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High Throughput Newborn Screening for Severe Combined Immunodeficiency (SCID) Using the Automated T-cell Receptor Excision Circle (TREC) in situ Assay
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Objective: Modification and validation of an automated in situ DBS TREC Assay for high throughput newborn screening (NBS) for Severe Combined Immunodeficiency (SCID) in Virginia.

Background: In September 2013 Virginia was awarded a CDC grant to implement SCID NBS.

Method: The automated in situ DBS real time PCR TREC Assay developed at CDC was adopted, using a robotic liquid handler with a selective pipetting head that can deploy 1-96 tips simultaneously. The 96 format was used for washing the dried blood spots (DBS) to achieve maximum throughput, and a single row of 8 tips was used for dispensing PCR reagent to minimize dead volume waste. One to four plates can be processed simultaneously in <30 minutes prior to PCR. Assay modifications include the use of a 1.5-mm DBS sample punch, new lab ware to reduce electrostatic charges, liquid volume adjustment and tip conditioning to improve precision of the multi-dispense, and the addition of partial plate processing within run. A platform consisting of two liquid handlers and four real time PCR instruments with 96-well format was used to meet the throughput. Reference materials from the CDC Model Proficiency Evaluation Survey (MPES), blinded samples from the New England Newborn Screening Program (NENSP) and the New Jersey (NJ) NBS program were used for assay validation.

Results: Precision and reproducibility, the major criteria for reliability of the robotic platform, were affirmed. Pipetting calibration results showed 4-plate averages were within 2.0% and 3.0% accuracy for the 125-μL (wash buffer) and 15-μL (reaction mix) volume dispense, respectively. Diagnostic accuracy of the assay scored 100% using MPES panels. Blinded samples from NENSP (n=5) scored 100% for accuracy, while the NJ sample set (n=302) scored 100% for all presumptive positive results. The assay was launched initially with provisional conservative TREC cut-offs until a large dataset could be accumulated to be used for adjustment. The first two days of screening (n=578) revealed a TREC median of 32.00 Ct with a high flag rate. Validation data were re-analyzed using less conservative cut-offs (34.5/35.8; flag rate ~7%) and an addendum to the validation was approved prior to releasing of results. Within 2.5 weeks cut-offs were further adjusted using data from 5,217 samples (TREC ABNORMAL ≥35.5 Ct, 97.7 percentile; and TREC CRITICAL ≥38.4 Ct, 99.8 percentile), reducing the TREC flag rate to 2.3%. Routine state-wide SCID NBS using this assay method has been ongoing in Virginia successfully since June 2015. Updated data will be presented in the symposium.

Conclusion: Virginia has met the goal of implementing high throughput screening for SCID using the automated in situ TREC Assay based on a new and improved robotic liquid handler with a selective pipetting head. This assay platform provides superior throughput compared to many other systems in use, as well as economy in conserving expensive reagents.

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P-2

Saving Virginia's Babies – The Virginia NBS Transit Time Project
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In 2013, in response to the heightened awareness and interest around NBS sample transit time, VA NBS Program created database queries to capture sample submission data for all submitting hospitals, statewide. These reports were reviewed and hospital submission data was analyzed. Through these findings, areas of improvement were identified and strategies were planned that would make a positive impact as quickly as possible. These strategies included changes to shipping protocols, changes in courier routes and schedules, statewide distribution of instructional packets and communication with hospital staff through direct calls, quarterly report cards and NBS team site visits to hospitals. At the same time, through this project it has become apparent that there is a tremendous need to provide more education to the parents and the healthcare professionals involved in newborn screening. The VA NBS Program has responded to that need by revising the parent informational flyer, updating/adding information to our program websites and creating educational web modules that cover all aspects of newborn screening in Virginia.

The VA NBS Transit time poster will demonstrate the successes of Virginia’s activities thus far and will discuss the program’s ongoing efforts and challenges to improve on newborn screening sample transit time and overall timeliness across the Commonwealth.

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P-3

To Mandate or Not to Mandate? That is the Question...A Collaborative Decision Making Approach in Virginia

Virginia newborn screening regulations state that the Virginia Genetics Advisory Committee (VaGAC), under the Virginia Department of Health (VDH), will consult and provide advice to the Commissioner of Health on proposed changes to Virginia’s newborn screening panel; however, every so often a state legislator will attempt to mandate screening for a disorder(s) that may not be on the Recommended Uniform Screening Panel (RUSP) and this recently occurred during the 2015 Virginia General Assembly session. This proposed mandate offered a unique opportunity for the Virginia Newborn Screening Program (VNSP) to educate and collaborate on many levels within the Commonwealth to thoroughly and thoughtfully review adding Krabbe Disease to the Virginia NBS panel instead of it becoming an unfunded mandate. These collaborations included the state legislative branch, families, medical specialists, institutions of higher education and other state newborn screening programs. A workgroup was brought together to review the science and feasibility of screening for Krabbe Disease and a formal report was sent to the Commissioner of Health to submit to the Virginia General Assembly for review. The workgroup recommended unanimously to not add Krabbe Disease to the Virginia
This poster details the collaboration and formal decision making process of answering the question of whether or not to add Krabbe Disease to Virginia’s newborn screening panel.

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**P-4**

**Sequence Analysis of TREC δRec-ΨJα Signal Joint Region of the most common ethnicities in New York State Newborn Population**


**Introduction:** Severe combined immunodeficiency (SCID) denotes a heterogeneous group of genetic disorders characterized by defects of the cellular and humoral immune responses. SCID babies are asymptomatic at birth but can die of infections during the first year of life. Early intervention by bone marrow transplant (BMT) reconstitutes immunity in 95% of patients. Absence or very low T cell counts are common features of SCID. Normal maturation of T cells requires recombination of the T-cell antigen receptor (TCR) genes generating T-cell receptor excision circles (TRECs) as a by-product. Quantification of TRECs by a multiplex quantitative real-time PCR assay (qPCR) in dried blood spots (DBS) identifies infants with SCID. In the New York State (NYS) Newborn Screening (NBS) Program, DBS with <125 TRECs/µL (whole blood) are referred for evaluation by an immunologist and flow-cytometry, and with >125 to <200 TRECs/µL are considered presumptive-positive (PP) and a second sample is requested. Since NYS started NBS for SCID >1.2 million babies have been screened, and >1800 were PP and >900 referred for diagnostic evaluation. A case with no detectable TRECs but normal T-Cell counts on flow-cytometry and an overrepresentation of the African-American population on the PP and referred populations, led us to study the genetic variability of the targeted TREC δRec-ΨJα Signal Joint Region (SJ-TREC).

**Objective:** To determine the genetic variability of SJ-TREC in NYS most common ethnicities and in NYS NBS presumptive-positive and referred SCID population.

**Methods:** TRECs and g.DNA are extracted from 3-mm diameter DBS punches as part of the SCID NYS NBS algorithm. Sequencing primers were designed outside of the screening primers-probe SJ-TREC in order to identify variants within the targeted region. Standard PCR and BigDye® Sanger sequencing (ABI 3730 DNA Analyzer) of the extended SJ-TREC were performed on one-thousand samples representing the referred, PP and the 4 most common ethnicities in NYS. Sequences were analyzed with SeqScape® 2.5.

**Results:** Several variants under the SJ-TREC TaqMan probe region were identified. Two very common SNPs, both C>T changes at the second and eighth base pair within the probe presented high frequencies and ethnic differences. Both SNPs are reported in dbSNP. The first (rs76132819) has a minor allele frequency of 48% in Caucasians versus 63% in African-Americans. The second (rs79211180) is rare. Our case with normal flow cytometry results, but no detectable TRECs had both SNPs.

**Conclusions:** SNPs under the SJ-TREC probe or primer regions increase the retest and false positive referral rates in NBS for SCID. The SNP frequencies vary within ethnicities generating an increase in false positive retesting and referral rate in some populations. By moving the SJ-TREC probe away from one of these SNPs, NYS reduced its PP retesting by > 50%.
P-5

Evaluation of Birth Hospital Compliance with CLSI Guidelines to Collect a Specimen Prior to Transfusion
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Objective: To evaluate compliance of birth hospitals with CLSI guidelines for newborn screening for preterm, low birth weight and sick newborns and to determine if outreach and education improved compliance.

Methods: Hospital-level data was collected for specimens received in 2013 with a transfusion indicated on the blood collection form and no prior specimen on record (AF specimens). All hospitals were invited to training webinars on the CLSI guidelines. Hospitals with a high number of AF specimens that did not comply with the CLSI guidelines were required to submit a corrective action plan and received additional training and education. Data for specimens received in 2014 was collected and compared to the 2013 data to evaluate the effectiveness of the educational campaign.

Results: Overall in 2013, the NYS NBS Program received 136 AF specimens. In 2014, the number of AF specimens increased to 203. In both 2013 and 2014, the same three hospitals had the highest number of AF specimens (2013: Hospital A = 26; Hospital B = 21; Hospital C = 14) (2014: Hospital A = 40; Hospital B = 24; Hospital C = 23).

Conclusion: Despite efforts to educate birth hospitals on proper NICU protocol for collection of Newborn Screening specimens, there still appears to be non-compliance with this protocol. Further educational efforts are needed to achieve better compliance.

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P-6

An Exploration of the Roles and Responsibilities of Genetic Counselors Working With Newborn Screening Programs
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Due to the hereditary nature of many of the conditions screened for, genetic counselors (GCs) are working closely with Newborn Screening Programs. The goal of this study was to elucidate the roles and responsibilities of these GCs. A 29-question survey was distributed via email to the Association of Public Health Laboratories (APHL) Newborn Screening Technical assistance and Evaluation Program (NewSTEPS) listserv as well as an international listserv administered by the National Newborn Screening and Global Resource Center. In total, 69 GCs responded, of which 97% were female and 71% were ages 25-44. In their NBS role, 77% of respondents had face-to-face patient contact, 97% had telephone contact with patients, and 69% have been involved in education via presentations and/or material production. Responding GCs worked with a variety of screened conditions, including Inherited Metabolic Disorders (72%), Cystic Fibrosis (65%), Endocrine (36%), Severe Combined Immunodeficiency
Hemoglobinopathies (32%), Lysosomal Storage Disorders (30%), Hearing Loss (14%), Critical Congenital Heart Disease (13%), X-Linked Adrenoleukodystrophy (12%), and HIV (3%). Work responsibilities included short-term follow-up (75% of respondents), long-term follow-up (43%), reporting (42%), research (30%), timeliness (29%), quality assurance (28%), grant writing (13%), unsuitable samples (13%), test development/implementation (10%), sample collection (9%), and accessioning (3%). 28% reported having supervision responsibilities in their NBS role. Genetic counselors utilize training and skill sets that make them uniquely suited for a variety of roles and responsibilities with Newborn Screening Programs.

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P-7

Revisiting the Follow-up Protocol for Initial Positive Results for Phenylketonuria (PKU)
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Introduction: The follow-up protocol for initial positive results for PKU requires a second heel-stick specimen for analysis before referral to a metabolic center. Recently, concern has been expressed that this second specimen inappropriately delays the provision of essential services.

Materials and Methods: Data from the Newborn Screening Program for 2014 were analyzed. Initial positive results for PKU were linked to the second specimen. Results were stratified by nursery type (reported as regular vs. NICU). In addition, patient data were explored using R4S tools for PKU, hyperphenylalaninemia, and TPN, including dual scatter plots to distinguish among these conditions.

Results: There were 236 initial positive results, of which 226 had a repeat specimen. Of these, 38 were from the regular nursery, and 198 were from the NICU. The second specimen gave a negative result in 187 of the 195 initial positives that resolved as false positives.

In the regular nursery, 27 of the infants were determined to have a diagnosis: 17 classical PKU, 6 variant hyperphenylalaninemia, and 4 benign hyperphenylalaninemia. Of the remaining 11, there were 5 where the R4S tools suggested TPN as the most likely condition.

In contrast, of the 198 infants from the NICU, only 5 were determined to have a diagnosis: 4 classical PKU and 1 benign hyperphenylalaninemia. Of the remainder, 180 were identified by the R4S TPN tool as being most consistent with TPN. Among the remaining specimens, 2 gave no positive results from the R4S tools, and the other 11 gave scores for both PKU and TPN. Use of the dual scatter plot resolved all but 2 of them as being most likely TPN.

Conclusion: Newborn screening programs that do not specifically derive an interpretation for TPN will have large numbers of false positives for PKU from the NICU. A second heelstick specimen when the newborn is not on TPN will often resolve the initial result without initiating a more complex follow-up protocol. Implementation of CLSI guidelines for newborn screening of babies in NICUs is one way to routinize this practice.

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California Goals for Timeliness in Newborn Screening Specimen Collection and Transit Time

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Objective: The California Newborn Screening Program joined Arizona, Colorado/Wyoming, Iowa, New Hampshire, Tennessee, and Texas in January 2015 to participate in the NewSTEPs Collaborative Improvement and Innovation Network (CoIIN) project to improve newborn screening specimen collection and transit timeliness. By the end of the project, in Spring, 2016, California’s Goal #1 is to have 95% of newborn screening specimens collected between 12 and 48 hours of life with a secondary goal that 100% of hospitals collect greater than 80% of their specimens in this time window. Goal #2 is to have the transit time for 85% of initial specimens from the hospital to the newborn screening laboratory within 48 hours of collection.

Materials and Methods: California initial newborn screening specimen data for Regular Nursery, Neonatal Intensive Care Units, and Midwife Birth centers from 2014 and 2015 were abstracted and analyzed by the California Newborn Screening Program and the CA NBS Area Service Centers (ASCs). Percentages of specimens collected in the time window were calculated for hospitals reporting at least 25 births in a given quarter. From July 2015-January 2016 the ASCs have been and will continue to be engaged in presenting this data to hospital staff and midwifery groups via telephone, e-mail and in person on-site visits and in providing support for facility quality improvement processes to reach target goals. ASCs provide assistance in analyzing hospital protocols for timeliness improvements.

Results: The CoIIN project began to show results in the third quarter of 2015. At the outset, 90.5% of CA screens were collected between 12 and 48 hours of age; the transit time was 2.10 days in the Regular Nursery and 2.01 in the NICU. Currently in the 3rd quarter over 94% of screens are collected in the desired timeframe and the average transit time is 1.96 in the Regular Nursery and 1.90 in the NICU.

Conclusion: Sharing of collection and timeliness data with birth facilities and focused education and attention on best practices can provide the basis for programmatic improvement, even in a state with a large diverse newborn practice network.

