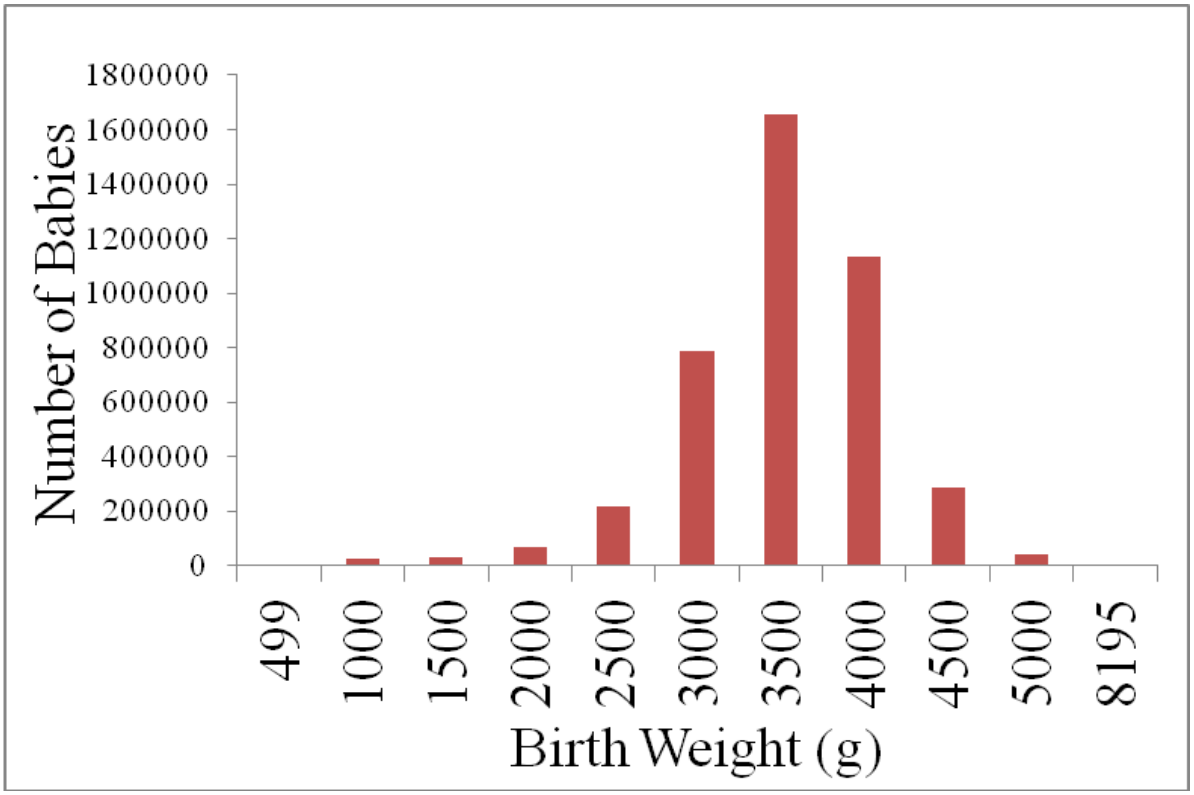
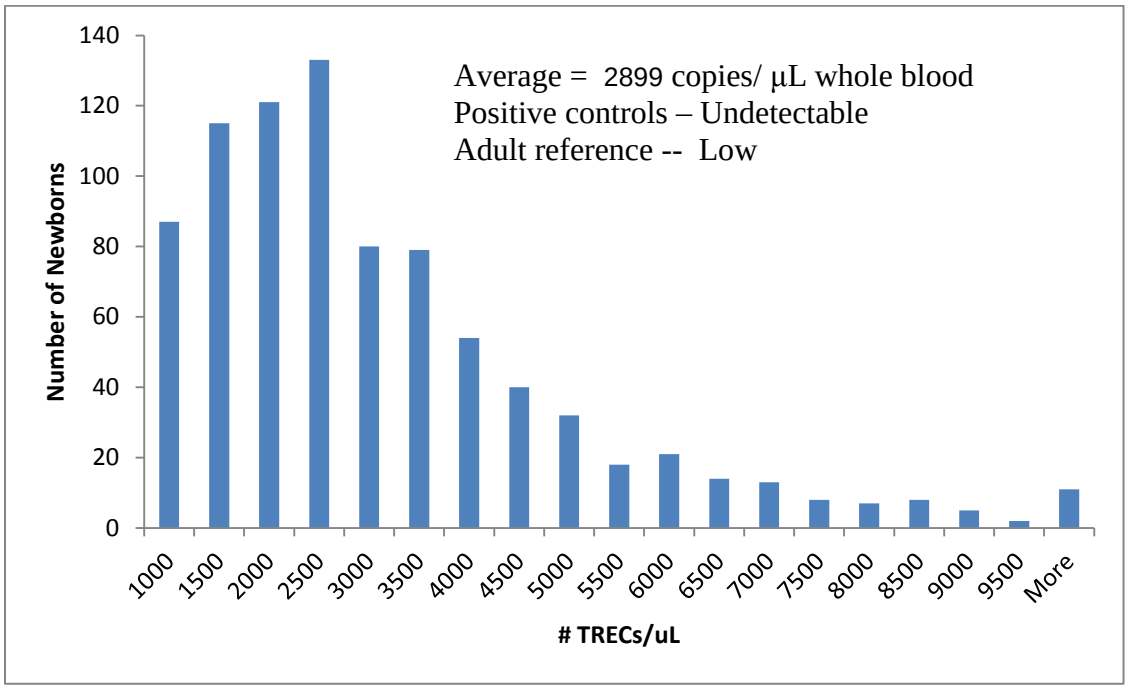