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California Thalassemia Fact Sheet & Provider Survey

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Goal: As part of the California Public Health Research, Education & Surveillance in Hemoglobinopathies project (CDC grant #DD12-1206), a Thalassemia Fact Sheet was developed to educate California physicians about Thalassemia diseases. The Fact Sheet provides an overview of Thalassemias, common symptoms, at-risk populations, and importance of newborn screening. A survey was sent to assess
provider responses to the Fact Sheet and to collect information about practice patterns, barriers to care and educational needs.

**Methods:** The Thalassemia Fact Sheet was mailed to 11,166 providers. Physicians were selected if they were “active” on the Medical Board of California Licensure Database and had a mailing address in one of the 30 cities in the state with the highest Thalassemia birth rates based on newborn screening data. The survey was sent to a subset of 7,521 providers in these cities including pediatricians (28%), family practitioners (25%), general medicine practitioners (18%), hematologists (2%), obstetrician/gynecologists (16%), and cardiologists (10%).

**Results:** 644 surveys were returned from pediatricians (34%), family or general medicine practitioners (29%); hematologists (4%); obstetrician/gynecologists (16%) and cardiologists (7%) and other (9%). Only 11% reported never having seen a patient with Thalassemia; 37% had 1-5 patients, 15% had 6-10 patients, and 34% had 10 or more patients. 90% or more of respondents rated the Fact Sheet as very good/excellent on overall appearance and clarity; 78% for usefulness in their practice.

When asked about additional information they would like to receive, 64% need guidelines for care; 45% need health education materials; 31% need information on complications and outcomes; and 28% need information on Thalassemia. Preference for receiving this information was: 44% mailed newsletter, 37% Thalassemia.com website, 35% emailed newsletter, 18% CME courses and 8% webinar.

Only 10% managed their patients independently; 16% relied on other specialists entirely; and 68% managed patients jointly with other specialist. Only 13% indicated being very familiar with Thalassemia guidelines for care, 58% were somewhat familiar; and 28% were not familiar. Among the providers not familiar with guidelines for care, 26% were family practitioners, 13% were general practice providers, 19% were obstetrician/gynecologists; 12% were pediatricians; and 11% were cardiologists.

**Conclusion:** The survey provided encouraging feedback about the acceptability and usefulness of the Thalassemia Fact Sheet and providers indicated a need for improved availability of Thalassemia guidelines for care and health education materials.

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**Findings from a Survey of Michigan Birth Hospitals: What Happens After a Failed Screen for Critical Congenital Heart Disease?**

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**Background:** Critical congenital heart disease was added to the Michigan Newborn Screening (NBS) panel in April 2014, with reporting of pulse oximetry screening results to the NBS Program. Excluding obvious data errors, hospitals reported 49 infants who failed their pulse oximetry screen in the first year following mandated reporting. Although several studies have assessed costs associated with conducting the pulse oximetry screens at hospitals, little is known about the steps that may occur after a failed screen for an asymptomatic infant and what costs a hospital may incur when caring for that infant.

**Methods:** Designated NBS coordinators for Michigan’s 114 birthing units (including regular nursery, NICU, and special care nursery) were sent a link to an online survey with questions about what processes may take place at their institution following a failed pulse oximetry screen. The survey included questions about what tests may be ordered, what medical providers may be consulted, and
under what circumstances or when the baby may be transferred to a different hospital. Results were compiled overall and stratified by both size of the hospital and its metro status.

**Results:** Coordinators from 40 of the 114 Michigan birthing units (35%) completed the online survey. All respondents were from regular nurseries, resulting in responses from 48% of Michigan’s 83 birthing hospitals. Overall, pediatricians were most likely to perform a clinical exam after a failed screen (selected by 83% of respondents), though larger hospitals reported that neonatologists or nurse practitioners may also examine the infant. Echocardiogram and four-point blood pressure were the tests most likely to be ordered after a failed screen (always ordered by 60% and 53% of hospitals, respectively). More of the larger hospitals reported that echocardiograms are performed (89%) and read on-site (61%) compared to their smaller counterparts (47% and 11%, respectively). Smaller hospitals and those in suburban areas reported having no access to pediatric cardiologists on-site, while approximately one-quarter of larger or urban hospitals had access. The majority of smaller hospitals reported transferring infants immediately after a failed screened or after a clinical exam, while larger hospitals reported transferring primarily after a diagnosis was obtained. According to NBS records, 13 of the 40 facilities with a response (32.5%) reported at least one failed pulse oximetry screen in the first year of screening.

**Conclusions:** Due to the small number of failed screens and the on-site capability for larger hospitals, the additional costs associated with failed pulse oximetry screens may not be as burdensome as originally anticipated. More information on the cost of and activities associated with failed screening for CCHD will be available as hospitals gain more experience with the process.

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**P-11**

**50 Years of Newborn Screening in Michigan: A Year-long Celebration**

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**Background:** Newborn screening (NBS) began in Michigan in 1965. To celebrate the 50th anniversary of this important public health program, the Michigan Department of Health and Human Services (MDHHS) planned a year-long campaign in 2015 designed to educate the general public, expectant parents, clinicians, and legislators.

**Methods:** Multiple approaches were used to increase NBS awareness. To maintain consistency throughout the campaign, a new program logo was developed. The NBS program identified a list of existing events for relevant target audiences including health fairs, baby fairs, and professional meetings, and assigned staff to attend as many as possible. While resources were limited, simple items (crayons and NBS coloring sheets developed by staff) were distributed at events geared for expectant parents and young families. Brochures and educational materials were distributed at professional events. Throughout the year, a variety of social media content, press releases and newsletter articles were shared with the MDHHS Communications office for dissemination both internally through the department and to external audiences. A governor’s proclamation was obtained and a day-long educational event was organized during September to coincide with NBS Awareness month. The event includes a tour of the NBS lab, recognition of hospitals that excelled in NBS bloodspot collection or hearing screening, and a symposium with a variety of speakers including lead clinicians at medical management centers and families. To promote awareness among policy makers, individualized reports were created for each state legislator. These reports listed the number of babies screened and number
identified with disease over 50 years of screening in Michigan, as well as the numbers screened and identified from birthing facilities in his/her district in the last 5 years. Individualized reports were also created for birth hospitals listing the number of screens submitted and number of babies identified with disorders in the last 5 years from that facility. An informational booklet reviewing the beginnings of Michigan’s program and special milestones throughout the past 50 years was developed.

**Results:** Staff participated in approximately 14 events during 2015. A total of 148 legislator reports and 83 hospital reports were distributed. Currently, 141 people have registered to attend at least one portion of the day-long educational event.

**Conclusions:** This year-long celebration of Michigan’s 50th NBS anniversary provided an opportunity to highlight the program in multiple ways and recognize all of the key partners who collaborate to make the NBS system successful. One of the many benefits included enhanced collaboration with the Early Hearing Detection and Intervention Program. The individualized legislator and hospital reports allowed for personalizing the impact of the program.

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**P-12**

**Use of State Administrative Data Sets for Newborn Screening Long-term Follow-up: Michigan’s Experience**

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**Background:** Michigan’s Newborn Screening (NBS) Program currently performs limited long-term follow-up of children confirmed as having disorders on the NBS panel. An increasing national focus on improving long-term follow-up efforts encouraged us to explore the utility of using linkages with pre-existing state administrative databases as a cost-effective method to enhance population-based follow-up efforts.

**Methods:** Since 2007, Michigan’s NBS Program has been routinely conducting a probabilistic linkage of NBS and birth certificate (BC) records to identify infants potentially missed by screening. BC records are linked to other state administrative databases, so the NBS/BC linkage allows for deterministic linkages across the data sets using the BC master record number. We examined a cohort of all individuals with confirmed-NBS disorders born in Michigan from 1997-2013 and linked their records with the following databases: birth certificates, death certificates, Michigan Inpatient Database (MIDB), Michigan Care Improvement Registry (MCIR), the Michigan Birth Defects Registry (MBDR), and public insurance claims data. We examined what percent of the cohort was found in each database, both overall and stratified by disease categories. Additionally, select outcomes of interest were assessed for each database.

**Results:** In total, 3,265 infants were identified with NBS diseases during these years and 3,248 (99.5%) were linked to BC records. The demographic characteristics displayed wide variability between disease categories. A total of 76 infants had died, with the highest mortality seen among those with a primary immune disorder. Approximately 82% of the NBS-confirmed cases were found in the MBDR for birth years 1997-2011. Those with cystic fibrosis had the highest average number of co-morbid ICD codes listed. Approximately 68% of cases had been enrolled in a public insurance program at some point in their lives and 50% were enrolled in 2013. Those with hemoglobinopathy or a primary immune disorder had the highest enrollment in public insurance (63% and 66%, respectively), while those with congenital...
adrenal hyperplasia had the lowest (39%). Overall, 97% of the study population had immunization records available in MCIR. Individuals with cystic fibrosis had the highest uptake of flu vaccine during fall 2014/winter 2015 season (71%), while those with galactosemia had the lowest (18%).

**Conclusions:** Our findings support the use of state administrative databases for NBS long-term follow-up. Since the majority of confirmed cases had records in these databases, they can be utilized to document the effectiveness of NBS and identify areas for improvement. Additionally, the study findings will serve as a baseline moving forward to help determine the appropriate database to use when particular long-term follow-up questions arise.

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**P-13**

**Use of Quarterly Quality Assurance Reports to Improve Hospital Performance**

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**Background:** In 2002, Michigan’s Newborn Screening (NBS) Follow-up Program began sending every birthing facility in the state a quality assurance report. These reports are distributed quarterly and include metrics that monitor different aspects of the NBS process that the facility has control over. The metrics and goal for each metric were selected by NBS Program staff and are modified as needed. The reports are designed to provide a tool for both NBS and hospital staff to quickly identify problem areas, monitor performance over time, and assess the effectiveness of any changes implemented.

**Methods:** In order to encourage the use of the quarterly reports, we provided a 30-minute overview during five regional NBS trainings for hospital staff in 2014. The presentation focused on how to use the performance metrics to improve the NBS process and described each metric in detail. The quarterly NBS newsletter contains a section that lists and defines the metrics in the quarterly report. In 2011 the newsletter began listing Michigan hospitals that are “Stellar Performers”, meaning they met all of the performance metrics during the previous quarter.

**Results:** Since including the list of high performing hospitals in the newsletter, we’ve received more feedback after the quarterly reports are distributed. Anecdotally, more hospital coordinators have asked for a list of specimens not meeting particular measures, so they could review and share the findings with staff. Several hospital NBS coordinators have requested technical assistance from the state NBS nurse consultant to improve one or more metric. In recent years, we’ve seen overall improvement in many of the metrics. In 2014, approximately 3-5 hospitals per quarter met every metric. During the second quarter of 2015, 9 of Michigan’s 113 birthing units met every metric.

**Conclusions:** The quarterly report metrics provide feedback to hospitals on whether blood specimens are collected appropriately and sent via courier in a timely manner; parental response is documented regarding the release of residual blood spots for research; pulse oximetry screening has been completed and documented; and the NBS card number is recorded on the birth certificate. We’ve found that these reports are an effective way to communicate with birthing facilities routinely with minimal time required from NBS Program staff. Additionally, the most improvement in hospital performance was seen after thoroughly explaining the metrics and highlighting stellar performers in a public manner.
A False Positive for Severe Combined Immune Deficiency (SCID) Caused by a Mutation for the MI T-cell Receptor Excision circle (TREC) Assay

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Michigan (MI) has been screening for SCID and other primary immune deficiency syndromes since October 1, 2011 with over 440,000 babies screened. To date there have been 9 babies identified with SCID. SCID is the most severe type of primary immunodeficiency. The MI method includes an automated DNA extraction from a 3.2 mm dried blood spot (DBS) followed by a duplex real time PCR assay for T cell receptor excision circle (TREC) and β actin. All automation was performed using an epMotion 5075 TMX from Eppendorf. Infants with SCID have been shown to have TREC values near zero or undetectable. When a baby has a result of zero TREC's this is considered a pediatric emergency. The baby is referred to the clinic and follow up testing is performed.

A result was reported as zero TREC's (strong positive) to the clinic for a NBS sample. Follow up testing studies including flow cytometry and mitogen stimulation studies were all normal. Because this is an abnormal situation several QA/QC steps were performed to rule out a laboratory or clinical error. These measures will be discussed in detail. It was determined after repeat testing by both the lab and the clinic the TREC assay result was a false positive. This type of false positive is the first of its kind observed in MI. A similar case was reported by New York in their manuscript detailing their first two year experience in SCID screening (2).

The purpose of this project was to determine if the MI TREC assay resulted in a false positive due a mutation preventing amplification of TREC. Different combinations of primers and probes used by MI and the CDC NBS and molecular branch (1) were used to help determine if the TREC assay failure was due to one of the primers or probes not binding due to a mutation. It was determined the mutation fell within the first 12 base pairs underlying the MI probe. Results will be presented for these experiments along with sequencing data.

MI NBS identified this case after screening over 400,000 babies. The incidence appears to be very low. The TREC region is a conserved region in which labs have designed the primers and probes. However, in very rare cases a mutation could be present prohibiting the reaction from occurring. This is a known limitation of the assay. Based on this data, it can be concluded that the mutations observed in the case in New York (2) are not in the same region of the suspected mutation in the MI case.

P-15

Glutaric Acidemia Type II and LCHAD Deficiency Cases Identified by Newborn Screening Short-term Outcome
A. Serrano Russi, University of Iowa Hospitals and Clinics, Iowa City, IA

We describe a case of Glutaric acidemia type II and a case of long hydroxyacyl CoA dehydrogenase deficiency identified by newborn screening and confirmed with molecular genetic testing (ETFA/HADHA) and in vitro testing of cultured skin fibroblasts. These cases are the first of their kind to be identified in our state by newborn screening. We describe the initial screening data and clinical follow up. None of the two cases have presented with severe illness associated with the primary condition. Long term follow up of this type of cases is recommended to determine natural history and to determine if preventive interventions will decrease morbidity and mortality for identified patients and relatives at risk.

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2 Year Clinical Follow up of a Patient with Methylbutyrylglycinuria Identified by Newborn Screening
A. Serrano Russi, University of Iowa Hospitals and Clinics, Iowa City, IA

We present the case of a girl born premature at 29 weeks gestation with a birth weight of 777 grams whose newborn screen collected at 27 hours was significant for elevation of C5 isovalerylcarnitine of 2.57 uM with a cut off of 0.6 uM and corresponding elevations of C5/C2 0.09 (<0.03) and C5/C3 1.9 (<0.49). Repeat newborn screening 7 days later showed persisting abnormalities. Clinical course up to that point was not remarkably different than other preterm newborns. There was no evidence of metabolic acidosis or hypoglycemia during her hospital stay. Acylcarnitine profile at 19 and 39 days of life showed elevated C5 acylcarnitine of 1.58 uM (<0.63) and 1.53 uM respectively with a plasma free carnitine level of 10 nmol/mL at 19 days of life. Supplementation with levocarnitine was recommended along with continued feeding with RDA for protein. Urine organic acids at 10 days of age showed elevated excretion of 2 methylbutyrylglycine of 49 ug/mg of creatine with a reference range of <7.5 suggesting the diagnosis of Methylbutyrylglycinuria. Repeat urine acylglycines at 21 days of life showed decreased methylbutyrylglycine levels to 10 ug/mg cr. ACADSB molecular genetic testing demonstrated an apparent homozygous variant of unknown significance denominated c.1186C>A (p.K396E). She was discharged at 72 days of age with no major complications. Only major illness after discharge was a severe pneumonia resulting in severe respiratory distress and hypoxemia at 22 months that improved within 24 hours without the need of invasive ventilation. Emergency protocol for the management of an organic acidemia was established and there was no evidence of a metabolic decompensation. Urinary methylbutyrylglycine level 24 hours after her acute illness was 70.90 ug/mg creatinine.

Case reports in the past have associated this condition with autism spectrum disorder and in some instances it has been found in completely asymptomatic adults. We present her clinical progress at the age of 28 months.
Cross-Sectional Survey on Newborn Screening in Wisconsin Amish and Mennonite Communities
S. Romoser, University of Iowa Hospitals and Clinics, Iowa City, IA

Low rates of newborn screening amongst Amish and Old Order Mennonite (collectively referred to as Plain communities) in Wisconsin have led to a major public health issue. Review of vital records data suggest up to 50%, or approximately 200 Plain infants, do not receive newborn screening each year, likely due to a number of variables related to their high rate of out-of-hospital deliveries and cultural practices. Given the increased prevalence of certain genetic disorders from founder mutations in this population, newborn screening is especially important for Plain infants. Unfortunately, several Plain children have developed long-term disability and fatal outcomes after costly hospitalizations in Wisconsin.

The primary aim of this research study was to gain a sense of the rate of newborn screening amongst Plain families in Wisconsin. Secondary aims were to improve our understanding of the Plain community’s knowledge and views of, as well as access and barriers to, newborn screening. Paper surveys were distributed to Plain families with assistance from Plain community members, midwives, traditional birth attendants, public health nurses and local health care providers across the state. With a response rate of 25.4%, a total of 315 households responded representing 2,010 total Plain children in Wisconsin. Results indicate a newborn screening rate of 73.4% amongst Plain respondents with 26.6% reporting never receiving newborn screening. 94.3% of households indicated having knowledge or recognition of newborn screening, 78% report favorable views of newborn screening in their community, and 81.3% perceive having access to newborn screening. The most common barriers identified include a lack of awareness about newborn screening at the time of birth (32.2%) or screening was not offered by their provider (22.9%). A statistically significant difference in the mean age of parents that had children who received newborn screening suggest an improvement in access to and acceptance of newborn screening over time. While the majority of families report having knowledge of newborn screening, still 19.8% were unsure of its importance or felt newborn screening was not important. 22.5% felt it was unlikely for them to accept newborn screening for future children.

We conclude that while the majority of surveyed Plain families report having knowledge, favorable views of, and access to newborn screening, there are still many families not receiving screening. This may be due in part to a lack of focused education regarding the importance of newborn screening in preventing major disability or death. Further research is necessary to ascertain representation from additional Plain settlements across the state for analysis with the ultimate goal of developing better outreach, educational tools, and strategies for increasing the rate of newborn screening amongst Amish and Old Order Mennonite families in Wisconsin.
Collaborative Improvement and Innovation Network (COIIN) for Timeliness in Newborn Screening - the Iowa Experience to Date
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The Iowa NBS is one of seven states working with a HRSA/APHL COIIN for Timeliness in Newborn Screening. Iowa’s COIIN Team is comprised of 5 core members from the screening lab, Follow-Up, the Iowa Hospital Association, a birthing hospital, and the Iowa Department of Public Health. We ID’d our state goal for timeliness in NBS: 95% of NBS specimens will be received by SHL within 60 hours after birth. We formed a plan to increase awareness of the importance of timely NBS and to inform birthing facilities of their performance toward meeting the goal. We designed an infographic to be sent to birthing facilities to provide a visual tool that would indicate to them how close they were to meeting our goal. The infographic shows where they were at the start of the project, where they are now, and compares it to state average at the beginning of the project as well as the current state average. We ID’d birthing centers to serve as pilot sites and to provide feedback on our infographic. These sites represented different sized birthing facilities and also encompassed the 3 major health systems in our state. In three months’ time, all pilot hospitals have improved their timeliness rates toward meeting the goal. We have formalized our infographic and are getting ready to implement it statewide and provide information about the COIIN project/timeliness in NBS via an educational webinar. Lessons learned: 1) garner support from risk managers at hospitals/health systems as well as mother/baby and lab managers; 2) partner with your state’s perinatal team if you have one; 3) be prepared; once word got out about the COIIN overwhelming response; 4) competition is a good — birthing facility staff want to be number one in the state for timeliness; 5) utilize COIIN to provide QI education on timeliness and general NBS issues. Findings: 1) people really want to do the right thing; 2) our statistics have improved from XX to YY in (time). Next Steps: 1) an educational webinar to introduce the COIIN concept/state goal to birthing facilities; 2) posting the infographic on the SHL web portal; 3) providing feedback and technical assistance; 4) site visits to provide education on NBS/timeliness. Conclusion: Iowa’s participation in the COIIN for timeliness in NBS has already proven to be beneficial to our program’s timeliness goals as well as educational and QI goals. Iowa strongly encourages that all programs participate in a COIIN for timeliness in NBS.

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Beyond Implementation: Developing Collaborative Partnerships for SCID Follow-up, Treatment, and Management

S. Mann¹, L. Bonilla², J. Boomsma¹; ¹Hawaii Department of Health Genomics Section, Honolulu, HI, ²Kapi’olani Medical Center Women & Children, Street, Honolulu, HI

In order for the state of Hawaii to responsibly add Severe Combined Immunodeficiency (SCID) to the state newborn screening panel, identifying and securing resources outside of the state were critical. The only public children’s tertiary care hospital in Hawaii, Kapi’olani Medical Center for Women and Children (KMCWC) does not have enough volume to support a Pediatric Immunologist. The Bone Marrow Transplant program at KMCWC has not completed transplants for immunodeficient children and often does not complete transplants for infants. There are no gene therapy trials available in Hawaii for SCID. Whereas, some large multidisciplinary children’s hospital, such as the University of Los Angeles Mattel Children’s Hospital, have multiple Pediatric Immunologists with well-established bone marrow transplant programs for infants with SCID and multiple gene therapy trials for newborns with SCID. The combination of its capabilities and expertise, its location near to the state of Hawai’i, and the established relationships with local physicians made a collaboration with UCLA and KMCWC an ideal arrangement. Therefore, the Hawai’i Department of Health Genomics Section sought and received funding for an Implementation Award from APHL for a collaboration between the state of Hawaii, KMCWC and UCLA. This funding is designated for UCLA to aid in the development of protocols for confirmatory testing following newborn screening, initial evaluation and management by our Infectious Disease Pediatricians, education for our community health care providers, and guidance for our Bone Marrow Transplant Program.

Information will be presented on the development and implementation of this collaborative clinical follow-up, management, and treatment model. As more disorders are being added to the Recommended Uniform Screening Panel requiring additional specialists and treatments that may not be well developed in some states, collaborative partnerships similar to the one Hawaii has created for SCID will be necessary.

Presenter: Jennifer Boomsma, MS, Hawaii Department of Health Genomics Section, 741 Sunset Ave, Honolulu, HI, 96816, Email: jennifer@hawaiigenetics.org

Developing Data Collection for Critical Congenital Heart Disease Newborn Screening

S. Mann, J. Boomsma and G. Palmer, Hawaii Department of Health Genomics Section, Honolulu, HI

Universal newborn screening for Critical Congenital Heart Disease (CCHD) was accomplished voluntarily by all birthing facilities in Hawaii by January 2014. A state mandate for newborn screening for CCHD and data collection by the Department of Health (DOH) was passed in the 2015 Hawaii legislative session and took effect July 1, 2015.

The DOH has the authority to request, collect and analyze data on CCHD newborn screening. The responsibility for the development of tools and methodology, collection, analysis, and dissemination of
the data has been assigned to the Genomics Section of the DOH. The Genomics Section houses the Newborn Metabolic Screening, Newborn Hearing Screening, Birth Defects, and Genetics Programs. Information will be presented on the successes and challenges of developing data collection (what type of data, how often data is collected, how data is transmitted), data analysis, and data dissemination. Examples will be presented to illustrate how the data analysis and dissemination supports the quality improvement activities for newborn CCHD, metabolic, and hearing screening in the birthing facilities.

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**Screening for SCID: The Colorado Experience**
E. Fields, Colorado Department of Public Health and Environment, Denver, CO

Severe Combined Immunodeficiency (SCID) includes a group of rare but serious and potentially fatal inherited immune disorders in which T lymphocytes fail to develop and B lymphocytes are either absent or compromised. Impairment of both B and T cells leads to the term “combined.” Affected individuals develop life-threatening infections due to bacteria, viruses and fungi. The newborn screening test for T cell receptor excision circles (TRECs), a byproduct of normal T cell development, identifies SCID as well as other related conditions with low T cells. For example DiGeorge Syndrome with impaired thymus development may cause low T cells and low TRECs.

Colorado was one of the first states to begin screening for SCID. In the 48 months since Colorado added SCID to its panel, approximately 270,000 babies have been screened resulting in 70 abnormal TREC results, three SCID diagnoses as well as several diagnoses of other disorders with a low T cell count. This poster will describe Colorado’s four years of data and experience with newborn screening for SCID. This includes a data analysis determining the positive predictive value and prevalence of SCID as well as the number and details of true and false positives. Additionally it will include outcome measures pertinent to SCID and some critical quality indicators, i.e., time from screening to diagnosis and time from abnormal result to involvement of a pediatric immunologist.

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Pulse Oximetry Screening for Homebirths in Oklahoma: A Collaboration between the Newborn Screening Program, the University of Oklahoma College of Medicine, and the Oklahoma Midwives Alliance

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In July 2013, HB 1387 mandated pulse oximetry screening which required all licensed birthing facilities to perform a screen prior to discharge from the facility. Homebirths are not mandated by legislation to have a pulse oximetry screen; however there is a growing interest in the lay midwife community to provide pulse oximetry screening to the families they serve. Barriers for lay midwives to provide the pulse oximetry screen include proper training of the screening procedure, selection of appropriate equipment and its cost, interpretation of results and lack of a tangible referral process.

Annually approximately 100 infants are identified with a life threatening critical congenital heart defect in Oklahoma. Approximately 800 -1000 births will be a home birth. Newborn born at home could have improved outcomes related to early identification and referral for testing through a more comprehensive pulse oximetry screening program. This project defines the collaborative efforts of the Oklahoma Nurse Midwives Alliance, the University of Oklahoma College of Medicine and the Newborn Screening program to expand pulse oximetry screening to the home birth population by providing education, developing policies and procedures and ascertaining a referral system for newborns identified at risk for critical congenital heart disease.

**Presenter:** Lisa Caton, Oklahoma State Department of Health, 1000 NE 10th Street, Oklahoma City, OK, 73117, Email: lisarc@health.ok.gov

The Oklahoma "Every Baby Counts" Quality Improvement Program

T. McCallister\(^1\), L. Caton\(^1\), L. Halstead\(^2\), S.T. Dunn\(^1\), S. Vaz\(^1\); \(^1\)Oklahoma State Department of Health, Oklahoma City, OK, \(^2\)Oklahoma Hospital Association, Oklahoma City, OK

Oklahoma law requires collection of a newborn screening specimen as soon as possible after 24 hours of age and submission of this specimen to the State Public Health Laboratory within 48 hours of collection. Unfortunately, many submitted specimens are received well beyond this time requirement, which potentially jeopardizes the health and wellbeing of Oklahoma’s babies. The Oklahoma Newborn Screening Program partnered with the Oklahoma Hospital Association and the Oklahoma Chapter of the March of Dimes to develop the "Every Baby Counts" Quality Improvement program in order to improve transit time efficiencies. The goal of the program is to improve overall statewide transit time for submission of initial newborn screening specimens to 90% compliance by March 31, 2016. Quarterly reports providing details on specimen submission times were created listing birthing facilities from the least compliant to the most compliant. Reports were posted publically on the State Department of Health website. Birthing facilities were encouraged to form teams comprised of members of all departments involved in the collection or processing of specimens, and for them to examine internal
processes. Teams participated in two facilitated WebEx sessions and a Q&A conference call to explain the program and share ideas for improvement. Currently, the State testing laboratory operates 5 days per week but allows for the state-contracted courier to deliver specimens 7 days per week. Weekend pick-up of specimens by the courier was initiated for Tulsa and Oklahoma City metro areas in January 2015 and is expected to expand to 40 of 55 birthing facilities by the end of 2015. To date, overall non-compliance has decreased from 64.13% for 2014 to 29.43% for July 2015. Improvements are expected to continue as hospitals evaluate internal processes and we strengthen communication with our newborn screening partners across the state.

**Presenter:** Tonya McCallister, MS, MPH, Oklahoma State Department of Health, 1000 N.E. 10th Street, Oklahoma City, OK, 73117, Email: tonyaj@health.ok.gov

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**A Picture Worth 1000 Words: The Use of Infographics to Convey Complex Information**

A. Gaviglio, B. Roby, S. Rosendahl, P. Constant and Mr. McCann, Minnesota Department of Health, St. Paul, MN

The advent of the “activated patient” age brings both opportunities and challenges for newborn screening. Not too long ago, most parents were unaware that their child was being screened and only received information in the case of a positive result. Today, however, parents desire information, discussions with their providers, and above all, choices. With the changing times, newborn screening has the opportunity to make all parents aware of the life-saving benefits of screening. With greater awareness and support, we can strengthen our systems, processes, and follow-up of positive results. Yet the complexity of the newborn screening process and the numerous options available to parents challenge programs to simply, transparently, and accurately educate the public without overwhelming parents or creating anxiety. We are also tasked with the challenge of educating busy providers, who often serve as the program’s front line of communication, about an ever-growing and changing program.