Figure 6. TREC Counts for First-Screen Specimens. a) DSHS b) U.Mass.

a)



b)

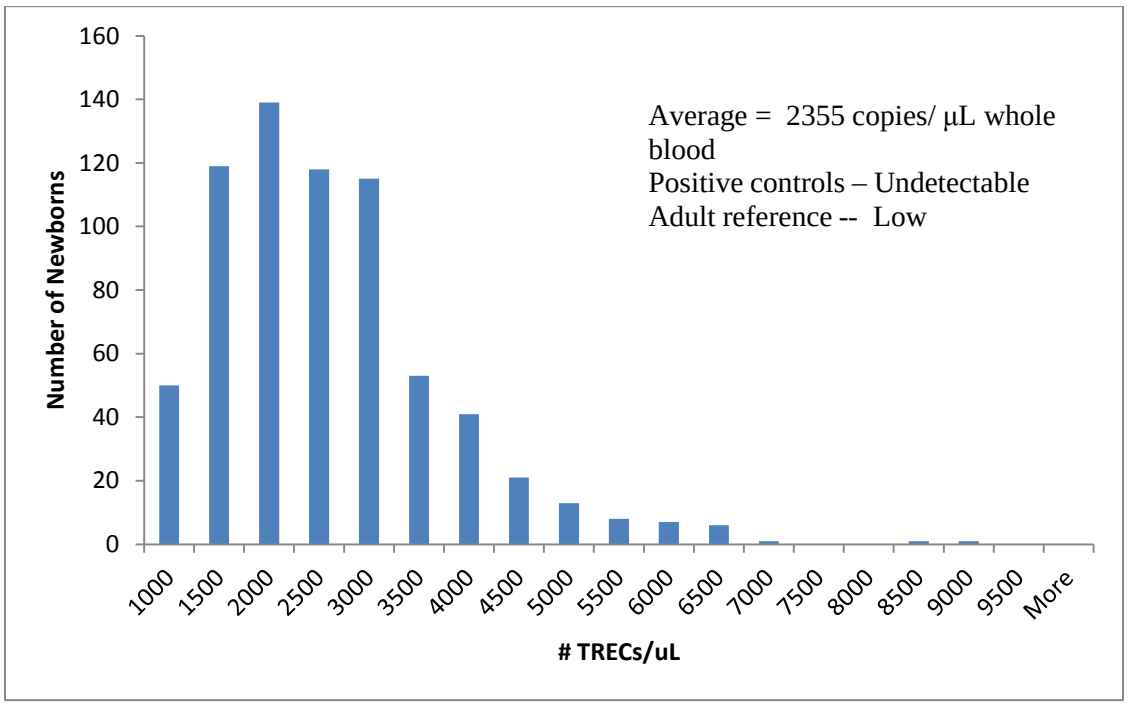
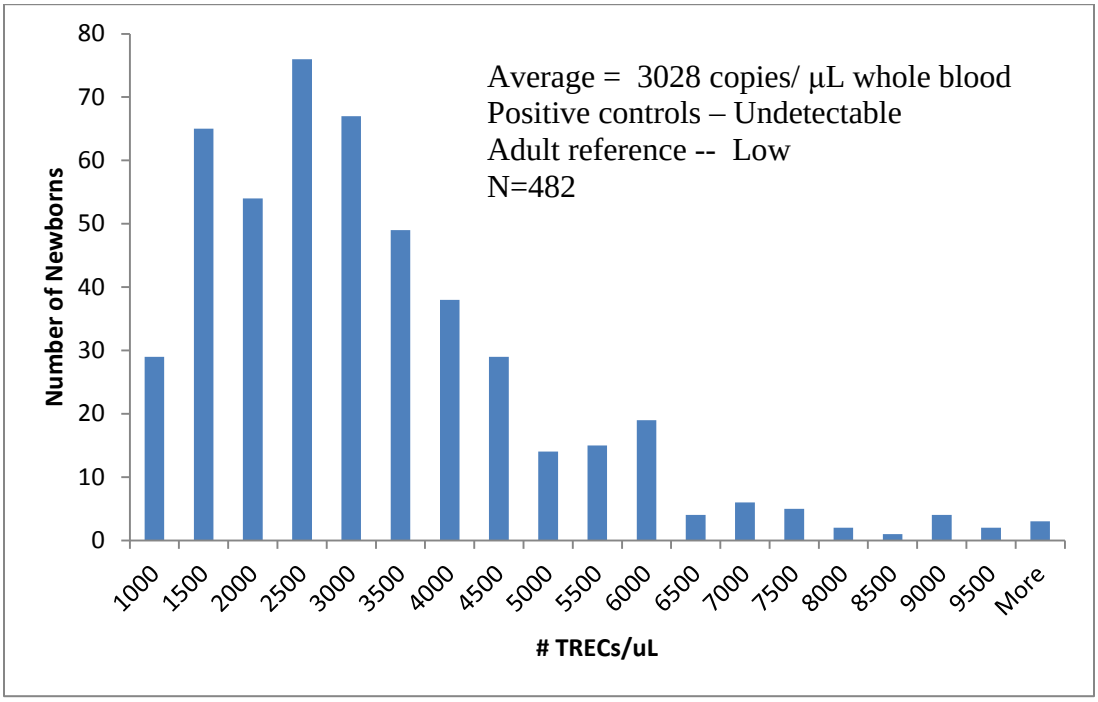


Figure 7. TREC Counts for Second-Screen Specimens. a) DSHS b) U. Mass.

a)



b)