Recognizing the need for clearer information in government communication, the Federal Plain Language Act was signed in 2010, which directs government agencies to use clear communication that the public can understand and use. One way to incorporate plain and clear communication is through the use of infographics. While informational graphics are not a new concept, their use as a means to express and engage consumers has become more commonplace. Indeed, infographics are considered to be effective worldwide as a means of communication. As such, the Minnesota Newborn Screening Program sought to develop an infographic to help parents and providers readily understand the process of newborn screening and parental options for dried blood spots and test results after screening is complete. We will discuss the process of creating an infographic about newborn screening and parental options from ideation to finished product. We will also present our work on disseminating the product in order to ensure the information is provided to parents before screening occurs. Our process of dissemination included contacting various advocate groups and utilizing existing state listservs to reach birth facility and midwifery staff; hosting free training webinars for providers on the importance of the infographic and how best to share it with families; follow-up with birth facilities to learn parental reactions; and tracking of parental choices regarding the storage and use of dried blood spots.
**Newborn Screening Education in the Prenatal Period: New Parent Experiences and Desires**

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**Background:** Newborn screening has a long standing history of simply occurring, without significant discussion, disclosure, or consent. Minnesota has long engaged the postnatal community in active education regarding newborn screening, but began to recognize the importance of education earlier in obstetric care on this topic to allow parents more time to become informed. These internal efforts were compounded by a legislative mandate for the Minnesota Department of Health to assist prenatal providers in efforts to address newborn screening in the prenatal timeframe.

**Methods:** We surveyed 1170 new parents about their recent experiences and desired experiences for newborn screening education in the prenatal period. The survey was paper-based with a QR code and website available. Survey distribution included a one-time mailing to all individuals who experienced a birth within the previous 45 days.

**Results:** Prior awareness of newborn screening was high (89%), but obvious differences exist between current and desired educational efforts for expectant parents on this topic. The majority of expectant parents are learning about newborn screening from a nurse (51%) while parents desire to hear this information from higher level practitioners as well, such as OB/GYNs and family physicians (68% and 24% respectively). Parents report this information is being provided most often in the labor and delivery time period (63%), but desire this information in the prenatal period (93%), with an emphasis on the 3rd trimester (64%). More parents desire a conversation with their provider than what is currently being reported to occur (70% and 61% respectively). Lastly, content of these discussions is currently lacking key pieces of information parents desire, such as how parents can get results (76% desired, 27% received) and what happens with abnormal results (75% desired, 26% received).

**Conclusions:** These data suggest that parents are not getting the desired information, both in terms of quality and timing, regarding newborn screening education in the prenatal time frame. These findings have important implications for future medical practice regarding newborn screening education. They also illuminate areas newborn screening programs might be able to impact most through focused efforts.

**Presenter:** Maggie Dreon, MS, CGC, Minnesota Department of Health, 601 Robert St N, St. Paul, MN, 55164, Email: maggie.dreon@state.mn.us
Medical Provider Practices and Desired Assistance: Newborn Screening Education in the Prenatal Period
M. Dreon¹, S. Rosendahl¹, P. Constant¹, A. Eames², B. Roby¹, Z. Rezania¹, A., Dahle¹, M. McCann¹;
¹Minnesota Department of Health, St. Paul, ²Icahn School of Medicine at Mt. Sinai, New York, NY

Background: Newborn screening has a long standing history of simply occurring, without significant discussion, disclosure, or consent. Minnesota has long engaged the postnatal community in active education regarding newborn screening, but began to recognize the importance of earlier education on this topic to allow parents the opportunity to better feel informed. These internal efforts were compounded by a legislative mandate for the MN Department of Health to assist prenatal providers in efforts to address newborn screening in the prenatal timeframe.

Methods: We surveyed 396 providers of prenatal care about their current practice of education of newborn screening in the prenatal period and inquired about additional assistance that could be provided to providers encourage and support prenatal education. Survey distribution included both paper-based and electronic-based options distributed in a variety of methods, including mailings, email, and utilizing state based medical organizations for membership E-blasts.

Results: The most common time for patient education about newborn screening reported by prenatal providers occurs when patients are in the hospital, labor/delivery and postpartum timeframe (85%), while 6% report they never discuss newborn screening with their expectant patients. A large majority report they both discuss and provide written materials about newborn screening (74% and 71% respectively). A significant number of respondents (35%) report feeling un-informed regarding newborn screening. Providers indicated additional provider-specific aids desired including: written materials (54.8%), website links (43.9%), pocket cards (39.9%), and phone applications (33.8%). For patient-specific aids desired they indicated: written materials (78.3%), tear-off fact sheets (53.0%), short video (46.0%), and website links (45.2%). With reference to the option of a short video for patient education, 36% indicated they would show this video in a clinic visit and 42% indicated they would provide a link to expectant patients.

Conclusions: These data suggest that while prenatal providers report education is occurring, there are a significant number who do not feel informed about newborn screening public health practices and there are a number of resources health departments could develop to better assist their community partners in educating their expectant patient population about newborn screening. These findings have important implications for future health department practices regarding intentional development of materials for their prenatal provider audience.

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Pre-Analytic Operational Improvements: Minnesota's Experience with UPS' CampusShip®
J. Simonetti, H. Brand, M. McCann and J. Riederer, Minnesota Department of Health, St. Paul, MN

Improved sample tracking and information regarding timeliness of sample delivery has become increasingly important. Minnesota’s newborn screening program has offered all of our specimen
submitters UPS next day air service as an option for delivery of newborn screening specimens via CampusShip®.

This initiative has been designed to do the following:
- Quicker time in transit for specimen shipments
- Oversight for specimen shipments with innovative tracking solutions
- Online integration and dedicated sample pickups for newborn screening affiliate accounts
- Supplies and materials provided to these newborn screening affiliate accounts
- No cost for the shipper

UPS’ CampusShip® allows preconfiguring the online shipping platform to be a turnkey solution where the user just makes three clicks and a shipping label is ready to be sent to the testing lab. From that point, CampusShip® allows the monitoring of the sample shipment(s) with tracking tools which also provides real-time updates for transparency.

We have successfully implemented and integrated over 60 hospital based accounts within the State of Minnesota and are adding more daily. This experience includes the successful implementation for out-of-hospital (OOH) births (22 midwives in Minnesota). Overall we have seen improved visibility regarding specimen tracking and timeliness as well as improvement in ease of invoice reconciliation due to billing fields being locked down in CampusShip®. This newborn screening operational initiative to move submitters to CampusShip® has been successful. We have reduced costly errors and avoided mistakes that lead to service charges or undeliverable specimens by using shared address books and address validation. More detailed numbers will be shared.

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**P-30**

**Collaborative Partners Project: A Regional Approach to Dissemination of Newborn Screening Information**

A. Herrera Morales, Heartland Genetics Services Collaborative, Little Rock, AR, Arkansas Children’s Hospital Research Institute, Little Rock, AR

The Heartland Genetics Services Collaborative (Heartland) is one of seven regional groups funded by the Maternal and Child Health Bureau of the Health Resources and Services Administration (MCHB/HRSA), Genetic Services Branch, to develop approaches to address maldistribution of genetic resources. Heartland serves Arkansas, Iowa, Kansas, Missouri, Nebraska, North Dakota, Oklahoma, and South Dakota, and engages its partners (clinicians, researchers, laboratorians, public health officials, patients, parents, industry representatives) at a regional and national level to increase access to and improve quality of genetic and newborn screening (NBS) services in the region, and at the same time, contribute to projects of national significance.

Based on the experiences of the Heartland NBS workgroup, there is limited knowledge of NBS in the health care communities of the Heartland region. The purpose of this project is to increase awareness of NBS and ultimately affect change in practices.
Heartland piloted the Collaborative Partners Project (CPP) to connect PCPs with a state resource partner at the 20—Newborn Screening and Genetic Testing Symposium (NBSGTS). Following the meeting, PCPs and their state partners developed and implemented mutually agreed upon projects based upon an identified need. Five states participated in the pilot and qualitative results indicated increased knowledge and dissemination of the knowledge. Based on the pilot data, the CPP was conducted for a second time in 2014. This second cohort followed the same intervention procedures with the exception that the selected participants were not required to be PCPs and, along with their state partners, developed their action plans prior to the 2014 NBSGTS. Four states participated in the second cohort and completed all of the evaluation measures. The participants included a neonatologist; the Birth Center Administrator and Board member of the Midwives Association; a Director of the Program Services Advocacy Government Affairs at the March of Dimes; and a pediatrician.

The results of the baseline survey suggested that the participants’ strengths were their understanding of NBS but knowledge of genetics was less. The participants viewed themselves as change agents and that in partnership with their state representative they could further the states’ efforts to disseminate information about genetics and NBS. Post-intervention survey results showed increased knowledge and the participants continued to learn about clinical genetics and NBS after participating in APHL NBSGTS and implementing their projects.

The CPP demonstrated that a low cost intervention for the states resulted in both increased knowledge of participants as well as meaningful implementation of strategies that made a difference at the local level. Several of the projects had potential to create lasting change at the system level.

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**Identification of Rare Folate and Remethylation Defects Due to Elevated C4 Due to FIGLU in Newborn Screening**

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Butyrylcarnintine (C4) is one of the acylcarnitines analyzed by tandem mass spectrometry as part of the panel of markers screened for by the New England Newborn Screening Program. Usually a positive screen is associated with short chain acyl-CoA deficiency (SCAD) or isobutyryl-CoA dehydrogenase deficiency [IBCD]. We report 2 unusual cases identified due to a positive signal for C4 secondary to FIGLU in their newborn screening specimen (NBS).

NENSP utilizes MRM to screen for all acylcarnitine routinely including C4. An increased signal for C4 [m/z of 288] prompts a second tier scan to determine if signal was secondary to increased formiminoglumatic acid (FIGLU) with its primary signal at m/z 287.
All neonates with positive C4 are referred to the specialist for further management including those in which the increased signal at C4 was due to FIGLU. NENSP screened 939 245 neonates during 8/1/07-8/1/15. Among them 268 neonates had C4 elevations on valid NBS; 72 were confirmed SCAD cases & in 27 a diagnosis is still pending.

In 2 cases the high signal at m/z of 288 was secondary to an elevation of FIGLU. Of the 2 neonates identified with high concentrations of FIGLU, one was diagnosed with glutamate formiminotransferase deficiency (folate synthetic pathway), & the second with a homocystinuria due to a remethylation defect as a consequence of possible synergistic heterozygosity of heterozygous mutations in two genes involved in remethylation (MTR associated with Cbl G and MMACHC associated with Cbl C).

Case 1. Premature ex-27 weeker noted to have high signal at m/z 288 (2.67; Range <1.9) on the initial NBS on DOL 3 & 9.56 on repeat NBS on DOL 7. Diagnostic testing at one month of age revealed high levels of plasma FIGLU suggesting a diagnosis of glutamate formiminotransferase deficiency. The perinatal history was notable for neonatal abstinence syndrome, premature lung disease, PDA, sepsis and hyperbilirubinemia; withdrawal symptoms including some occasional myoclonic jerks, but had a normal EEG. At a recent PCP visit, at age 8, the child was noted to have hearing loss, toe walking, muscle spasms, unspecified visual disturbances, ADHD, sleep disturbance. Infant is on folic acid supplements.

Case 2. Former 35-weeker, low birth weight (1/9 kg) with a h/o necrotizing enterocolitis (NEC) and 12 cm ileal resection was noted to have high C4 signal (2.5; Range <1.9) on the initial NBS on DOL 8, and 2.66 on repeat NBS on DOL 21, following a normal screen on DOL 3. Diagnostic testing initiated on DOL 12 revealed elevated homocysteine & slightly low methionine. In view of the neonate’s history of NEC a B12 deficiency was suspected. However no improvement was noted with B12 treatment. A Cobalamin Homocysteine Methionine NGS panel -20 gene sequencing & Del/Dup panel revealed heterozygous mutations on 2 genes involved in remethylation were found (MTR associated with Cbl G and MMACHC associated with Cbl C). Currently infant on Betaine and is doing well.

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**Quality Assurance Highlights and Educational Efforts in Washington State**

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**Background:** The Washington State Newborn Screening Law was revised by the State legislature in June 2014 to include mandates on the timeliness of newborn screening specimens and to require a publicly published annual report on submitter compliance and performance. In response, the Newborn Screening Program established new systems for tracking submitter performance and identifying providers and facilities in need of targeted education and outreach. In addition, new reports and educational materials were developed to improve and expand understanding of newborn screening and the guidelines in Washington State.

**Objective:** Highlight NBS activities in monitoring and reporting submitter performance as well as education and outreach efforts used for improving timeliness and quality of newborn screening in Washington State.
**Monitoring and Reporting:** Describe changes and updates made to the NBS database system to monitor submitter performance and produce meaningful reports. Display new and updated reports and materials used to inform and improve submitter performance and compliance on newborn screening policies and guidelines.

**Education and Outreach Activities:** Follow the new NBS mascot “Storkie” as he visits hospitals and clinics throughout Washington, attends prenatal conventions, and hosts tours and training sessions at the NBS laboratory. Display new educational materials developed for healthcare providers such as Tips for Timeliness, and educational materials geared towards new and expecting parents as well as the general public.

**Next Steps:** Efforts to improve timeliness and quality are ongoing. Individual submitter performance varies quarter to quarter, indicating continual education and outreach is necessary to implement lasting improvements. At a program level, additional efforts are underway to streamline and target outreach activities. On-line training sessions are being explored in order to maximize limited staff resources. Within the NBS database, on-demand performance reports are in development, which will allow for real-time intervention. Enhancements to the NBS website are underway to improve functionality and access to educational materials.

**Presenter:** Ashleigh Ragsdale, MPH, Newborn Screening Program, 1610 150th St NE, Shoreline, WA, 98155, Email: ashleigh.ragsdale@doh.wa.gov

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**P-33**

**Utilization of Lean Six Sigma to Expedite the Initiation of Testing Processes in Texas**

B. Reilly, P. Trevino Gonzales, T. Odoms, A. Vinyard and R. Lee, Texas Department of State Health Services, Austin, TX

**Objective:** Using a Lean Six Sigma approach, reduce overall laboratory turnaround times by developing a system workflow that maximizes the laboratory’s ability to complete the punching process and initiate testing on the day of specimen receipt.

**Background:** In the current Texas laboratory system for completing Newborn Screening (NBS) testing, presumptive positive results are communicated to Clinical Care Coordination between 2 and 5 days after specimen receipt depending on the disorder. Final results for the entire panel are reported between 4 and 6 days after receipt. A constraints analysis of the laboratory system was performed. Multiple bottlenecks were identified, including a significant constraint to the system due to the practice of initiating the punching process 1 day after receipt of specimens.

**Methodology:** A project following the Lean Six Sigma process (Define, Measure, Analyze, Improve, Control) has been performed to remove the delayed punching barrier to timeliness improvements. During the Define phase, the primary metric was identified as:

- Percent of initial sample punches completed the same day received in the laboratory

Secondary measures will track the effect of improvements on Texas’s ability to meet ACHDNC recommendations for timeliness measuring the times from receipt of the specimen in the laboratory to:

- Release of presumptive positive results for critical disorders
- Release of presumptive positive results for non-critical disorders
- Reporting of complete results
**Results:** The Texas NBS Laboratory will examine the defined goal, purpose, scope, recommendations, implementations and outcomes of this project. The projected and actual impact on Texas’s ability to meet timeliness measures will be evaluated.

**Presenter:** Brendan Reilly, Texas Department of State Health Services, PO BOX 149347, Austin, TX, 78714-9347, Email: Brendan.Reilly@dshs.state.tx.us

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**P-34**

**The Road to 95% – Texas and the NewSTEPs CoIIN**

R. Lee, B. Reilly, S. Arreola, K. Hess, A. Arreola, C. Chapa and S. Tanksley, Texas Department of State Health Services, Austin, TX

**Objective:** To review ongoing efforts and evaluate the effectiveness of new specimen transit time improvement activities implemented by the Texas Newborn Screening Laboratory as part of the NewSteps Collaborative Improvement and Innovative Network.

**Background:** Over the last decade, the Texas Newborn Screening Program has launched various initiatives in a continuing effort to assist submitting facilities in improving pre-analytical specimen quality measures. In 2013, Texas implemented a Transit Time Workgroup that leveraged enhanced national awareness and media attention to implement educational efforts and to initiate a series of new activities aimed at reducing specimen transit delays in Texas. Efforts under this project improved the percentage of initial screen specimens received in the laboratory within 3 days of collection from ~70% to ~90%. Despite this improvement, over 3,000 1st screen specimens per month continued to experience delayed delivery to the laboratory.

In January 2015, Texas joined 7 other states in working together as part of a NewSTEPs (Newborn Screening and Technical assistance and Evaluation Program) Newborn Screening Timeliness CoIIN (Collaborative Improvement and Innovative Network) to identify barriers to timely newborn screening. As part of CoIIN, Texas aimed to improve the percentage of initial screen specimens received in the laboratory within 3 days of collection from ~90% to 95%.

**Methodology:** The Texas CoIIN Team evaluated the effectiveness of ongoing laboratory activities implemented by the Transit Time Workgroup, reassessed efforts, and developed new strategies and approaches to address delayed specimen transit times. Texas developed and monitored various measures to estimate the effectiveness of each of these new approaches. In addition, Texas monitored and reported to CoIIN quality indicators measuring:

- Time between birth and specimen collection
- Time between specimen collection and receipt by lab
- Time between specimen receipt and reporting of complete results
- Time between birth and reporting of complete results

**Results:** Texas will present a summary and analysis of newly initiated activities. Data will demonstrate the estimated effectiveness of each new approach and activity as well as the overall trend for statewide compliance with national recommendations for timeliness in newborn screening.

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Second-tier Newborn Screening DNA Testing for VLCAD Deficiency in Texas
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Objective: To develop, evaluate, validate and implement a new method for sequencing analysis of all 20 exons and adjacent intervening sequence (IVS) splicing regions of the ACADVL gene for 2nd-tier newborn screening of Very Long Chain Acyl-CoA Dehydrogenase (VLCAD) deficiency in the Texas Newborn Screening Program.

Methodology: Each newborn in the state of Texas is screened for VLCAD deficiency by Tandem Mass Spectrometry (MS/MS). Specimens with elevated tetradecanoylcarnatine (myristoylcarnatine), tetradecenoylcarnatine, hexadecanoylcarnatine (palmitoylcarnatine), octadecenoylcarnatine, and/or the ratio of tetradecenoylcarnatine to acetylcarnatine are reported as possible VLCAD and will be reflexed to 2nd-tier DNA testing. For this test method validation, 13 samples will be evaluated. Twelve sets of primers covering the 20-exons and adjacent IVS regions of the ACADVL gene were developed to allow simultaneous amplifications using the same reaction conditions in a 96-well plate format. Additionally, forward and reverse sequencing is performed at the same time using all 12 primer sets. Life Technologies BigDye Direct Sequencing kit is used for PCR amplification and cycle sequencing PCR. To further streamline the testing process, improve efficiency, and reduce turnaround time, this sequencing procedure is semi-automated. Biomek 4000 liquid handler will dispense master mix for PCR into the 96-well plate, and a second liquid handler will dispense sequencing primers and cycle sequencing master mix to the first PCR product. A modified clean-up method was developed using Zymo Research Sequencing Clean-up kit. Samples are loaded on to the Life Technologies 3130 Genetic Analyzer which employs a 4-capillary array to analyze four samples simultaneously. Mutation Surveyor software rapidly analyzes sequence files.

Results: The sample preparation procedure can be completed within one working day followed by an overnight sequencing analysis run using the Life Technologies 3130 Genetic Analyzer. With mutation analysis using Mutation Surveyor, the total turnaround time including sample preparation and data analysis is expected to be 2-3 working days. The accuracy, precision, clinical specificity, clinical sensitivity, reportable range, reference range, and mutations identified will be presented. Literature reviews have been conducted to investigate potential clinical consequences of mutations identified and determine appropriate result interpretations and recommendations.

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GALT Mutation Analysis in New Jersey: Development, Implementation, and a Year of Babies
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Background: Classic galactosemia is an autosomal recessive disorder characterized by elevated galactose in the blood due to the absence or dysfunction of an enzyme responsible for the transformation of galactose to glucose, galactose-1-phosphate uridylyltransferase (GALT). Treatment
consists of removal of galactose and its major precursor, lactose, from the diet, and should be initiated as early as possible and maintained throughout an individual’s lifetime.

**Current Approach:** The New Jersey Newborn Screening (NJ NBS) Laboratory screens all babies for galactosemia by measuring levels of GALT activity and concentrations of total galactose in dried blood spots. Babies with low enzyme activity are usually switched to soy formula by their physicians while diagnostic testing, including mutation analysis, is performed.

**Quality Improvement Opportunity:** Diagnostic testing is expensive and can take a few weeks to complete. In addition to identifying babies with classic galactosemia, screening may also result in false positives or identify babies with reduced GALT activity due to carrier status or non-pathogenic mutations. These babies may be switched unnecessarily to soy formula while awaiting diagnosis. Performing mutation analysis during screening represents a quality improvement for the NBS system that benefits the physicians and affected families.

**Action:** To minimize the need for costly and time consuming diagnostic testing and to lessen the likelihood of removing a child from breast milk, the NJ NBS Laboratory modified and implemented a second tier molecular assay to test babies with low GALT activity for 3 common pathogenic mutations (Q188R, K285N, and S135L) and one mutation that leads to a reduction in GALT levels (Duarte D2N314D).

**Results:** GALT mutation analysis was added in October 2015; however, all babies with low GALT activity in 2015 were analyzed during the pilot study step of the method modification. Mutation frequency for all of 2015 will be presented.

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**P-37**

**Newborn Screening for Critical Congenital Heart Defects (CCHD) in New Jersey**

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**Discussion:** Congenital heart disease (CHD) is the most common birth defect in the United States and the leading cause of infant deaths due to birth defects. Approximately 9 out of every 1,000 infants are born with CHD, 25% of which are considered to be critical congenital heart defects (CCHD) requiring either a catheter intervention or surgery in the first year of life. Early diagnosis of CCHD allows for optimal treatment and best outcomes. Infants with undetected CCHD are at risk for death or significant disability.

While unable to identify all infants with CCHD, pulse oximetry has been shown to be an effective tool in the detection of previously unrecognized CCHD. Pulse oximetry screening is most likely to detect seven of the CCHDs. These seven primary screening targets are hypoplastic left heart syndrome, pulmonary atresia (with intact septum), Tetralogy of Fallot, total anomalous pulmonary venous return, transposition of the great arteries, tricuspid atresia, and truncus arteriosus. Pulse oximetry screening may also detect other significant medical conditions which cause low blood oxygen levels.

In August, 2011, New Jersey became the first state in the nation to implement legislatively mandated CCHD screening using pulse oximetry. Shortly thereafter, the Secretary of Health and Human Services
recommended that screening for CCHD be added to the recommended uniform screening panel (RUSP). The majority of states have since followed suit in mandating or requiring pulse oximetry screening.

**Objective:** To evaluate implementation of the statewide mandate for newborn CCHD screening.

**Methods:** Evaluation of screening coverage for a new statewide mandate is important to identify challenges in implementation and to ensure that all eligible births are being screened. Aggregate and individual level data reporting mechanisms were established to assess statewide surveillance efforts. A template was provided to birthing facilities to record live births, number screened, explanation of discrepancies, and number of failed screens. Detailed information regarding the failed screens is reported to the New Jersey Birth Defects Registry (NJBDR). Analysis of the reported data allows determination of the unique contribution of the mandated screening to detection of previously unrecognized critical cardiac defects and other significant medical conditions.

**Results:** Screening coverage rate is high. From August 31, 2011-December 31, 2014, 99.7% of eligible births were screened using pulse oximetry. According to NJBDR data from August 31, 2011 to June 30, 2015, 19 infants with previously unrecognized CCHD were detected. Screening also identified infants with other congenital heart defects and significant medical conditions.

**Conclusion:** Pulse oximetry screening has the potential to identify a number of asymptomatic infants with CCHD that otherwise might be discharged from the hospital prior to diagnosis.

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**P-38**

**NewSTEPs Site Visits Have Tangible Impacts**

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**Background:** NewSTEPs (Newborn Screening Technical assistance and Evaluation Program) is a national newborn screening program designed to provide data, technical assistance, and training to newborn screening programs across the country and to assist states with quality improvement initiatives. At the request of the state newborn screening program, NewSTEPs provides a non-regulatory Evaluation Site Visit aimed at assessing various components of a newborn screening program including the laboratory system, birth facilities, and follow-up system for quality improvement purposes. The comprehensive Evaluation Site Visit is facilitated by a team of experts that evaluate programs in a customizable manner, with a focus on assessing programmatic areas including state legislation and policy, ethics, funding models, organizational structure, point of care testing, education, and more.

New Jersey: A comprehensive evaluation site visit to the New Jersey Department of Health NBS Program was conducted in April 2013. The evaluators commented on dedicated Program leadership having a strong relationship with the subspecialists throughout the state. They identified improvement opportunities with system communication and certain quality assurance activities.

Florida: A comprehensive evaluation site visit to the Florida Department of Health NBS Program was conducted in February 2014. As a result of this visit, Florida made modifications to the program such as changing a specimen collection requirement and changing the specimen tracking time. The review team confirmed Florida’s best practices and shared suggestions from other states’ program practices.

**Results:** Based on findings from the evaluation site visits, a strengths, weaknesses, opportunities and threats (SWOT) analysis was conducted in each state to capture high level perspectives of evaluator
perceptions of the respective NBS Program. In addition, lists of recommendations were provided for the Programs to consider. Results from the SWOT analyses and changes implemented as a result of the reports’ recommendations will be presented.

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Timely Newborn Screening Status Assessment
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Background: Newborn screening (NBS) is a vital public health service. An NBS program is “successful” when all eligible infants are timely screened, and all affected children are timely identified and treated. There is a need to timely assess the percentage of eligible infants not receiving a valid newborn screening test, one of the NewSTEPs quality indicators.

Objectives: As a part of the Wisconsin NBS Program’s continuous quality improvement efforts using NewSTEPs quality indicators as a guideline, we established a policy of “Report Newborn Screening Status on Every Wisconsin Birth” to ensure that all eligible newborns in the state have access to newborn screening in the required timeframe. To facilitate the practice of this policy, we also established a procedure of recording and tracking NBS status on newborns.

Methods: First, we distributed the policy letter at the end of January, 2015, five months before the official implementation date of July 1, 2015. Second, we modified the demographic information section of NBS specimen collection cards to enable submitters to report “not screened” status, including deceased, refused, or transferred. Those revised cards were made available to all NBS submitters two weeks after the policy announcement, and all NBS submitters were encouraged to use the revised cards immediately, and were directed to exchange old cards for the revised ones. Cards submitted as “not screened” without blood sample collection were replaced without charge on a monthly basis.

Results: The NBS submitters began to use the revised cards in February. The proportion of the revised cards in total submitted cards increased steadily each month, and reached 98% in August, the second month of official policy implementation. Between March and August, we had 316 cards submitted as “Blood Not Screened”: 56 refused, 16 deceased, 234 transferred, and 10 early discharged. All the early discharged newborns received NBS later. All the transferred newborns except one received NBS in receipt hospitals, and 89% of them had NBS specimens collected within the recommended collection time of 24-48 hours after birth. Because of the established recording, tracking, and follow-up process, a twice-transferred newborn got NBS done, and was identified and confirmed to have congenital hypothyroidism. The newborn received treatment, and his hypothyroidism has been well managed.

Conclusions: We have successfully established and implemented a policy of “Report Newborn Screening Status on Every Wisconsin Birth”. The accompanied procedure allowed us to timely record and track NBS status on newborns, and to take follow-up actions when it is necessary to ensure that NBS eligible
newborns receive NBS. The continuous effort will be focused on an electronic notification, submission, and reporting system that allows a real-time NBS status assessment, and improves timeliness in the NBS system.

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**P-41**

**Assessing Residual Blood Spot Volumes in the Midst of Newborn Screening Expansion**

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Newborn screening (NBS) is one of the most successful public health programs of the past fifty years. It has allowed the identification and early treatment of infants before significant and often irreversible consequences have set in. Most tests included in NBS in the United States are performed using dried blood spots on specially designed filter paper. Currently, the Recommended Uniform Screening Panel (RUSP) in the United States has 29 primary conditions, and 31 secondary conditions. Recently, the ACHDNC has recommended the addition of MPS-I and X-ALD. Other conditions (e.g., Krabbe Disease) have been formally proposed but not yet added, and a still larger group has been named as potential targets for screening, with methods being developed and pilot studies undertaken. One concern is that as more conditions are added to NBS panels there may be insufficient specimen to perform testing for all of the conditions.

The volume of blood collected on a NBS card is approximately 375 μL (i.e., 5 spots of 75 μl each). Some specimens are not satisfactory for testing, and others have a mixture of satisfactory and unsatisfactory spots. We examined existing Georgia Department of Public Health (DPH) records for the number of newborn screening specimens submitted that were deemed to have insufficient blood volume for testing (QNS), based on the current requirements. We then examined a selection of cards on which all testing was completed. We determined how many circles had blood applied to them (maximum = 5), how many circles were satisfactory for testing (maximum = 5), how many punches had been made for all required tests on the specimen (minimum = 9), and how many potential punches were left (maximum = 21). The maximum number of punches (3.2 mm diameter) on a properly collected card is 30. Based on the average number of spots punched from a card, we calculated an average repeat rate of 25% for the entire panel. Any repeat analysis is done in duplicate, and in some cases more than one test needs to be repeated from a single card. The predicted repeat rate was used to determine the rate at which additional tests added to the screening panel would result in specimens being insufficient to complete the full testing menu.

By examining the false positive rate of existing tests in the NBS setting, and the current rate of QNS specimens, we have projected out the increase in QNS samples with each additional test added to the panel. Based on our current QNS rate of 0.3%, Georgia could add an additional two conditions requiring a 3.2 mm punch before reaching an internally unacceptable level of QNS specimens (i.e., 1%), or modifying the amount of blood required on the NBS card, which is not desirable.
Timeliness of Metabolic Screening in Tennessee - A Progress Report

Y. Li, S. Guerra, M.C. Dorley and T. Childs, Tennessee Department of Health, Nashville, TN,

**Introduction:** In responding to a 2013 report in the Milwaukee Journal Sentinel that revealed significant delays in newborn screening in many states in the US, Tennessee joined seven other states in the Newborn Screening and Technical Assistance and Evaluation Program (NewSTEPs) in January 2015 to identify areas of improvement in the newborn screening system with the aim of increasing timeliness of dried blood spot (DBS) collection and specimen transit. In this presentation we will share Tennessee’s experience and data.

**Method:** Tennessee adopted two quality indicators developed by NewSTEPs: 1. Time from birth to DBS specimen collection; and 2. Time from specimen collection to receipt by the lab. Intervention activities included conducting site visits to educate hospital staff, implementing courier services in all hospitals, adding Saturday schedules for both lab testing and follow-up of presumptive positives, revising the DBS form to include information on refusal and infant death, and sending hospitals monthly quality indicator reports and lists of infants not receiving screening. We used data for January-July 2014 births as the baseline and the monthly trends for 2015 births to monitor changes in the two quality indicators.

**Results:** DBS specimen collection time: At baseline, 85.7% of specimens were collected between 24-28 hours after birth, the ideal time for DBS collection. From January – July 2015, about 88% of specimens were collected between 24-28 hours after birth with slight variation from month to month. These were slight improvements from baseline, but results were still lower than Tennessee’s target of 95%. The average time from birth to DBS collection in 2015 by month ranged from 37.5-38.4 hours, a slight improvement from the baseline of 39.2 hours.

Transit time from specimen collection to receipt by the lab: At baseline, 18.7% of specimens were received on the same day or one day after the day of collection, i.e. the ideal transit time. This percentage increased to 27.5% in April, when the courier services were implemented for East and West Tennessee. The percentage further increased to 39.1% in July, when the courier services were implemented statewide. Despite doubling percentage of specimens received within one day after birth, results are still far behind the goal of 95%. The average transit time at baseline was 73.0 hours and dropped to 61.4 hours in April (12% drop) and 44.5 hours in July (39% drop).

**Conclusions:** Tennessee has seen slight improvement in DBS collection time and significant improvement in transit time after the implementation of intervention activities. Despite the demonstrated improvements, more efforts are warranted in order to reach our goals. We plan to initiate a pilot project with select hospitals that involves sending them weekly lists of no screening reports derived from linking raw birth certificate information with newborn screening data.

**Presenter:** Yinmei Li, MD, PhD, Tennessee Department of Health, 710 James Robertson Pkwy, Nashville, TN, 37243, Email: yinmei.li@tn.gov
Improvement in Transit Times for Newborn Screening Specimens in North Carolina
A. Grush, K. Sanderson and S. Zimmerman, North Carolina State Laboratory of Public Health, Raleigh, NC

Objective: Decrease NBS specimen transit times from collection to laboratory receipt to meet national guidelines. Clinical Laboratory Standards Institute (CLSI) recommends specimens should be mailed as soon as they are dry and within 24 hours of collection and practices established for daily transport to the testing laboratory.

Methods: The average transit time of 5 days (days from collection to receipt) for July-December 2014 initial samples was calculated using data derived from the LIMS. The data did not include specimens sent over weekends or holidays as NCSLPH currently tests Monday–Friday. Ten percent of hospitals have transported specimens using private or commercial carriers or state courier while the remaining 90% send specimens by USPS. Communications with hospitals revealed that specimens were held or batched before sending and some hospitals sent samples to a second location before mailing. In October 2014 a memorandum to NBS partners detailed the importance of timely transport and mailing within 24 hours of collection.

In February 2015, the NCSLPH initiated a dialogue with the North Carolina Hospital Association (NCHA) regarding transit time from birthing hospitals to NCSLPH. An initial meeting outlined the importance of timely transport and shared transport time data from hospitals. NCSLPH and NCHA agreed to collaborate toward a goal of reducing transport time of specimens from 5 days to 48 hours after collection. NCSLPH agreed to provide monthly transit time data on each hospital to the NCHA. In July 2015, the NCHA’s Quality Center hosted a webinar for member hospitals where information on the importance of NBS and timely transport was presented. The NCHA uses NCSLPH-provided data to create a dashboard detailing the average transit time of each birthing hospital. This dashboard is available on the NCHA website and serves as a “report card” for all birthing hospitals.

Results: Since the inception of the dashboard, average transit time for hospitals has decreased from 5 to 3 days; 90% of samples are received within 4 days. The reduction in transit time is partly due to birthing hospitals modifying their transport methods to overnight delivery of specimens. As of December 31, 2015, the percentage of hospitals converting to private or commercial carrier for delivery has increased to 93%. Due to the increase in the number of hospitals sending samples in a timely manner, the percentage of first test samples arriving within 2 days of collection has increased to 55%, with the percentage of samples arriving within 1 day of collection at 24%.

NCSLPH continues to communicate with non-compliant hospitals and to track compliance. NCSLPH has recommended the use of a tracking form to ensure that specimens sent by the hospital are received at NCSLPH. To further reduce the turn-around-time for NBS results, NCSLPH is making plans to implement 6-day/week operations in 2016.

Presenter: Ann Grush, BA, NC State Laboratory of Public Health, 4312 District Drive, Raleigh, NC, 27607, Email: ann.grush@dhhs.nc.gov
Quality Improvement Efforts in Arkansas
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Problem: Sample delivery times and the number of hours from birth to reporting of results exceeds the timeframe as determined by the Newborn Screening Quality Improvement Team in Arkansas.

Methodology: In 2013 the LHU courier service was utilized for sample delivery and a newborn screening toolkit and sample collection video were distributed. Monitors were put in place and some improvement was seen in sample delivery but there were areas identified that needed to be re-assessed in order to provide better education and technical assistance to the hospitals.

A QI team of interested stakeholders was formed. The team held quarterly meetings and reviewed processes and data. The NBS program and lab representatives took part in quality improvement meetings initiated at two local hospitals, piloting projects that focused on turnaround time at three points – birth to sample collection, collection to receipt at the public health lab and receipt to reporting of all results.

In the fall of 2014 the lab implemented a Saturday work schedule. Samples are received via courier and overnight delivery services from 8-10 AM. To date, roughly 25% of the birthing hospitals take advantage of Saturday specimen receipt and testing.

The nurse educator made annual visits to each birth facility and Arkansas Children’s Hospital and offered technical assistance as needed throughout the year. She shared demographic statistics and quarterly graphs on sample submission. In addition to quarterly graphs, a project that was started at the end of 2013, a trend chart was sent to each hospital CEO, nursery/NICU and laboratory managers each year showing placement of the facility compared to others in the state.

The Rules and Regulations Pertaining to the Testing of Newborn Infants were revised and implemented on May 1, 2015. Each birthing facility’s CEO, lab and nursery managers and licensed lay midwives received copies of the document. A letter was sent out with this and included a link to the web site.

Results: There was a significant increase in sample delivery to the lab within 48 hours. The first measurement taken in December 2013 was 15%. At the end of December 2014 this measurement increased to 45% and by the end of December 2015 this measurement had increased to 63%.

The average hours from birth to reporting decreased from 218 hours in January 2014 to 153.2 hours at the end of October 2015. An increase was seen in the month of November due to low humidity and increased static electricity causing QC problems for one assay that resulted in a delay in printing completed reports.

Conclusions: The implementation and activities of the QI team and the inclusion of appropriate stakeholders as active participants in this process will continue. We remain committed to working with our stakeholders in increasing the number of samples submitted in a 48 hour timeframe thereby decreasing the number of hours from birth to reporting results; assisting staff with identifying gaps and barriers to timely sample collection and delivery; offering suggestions for improvement; and working with each facility to set attainable goals.

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**Integrated Bloodspot-Hearing Newborn Screening Hospital Scorecards**
K. Petritis, T. Zhang, S. Aponte, R. Mehta, F. Altmaier, and W. Jacox, Arizona Department of Health Services, Phoenix, AZ

**Objective:** Most hospitals thrive for excellence through quality assurance and continuous protocol improvement; unfortunately, many hospitals lack the time and resources to achieve these objectives. With newborn screening, it is often difficult for the hospitals to establish and routinely monitor baseline key performance indicators since they might not have access to the appropriate data (e.g. insufficient quantity and unsatisfactory specimens etc). We have developed a hospital scorecard that provides a comprehensive assessment of hospital performance in several bloodspot and hearing key performance indicators. This scorecard will allow hospital management to track and compare key metrics over time and identify areas of improvement.

**Method:** The data necessary to compile the quality indicators and other statistics in the hospital scorecard were extracted with SAS or other query tools from Neometrics and HiTrack data management systems. Excel was used for most graphs and the hospital scorecards were developed in Microsoft Publisher.

**Results:** This first iteration of hospital scorecards has been generated for all Arizona hospitals with birthing facilities in 2013 and 2014, and benchmarks hospital performance for several bloodspot and hearing key performance indicators (KPI). The hearing KPIs include total newborns screened, total babies screened within 1 month, outpatient return rates, lost to follow up and inpatient pass rate. The bloodspot KPIs include the average age at collection for first and second screens, transit time (i.e., collection to receipt by the lab), as well as the percentage of unsatisfactory and insufficient quantity specimens that were received. Furthermore, the hospital scorecards depict a peer-to-peer comparison of KPIs across hospitals within the same peer group. Additional information is provided in the scorecard, including the number of first and second screens, confirmed cases per year, and number of transferred babies.

**Conclusion:** We have developed integrated bloodspot and hearing newborn screening hospital scorecards that provide a comprehensive assessment of hospital performance in several quality indicators, allowing the hospital management to track and compare key metrics over time and identify areas of improvement.

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**Regional Newborn Screening Laboratory Backup Planning: 2015 Snapshot**
H. Andersson and W. Perry, Tulane University School of Medicine, New Orleans, LA

Newborn screening laboratories have demonstrated vulnerability to local and regional emergencies. Hurricanes Katrina and Rita caused outsourcing of newborn screening of Louisiana samples to Iowa for over two years while the newborn screening laboratories were reestablished (Pediatr 2007, 120:e749). Local emergencies can impair sample delivery to laboratories or impair reporting of results to hospitals.
Emergency preparedness has become recognized as a necessary element of standard operating practice for newborn screening laboratories.

Several of the Regional Genetics Collaboratives have engaged state-based newborn screening (NBS) laboratories to develop contingency plans for backup capacity during a local or regional service outage. Florida and Texas have developed a backup plan wherein excess capacity in one state is available to the other state in the event of a disruptive event. Other regions have held EP activities to identify needs and capacity, undertake harmonization exercises to identify disparate NBS elements between states, and plan workshops and tabletop exercises. This presentation will define the current status of state NBS laboratory backup in various regions of United States and present a summary of a harmonization workshop. The methodology presented can be utilized by any state laboratory in developing an emergency preparedness backup plan.

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Diagnosing Prader-Willi Syndrome in Infancy Reduces Obesity and Associated Co-morbidities

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Prader-Willi syndrome (PWS), affecting 1/15,000 individuals, is a genetic disease characterized by lack of expression of genes on the paternal chromosome 15q11-q13 region with 70 percent caused by paternal 15q11-q13 deletions, 28 percent due to uniparental maternal disomy and 2 percent are caused by an imprinting defect. Clinical features include neonatal hypotonia, poor suck, feeding difficulty and failure to thrive, hypogonadism, characteristic dysmorphic features, mild intellectual disability, growth hormone deficiency with short stature, small hands and feet, and later development of excessive hunger and obesity and distinctive behavioral characteristics with temper tantrums, outbursts and self-injury. Growth hormone replacement has revolutionized the stature and body composition in individuals with PWS who have started treatment early. Our consortium is an NIH funded RDCRN (Rare Diseases Clinical Research Network) for Prader Willi research since 2006. The long term data accrued from 351 Prader Willi patients recruited for the RDCRN natural history studies represent the best characterized cohort in the world. Because of large variability in age of diagnosis (range from 1 month to 47 years old), the age of diagnosis was categorized into 3 categories. Despite significant advances in diagnosing PWS, the mean age for diagnosis was delayed with 84 (25%) diagnosed with PWS ≥ 3 yrs, 41 (12%) between 1 and 3 yrs. and 208 (62%) < 1 yr. Both the age of diagnosis (p<.001) and the race (p=.018) were significant factors for the age when the child first became heavy. The estimated median ages for when the child first became heavy were 10 years for age of diagnosis < 1 yr, 6 years for age of diagnosis between 1 – 3 yrs, and 4 years for age of diagnosis greater than 3 yrs. Non-white individuals exhibited earlier age of first becoming heavy than whites.
Molecular and genetic testing of newborns with PWS is robust and conclusive. We propose that technology currently exists to perform newborn screening using filter cards that are routinely in use at the state level. It is critical for PWS to be detected in the newborn period in order to avoid costly invasive diagnostic work-ups thus permitting early GH treatment to prevent obesity and associated comorbidities.

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**P-48**

**Newborn Screening for Mucopolysaccharidoses: Determination of Sensitivity, Specificity and Cutoff Values**

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**Introduction:** Mucopolysaccharidoses (MPS) are inherited lysosomal storage disorders caused by deficiency of the lysosomal enzymes needed to degrade glycosaminoglycans (GAGs), which include chondroitin sulfate (CS), heparan sulfate (HS), keratan sulfate (KS), dermatan sulfate (DS) and hyaluronan. Lysosomal accumulation of GAG molecules result in cell, tissue and organ dysfunction. Most MPS patients are asymptomatic at birth with subsequent onset of clinical signs and symptoms. Currently some therapeutic options are available for most MPS. Thus early diagnosis by newborn screening (NBS) is critical to achieve better treatment outcomes. Here we describe an accurate and sensitive method for measuring DS, HS and KS levels simultaneously in dried blood spots (DBS) using tandem mass spectrometry (LC-MS/MS).

**Methods:** DBS of 3,256 de-identified newborns were digested with chondroitinase B, heparitinase and keratanase II, and analyzed using API-4000 LC-MS/MS at Saint Louis University. Concentrations of HS subtypes (HS-6S, HS-0S, and HS-NS), KS, and DS were determined for each newborn. Cutoff levels of mean+2(SD) (97.5th percentile) and mean+3(SD) (99.85th percentile) were compared.

**Results:** The coefficient of variation was ranked as follows: NS>6S>0S>KS>4S. DiHS-NS had the greater variation or dispersion and Δ4S had the lowest variation among all the analytes. Overall there was very small intra-sample variation. 54% of newborns had HS-6S levels above the low level of quantitation (LLOQ), while 98.5%, 98.46%, 98.53%, and 98.40% of newborns had HS-0S, HS-NS, KS, and DS-4S levels above the LLOQ, respectively. The cutoff values (mean+2(SD)) were determined to be: 167.97ng/mL, 108.44ng/mL, 50.72ng/mL, 155.43ng/mL, and 62.77ng/mL for HS-6S, HS-0S, HS-NS, KS, and DS-4S, respectively.

**Conclusion:** This methodology provides high sensitivity and accuracy for simultaneous measurement of three disaccharides (DS, HS, and KS) in DBS.

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Current Status of Newborn Screening in Puerto Rico
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The Puerto Rico Newborn Screening (PR NBS) program has an important role in the early detection of hereditary diseases in PR. In 1987, the law that mandates NBS in PR was created and since that year more than 1,200,000 newborns have been analyzed for different conditions including rare metabolic diseases, endocrine diseases and hemoglobin disorders. In the last eight years, the PR NBS Program has evolved and new and modern methodologies have been incorporated to expand significantly the number of conditions for analysis. In our program, dried blood samples are analyzed using isoelectric focusing electrophoresis for hemoglobinopathies, tandem mass spectrometry for metabolic diseases, time-resolved fluoroimmunoassays for endocrine disorders and cystic fibrosis, and enzymatic fluorescence-based assays for galactosemia and biotinidase deficiency. More recently, a TREC assay was incorporated to test for SCID. Currently, the PR annual birth rate is ~33,000 and all the conditions under the Recommended Uniform Screening Panel (RUSP) are evaluated except for tyrosinemia type I and Pompe. In an analysis from 2008-2013, 248,645 samples were analyzed and a total of 168 positive cases were detected. The predominant conditions identified in the Puerto Rican population are hypothyroidism and hemoglobinopathies with estimated incidences of 1:4,000 and 1:5,000, respectively. Also, fatty acid oxidation disorders are of importance in our population. In the last five years, 8 cases of SCAD and 5 of MCAD have been identified. Estimated incidences for PKU, galactosemia, CAH, cystic fibrosis, biotinidase deficiency and SCID are: 1/26,000, 1/50,000, 1/18,000, 1/17,000, 1/25,000 and 1/60,000, respectively. In conclusion, the PR NBS program has been successful for more than 25 years in the identification of rare genetic conditions making possible to better understand the status of these diseases in our population. This important public health service has not only allowed the identification of different conditions but also an immediate intervention by the corresponding healthcare professionals improving the quality of life of these patients and their parents. Future directions in our program will be focused in the evaluation of new methodologies for screening and new conditions to be added to our NBS panel.

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Bridging the Language Gap: Creating Culturally Competent Newborn Screening Education for Spanish-Speaking Communities
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According to US Census Bureau projections, the Latino and Hispanic population in the United States is expected to double over the next 40 years. At such a rapid rate of growth, it is crucial new and expecting parents in Latino and Hispanic communities receive up-to-date, culturally competent, and easily accessible newborn screening information. In April 2015, Baby’s First Test, the nation’s newborn screening clearinghouse, launched the Spanish language version of its site (Spanish.BabysFirstTest.org)
in order to meet the educational needs of new and expecting parents from and within Hispanic communities.

Spanish.BabysFirstTest.org provides the up-to-date, adaptive, and culturally competent resources and materials needed to increase the amount of support available to and details necessary for expecting parents around newborn screening, specifically in a way that has not previously existed. The Spanish language version of Baby’s First Test was professionally translated by a contracted health translator service to ensure a high level of accuracy and linguistic accessibility. Paying attention to the particular needs of Spanish-speaking families, the Spanish site also addresses the implications of the Affordable Care Act (ACA), including healthcare access and costs that may affect families with a range of citizen status.

This presentation will discuss the implementation and evaluation of Spanish.BabysFirstTest.org, including lessons learned and strategies for creating community-focused and culturally competent educational resources. The presentation will also discuss opportunities for newborn screening education and outreach in Spanish-speaking communities.

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Couple Dynamics in Decisions About Elective Whole Exome Sequencing for Newborn Screening
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Objective: Whole exome sequencing (WES) may be used in newborn screening (NBS) programs in the future. Our objective was to explore decision-making processes in couples regarding the type of results they would choose to receive if WES was part of NBS. The goal was to understand their interactions to inform the development of a decision aid.

Methodology: We conducted 90-minute interviews with 33 couples. On average, participants were 32.4 years old and had been in their committed relationship for 5.9 years. Couples were either currently pregnant, had given birth in the past 12 months, or had postnatal genetic testing for a child five years old or younger.

We described WES and the North Carolina Newborn Exome Sequencing as Universal Screening (NC NEXUS) study. We then informed couples about five hypothetical results categories potentially available from newborn WES. These included gene differences signifying disorders that are (1) medically actionable with a childhood onset; (2) non-medically actionable with a childhood onset; (3) medically actionable with an adult onset; (4) non-medically actionable with an adult onset, or (5) carrier status.

Each parent completed a decision worksheet for each of the five categories. The couples then discussed their answers and how they might collaborate to reach a mutual decision for each category. We used a framework analysis method to code and analyze the qualitative data.

Results: Ninety-one percent of all of participants said they would definitely want newborn WES if presented with the option. Nearly 40% of parents answered the same choice consistently across all five result categories. This means just over 60% of parents varied their five decisions based upon category-specific considerations (e.g., onset age, actionability).
Many couples (42%) described a collaborative process before making joint decisions. More than one-third of partners (36%) said they would find the decision harder to make alone because their partner offered support or reassurance for their decision.

For some couples one partner was swayed by the other, often simply a result of their partner bringing up a new perspective. Sometimes, though, partners were “convinced,” deferred to the other who had a firm preference, or let them “win” (12%). The rate of mutual agreement between partners declined as result categories strayed further from standard NBS criteria (e.g., clear actionability, childhood onset).

**Conclusions:** Parent interest in receiving newborn WES is high. However, decisions about newborn WES are complex, especially when information is not clear or actionable. There was varying levels of agreement between partners in what results would be desired and how couples prefer to make these decisions. In light of these findings, a decision aid that focuses on supporting couples in learning about different potential results, facilitating conversations, and encouraging family-centered decision-making would be useful to couples considering WES for their child.

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**Values and Beliefs Important for Parental Decisions to Have Genetic Screening for a Child**

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**Objective:** There is the potential for more widespread use of whole exome sequencing (WES) in the future either via screening programs or parents electing to use direct-to-consumer tests. We interviewed parents to understand factors that might affect their decisions to have WES for their child. Insights from these interviews will inform the development of a decision aid to support parental decision making as part of the North Carolina Newborn Exome Sequencing as Universal Screening study (NC NEXUS).

**Methodology:** We conducted 90-minute interviews with 66 parents representing 33 couples. All couples were either currently pregnant, had given birth within the last 12 months, or had a child under the age of five that had undergone postnatal genetic testing.

We provided couples with information about NC NEXUS and explored what was most important to parents in deciding to have sequencing for their child. We also sought input on the five results categories potentially available from NBS WES including gene variants signifying disorders that are (1) medically-actionable with a childhood onset, (2) non-medically actionable with a childhood onset, (3) medically actionable with an adult onset, (4) non-medically actionable with an adult onset, and (5) carrier status. We used qualitative framework analysis to code and analyze the data to define common themes across parent responses.

**Results:** A variety of themes emerged across parent groups, with no meaningful thematic differences between groups. Improving quality of life was the most frequently mentioned benefit of sequencing. Parents also cited that knowing their child had any health conditions early on would allow time to prepare for raising a child with a genetic condition. Parents reported concerns about psychosocial impacts of testing, including stress and anxiety for both child and parent, and the potential impact of stigma and labeling on the child. Some parents believed it
was better not to know this type of information with a few citing spiritual or religious beliefs for not wanting to know.

A few parents mentioned skepticism and mistrust of potential ramifications regarding sequencing. Some parents also had concerns about privacy, confidentiality and accuracy of the test results. Many parents were in favor of having WES for their child based on previous experiences with genetic health issues, personally or secondhand. However, there were a few parents that were against screening based on previous experiences.

**Conclusions:** Parents shared a multitude of values, beliefs, experiences and reasons for and against having sequencing for their child. The multi-faceted nature of the decision to have sequencing for one’s child necessitates that the information parents receive address these values and concerns in order to support informed decision making.

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**P-53**

**Proficiency Testing for T-Cell Receptor Excision Circles (TREC) in Blood Spots to Detect Severe Combined Immunodeficiency (SCID)**

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**Background:** In 2011 The Newborn Screening Quality Assurance Program (NSQAP) initiated a pilot proficiency (PT) program for T-cell receptor excision circles (TREC) in dried blood spots (DBS) to detect severe combined immunodeficiency (SCID). The PT program, an extension of Newborn Screening Translational Research Initiative’s Model Performance Evaluation Survey, began with seven US participants. The program accepted international laboratories in 2014, and it has grown to 28 domestic and 17 international laboratories in 2015.

**Methods:** DBS specimens were produced from human blood, including cord blood from unaffected individuals and modified adult blood depleted of mononuclear cells or leukocytes. The materials represented normal TREC and reference gene levels, normal specimens with TREC at medium and below average levels (reference gene within standard range), SCID-like specimens (reference gene within standard range), and specimens where both TREC and reference gene levels are below reference ranges. Participating laboratories tested the PT specimens and reported the following for each specimen: categorical results ‘No follow-up required (Screen Negative)’ or ‘Follow-up required’; methods used to assess TREC levels; methods used to prepare DNA from DBS; reference gene used; and reference gene category (reference gene level within standard reference range or reference gene level outside standard reference range).

**Results:** We summarized qualitative PT results over five years. The U.S. misclassification rate for TREC was 0% false negative results and 1.7% false positive results. The combined misclassification rates (domestic and international) were 0.5% false negative results and 1.8% false positive results for the PT specimens. We also found that use of beta actin as the reference gene, along with a commercial kit method for TREC measurement, contributed to the majority of the reference gene misclassifications (67%). In 2015 most participants used laboratory-developed tests based on real-time PCR to quantify TREC, while seven laboratories used the commercial kit method, which is based on a single-point PCR assay.
Conclusions: The TREC PT program for SCID identified conservative practices where normal PT specimens with TREC at medium or below average levels were more likely to be classified as requiring follow-up. Use of beta actin as the reference gene contributed to more reference gene misclassifications. U.S. laboratories had no false negative misclassifications over the entire five year period.

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CDC Reference Methods and Materials to Support Expanded Newborn Bloodspot Screening for Pompe Disease and Other Lysosomal Storage Disorders
H. Zhou, P. Dantonio, R. Vogt and F. Lee, Centers for Disease Control and Prevention, Atlanta, GA

Pompe disease is a lysosomal storage disorder that causes progressive muscle weakness resulting from accumulation of glycogen in the lysosomes. Without treatment, babies with classic infantile-onset Pompe disease will die in infancy from cardiorespiratory failure. Enzyme replacement therapy approved by FDA has been shown to be effective and clearly improve clinical outcomes if patients are identified and receive therapy promptly before they are symptomatic. Early identification of patients with Pompe disease can be achieved by population-based newborn screening for acid α-glucosidase deficiency in dried blood spots (DBS). The US Discretionary Advisory Committee on Heritable Disorders in Newborn and Children recommended addition of Pompe disease to the national recommended uniform screening panel based on evidence accumulated with technical support from CDC. In March 2015, the secretary of HHS accepted this recommendation, and the number of newborns screened for Pompe disease is growing rapidly, reaching more than 400,000 in the US to date. CDC’s quality control samples and proficiency testing materials have become even more important with the expanded implementation of NBS testing to detect Pompe disease. In addition to DBS reference materials for QC, we have recently developed DBS that emulate the phenotype and genotype of newborns affected by Pompe disease or another specific lysosomal storage disorder. These reference materials were used to validate an alternative high throughput MS/MS assay and a digital microfluidic fluorescence assay that are undergoing regulatory approval for commercial availability. CDC’s quality assurance program for Pompe and other LSD continues to grow in order to meet the increased demands of expanded newborn bloodspot screening in the US and worldwide.

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P-55 – Withdrawn
Assessing DNA Extraction Methods Commonly Used in Newborn Screening Labs for the Detection of Cytomegalovirus in Dried Blood Spots
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Dried blood spots (DBS), collected universally from newborns in the U.S., may be valuable for the detection of asymptomatic congenital cytomegalovirus (CMV) infection. Previous studies have shown a significant association between increasing blood viral loads and risk for hearing loss and cognitive disability (>10,000 copies/ml blood). Currently, the “gold standard” for detecting CMV in DBS is the thermal shock DNA extraction method. However, this method requires an overnight freeze step and an input of a 6-mm DBS punch versus the standard 3.2-mm punch used in newborn screening. Our objective was to test DNA extraction methods commonly used in newborn screening laboratories to determine if any could achieve the same sensitivity as the thermal shock method.

DNA was extracted from 100µl of whole blood from CMV-positive organ transplant recipients with blood viral loads between 1,000-200,000 copies/ml. The remaining blood was spotted on filter paper. Real-time PCR for CMV detection was performed on DNA extracted from a DBS punch using the thermal shock method, a potassium hydroxide (KOH) Tris based buffer prep (published and now commercially available), and a commercial DNA elution buffer, alone or in combination with a DNA purification buffer prep commonly used in newborn screening laboratories. The thermal shock was performed with either an overnight or a 2hr. freeze step at -20°C. The commercial methods were performed with and without a 2 hr. -20°C freeze step. In addition, a novel in situ real-time PCR method without a DNA extraction step was performed on a 2-mm DBS punch.

Preliminary results show that the KOH Tris based buffer prep (3.2-mm punch) and the thermal shock prep (6-mm punch with overnight freeze step) provided the highest sensitivity for CMV detection in DBS, particularly when blood viral load was <10,000 copies/ml. Reducing the DBS punch from 6-mm to 3-mm and the freeze step from overnight to 2 hours in the thermal shock method resulted in a small, but not significant loss in assay sensitivity. The addition of a freeze step in any of the commercial methods had no impact on sensitivity. The CMV assay sensitivity using DNA extracted with a commercial DNA elution buffer, alone or in combination with a DNA purification buffer was significantly lower than either the thermal shock or KOH Tris based buffer prep. The in situ PCR produced variable results for sensitivity and yield, likely due in part to the 60% decrease in blood volume contained in a 2-mm punch. Based on preliminary data, the results from the KOH Tris based buffer prep was similar to the “gold standard” thermal shock method and resulted in the highest CMV DNA yield. Overall, the KOH Tris based buffer prep is better suited for newborn screening in that it is a simple, fast and inexpensive protocol that only requires a 3.2-mm DBS punch. The resulting DNA is also compatible with other molecular tests ongoing in newborn screening laboratories.

Presenter: Deborah Koontz, Ph.D., Centers for Disease Control, 4770 Buford Hwy. NE, MS-F24, Atlanta, GA, 30341, Email: duk5@cdc.gov
Validating CDC’s Cystic Fibrosis Molecular Dried Blood Spot Repository for Use with Next Generation Sequencing Methods
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Objective: Validate CDC’s Cystic Fibrosis (CF) Molecular Proficiency Testing dried blood spot (DBS) specimens for sequencing technologies including commonly used next generation sequencing platforms.

Background: There are a wide variety of molecular assays utilized by newborn screening laboratories to characterize the CFTR gene including Sanger sequencing technology as well as next generation sequencing technologies. In order to provide comprehensive quality assurance for all laboratory participants in CDC’s Newborn Screening Quality Assurance Program (NSQAP), it is essential to validate and characterize the CF Molecular DBS Repository samples using these methods.

Methods: DNA was extracted from one 3.2 mm DBS punch from all samples in the CF Molecular DBS Repository using a commercial assay that includes a DNA elution and DNA purification buffer. Samples were characterized using the two commercial genotyping assays that detect expanded mutation panels inclusive of the ACMG 23 recommended mutations as well as two next generation sequencing platforms. All results were validated with Sanger sequencing of CFTR gene coding regions, intron-exon splice junctions and non-coding regions known to contain CF causing mutations. An additional step in the validation process was to ensure all repository samples would yield adequate quantities of DNA for the various types of molecular analyses being used by NSQAP participants including next generation sequencing. To accomplish this, DNA was extracted from individual 3.2 mm DBS punches taken from multiple replicate samples using the same commercial assay as above. All DNA extractions were quantified using real time PCR of the RNase P housekeeping gene. Finally, microsatellite marker analysis was performed to ensure that there was no sample contamination or mix ups during the blood collection and spotting process. Repository samples were considered validated once each specimen produced acceptable results for all of the prescribed assays.

Results/Conclusion: CDC’s CF Molecular DBS Repository samples have been validated for use with commercial genotyping assays based on hybridization to specific mutations or normal sequence, next generation sequencing assays on commonly available platforms and Sanger sequencing. These assays all perform well when DNA is extracted using a commercial assay that includes a DNA elution and DNA purification buffer. Thus, these specimens are suitable newborn screening quality assurance materials for both simple and complex genotyping methods as well as all types of DNA sequencing including next generation sequencing.

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Monitoring Method Performance Through the Use of External QC Materials for Newborn Screening Analytes
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Background: Newborn Screening Quality Assurance Program (NSQAP) Quality Control (QC) dried blood spot (DBS) materials provide participants with external controls to monitor long term assay stability. The materials provide continuity that transcends changes in kit lot production. We routinely use data collected from participants testing these materials to monitor statistical parameters for method performance such as reproducibility, outliers, slopes, and trends. Methods with slopes between 0.8 and 1.2 are considered to have acceptable performance.

Methods: DBS specimens prepared from donor blood and enriched with predetermined levels of analytes were sent to participating laboratories for use as external QC. Participants submitted method information and at least nine data points per enrichment level. Basic statistical parameters including the mean, standard deviation, y-intercepts, and slopes were used to assess method performance. Using data collected in 2014, we plotted enrichment values vs. observed concentrations for various methods and compared slopes for phenylalanine (Phe), succinylacetone (SUAC), arginine (Arg), and acylcarnitines C0, C5OH, C5DC, C3DC. For immunoreactive trypsinogen (IRT) we plotted slopes from 2008-2014 for an enzyme immunoassay method (EIA) to show changes in recovery over time. Additionally, five years of slope values were charted for all analytes.

Results: QC material analysis by MS/MS for Phe showed minor quantitative differences between methods. SUAC methods yielded 20-60% lower recoveries, while Arg methods had 50-90% recovery. Higher slopes were seen for C0 (1.0-2.1), C5OH (0.8-1.4), C5DC (0.7-1.7), and C3DC (0.6-1.4). Recovery of IRT by EIA showed slope variation from year to year reflecting changes in kit lots. Plotting slope data showed stable Phe recovery for all methods; SUAC recovery was consistently low but stable; Arg improved when QC materials were enriched with D-Arg instead of L-Arg; C0 slopes for derivatized methods were higher, while low slopes for the majority of methods for C3DC were lower; a higher range of slopes for C5DC was due to a non-derivatized method. We observed steady slope improvement for C5OH since 2013 and a significant improvement in recoveries for IRT since 2013.

Conclusion: Our observation of low variability and high reproducibility of slopes by-method over time confirms the stability of NSQAP QC materials, particularly during lot number changes. The QC data provided in our annual reports provides participants with a means of comparing method performance. NSQAP will continue to assess statistical parameters of current and historical participant QC data to monitor performance trends.

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P-59 – Withdrawn
Minnesota Population Spectrum of Congenital Adrenal Hyperplasia Causing Mutations in the CYP21A2 Gene
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Objective: Determine the spectrum of CYP21A2 mutations in Minnesota (MN) patients with congenital adrenal hyperplasia (CAH) by allele specific PCR and gene sequencing.

Background: CAH due to 21α-hydroxylase deficiency is an autosomal recessive disorder of cortisol biosynthesis and has a frequency of 1:15,000 births in the U.S. The current newborn screening method for CAH uses an immunoassay to measure elevated 17-hydroxyprogesterone (17OHP), a metabolite elevated due to the enzymatic block. However, this assay has been well documented to have a high false positive rate and more recently, studies have shown that it also has a higher than previously expected false negative rate. We postulate that a second tier molecular assay would allow newborn screening programs to lower their 17OHP cutoff level to increase the test’s sensitivity while maintaining specificity by following up elevated 17OHP with CYP21A2 mutation detection. To design an effective molecular second tier screening assay, we characterized MN CAH patients and family samples for CYP21A2 mutations (N=73 diagnosed CAH cases and N=80 family members) using an in-house developed assay.

Methods: DNA was extracted from 250 µL of whole blood using Qiagen QiaAMP DNA Mini kit. Three allele specific PCR amplifications were performed. The first PCR was specific for the CYP21A2 gene in order to amplify the functional gene rather than the pseudogene which is 98% identical. The second and third PCR were to detect large scale gene rearrangements including the common 30kb deletion alleles and large scale gene conversions. The functional CYP21A2 gene from the first PCR amplification was sequenced to detect small internal insertions and deletions as well as point mutations.

Results and Conclusions: Analysis of the CAH positive cases found that all samples had two identifiable mutation in the CYP21A2 gene. In all of the CAH cases, we identified at least one mutation that is part of the diagnostic mutation panel (includes 12 most common CYP21A2 mutations, the 30kb deletions, and large scale gene conversions). In addition to mutations included in the diagnostic panel, we identified six additional salt-wasting or simple-virilizing mutations and one non-classic mutation. The spectrum of CYP21A2 mutations in the MN population identified by this study will be used to develop a MN population appropriate second tier mutation detection assay that can be used for newborn screening pilot testing.

Presenter: Zachary Detwiler, M.S., U.S. Centers for Disease Control and Prevention, 4770 Buford Highway, Atlanta, GA, 30341, Email: wtp4@cdc.gov
Long-term Stabilities of 17-Hydroxyprogesterone, 4-Androstenedione, Cortisol, 11-Deoxycortisol and 21-Deoxycortisol in Dried Blood Spots at Various Conditions

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Background: Congenital adrenal hyperplasia (CAH) is a heritable disorder caused by defects in the steroid biosynthesis pathway. CAH due to 21-hydroxylase deficiency accounts for about 95% of all cases whereas 11-β-hydroxylase deficiency accounts for around 5%. However, both enzymatic deficiencies cause the buildup of 17α-hydroxyprogesterone (17OHP) while reducing the efficiency of cortisol synthesis. First-tier testing for CAH is done by screening for 17OHP levels in dried blood spots (DBS). A second-tier testing CAH method was developed to improve the specificity of testing for CAH. The CDC’s Newborn Screening Quality Assurance Program provides blind-coded proficiency testing (PT) panels containing the five steroid hormones associated with CAH: 17OHP, 4-androstenedione (4AD), cortisol (CORT), 11-deoxycortisol (11D), and 21-deoxycortisol (21D). Materials produced for the PT program were used to investigate the long-term stability of the five analytes (17OHP, 4AD, CORT, 11D, 21D) in DBS stored under various temperatures.

Methods: DBS enriched with 17OHP, 4AD, CORT, 11D, and 21D were placed in Mylar foil bags containing desiccant packets to maintain humidity at <30%. Identical sets of these specimen bags were grouped and stored at different conditions: 37°C, ambient temperature, 4°C and, -20°C. Three specimens from each set of conditions were collected weekly and stored in at -70°C. DBS samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Recoveries were calculated as the percentage of initial concentration remaining after each storage interval using a simple Arrhenius model.

Results: DBSs stored for up to one year with humidity controlled at <30% retained an average of 107%, 100%, and 101% for 17OHP, 4AD, and 21D stored at 20°C, 4°C, and ambient temperature, respectively. At ambient temperature, CORT and 11D were recovered at an average of 86%. At 37°C the recovery of, 17OHP, 4AD, and 21D was an average of 83%, while CORT and 11D saw the greatest decline with an average of 63% recovery.

Conclusions: These results demonstrate steroid hormones measured in DBS have good stability and recoverability as determined by LC-MS/MS. The DBS enriched maintained with humidity <30% are stable at least up to 6 months if stored at 4°C and for at least 100 days if stored at ambient temperatures. The recommended storage conditions for NSQAP’s CAH DBS is at ~20°C, with the routine working bag stored at 4°C, both with desiccant.

Presenter: Gyliann Pena, BS, Centers for Disease Control, 4770 Buford Hwy NE, Atlanta, GA, 30341, Email: klx9@cdc.gov
Dried Blood Spot DNA Extraction Efficiency and Quantification Varies Greatly by Extraction and Measurement Method
S.R. Nikolova, G.K. Yazdanpanah, F. Lee and S.K. Cordovado, Centers for Disease Control and Prevention, Atlanta, GA

Objective: Evaluate efficiency of DNA extraction from dried blood spots (DBS) by various protocols and quantification methods.

Background: Molecular testing in newborn screening programs has greatly expanded in the past 10 years. There are a number of DNA extraction protocols currently used by screening laboratories that vary greatly in yield and purity, which may affect downstream applications. In addition, not all quantitation methods are appropriate to measure DNA concentrations from DBS extractions because of impurities associated with the protocol used and/or matrix effects.

Methods: To measure extraction efficiency, reference DBS were prepared from mononuclear cell-depleted blood spiked with 2000/µl transformed lymphocytes containing single copy of TREC. DNA was extracted from five 3.2 mm punches using several commercially available or home brew methods used in newborn screening laboratories. DNA was also extracted in duplicate with the same extraction methods from a 3.2 mm punch taken from 10 cord blood DBS and 10 adult samples from CDC’s Cystic fibrosis (CF) molecular repository. DNA concentration was measured using three methods, real-time PCR of the RNAseP gene, spectrophotometric absorbance and UV fluorescence of a DNA intercalating dye.

Results: The DNA extraction efficiencies were calculated by average % recovery of TREC measured by quantitative PCR based on standards quantified by digital PCR. Of the DNA extraction methods tested, the method using micro column was the most efficient with 57% TREC recovery, followed by commercially available boil preps. The home brew preps containing the detergent Triton-100 were significantly lower, and the methanol containing boil prep was the least efficient. DNA quantification using a DNA interacting dye and Real-Time PCR resulted in almost identical values when using a human genomic DNA standard curve. The spectrophotometric quantification yielded significantly higher values than the other methods for all DNA extractions, however the overestimation was less pronounced for the column-based method.

Conclusions: When selecting a DNA extraction method, it is important to consider the purity and DNA input requirements of each downstream application. While current molecular newborn screening assays do not require ultra-pure DNA, it is well established that the TREC assay requires a greater yield than commercial CF genotyping assays. When quantitation is necessary, not all methods are appropriate for use with DNA extracted from DBS. While the spectrophotometry is fast and simple, it results in a significant overestimation of DNA concentration. Both real time PCR and use of a DNA interacting dye are accurate quantitation methods for use with DBS extracted DNA.

Presenter: Stanimila Nikolova, PhD, Centers for Disease Control and Prevention, 4770 Buford Hwy, MS-F24, Atlanta, GA, 30341-3717, Email: sjn4@cdc.gov
Stability of Glucose-6-Phosphate Dehydrogenase Proficiency Testing Dried Blood Spot Materials
S. Flores, E.M. Hall, V. De Jesus, Centers for Disease Control and Prevention, Atlanta, GA

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency affects 400 million people globally and commonly causes episodes of hemolytic anemia. The Newborn Screening Quality Assurance Program (NSQAP) will begin offering G6PD dried blood spot (DBS) proficiency testing (PT) materials in 2015. Participants in the program have 30 days to report their PT data to the NSQAP. Prior to the first distribution of G6PD PT materials, we stored G6PD-normal DBS under various temperature and humidity environments to determine optimal storage conditions of G6PD PT materials and enzyme stability.

Methods: Three cord blood units were pooled, hematocrit adjusted to 50±1% and dispensed in 75uL aliquots onto grade 903 filter paper to prepare G6PD-normal DBS. Specimens were packaged in Mylar zip-seal bags and stored for one month at -20°C, 4°C, room temperature (RT) or 37°C with or without desiccant at low humidity (<30%). A set of identical bags were prepared with and without desiccant and were placed in plastic containers with damp paper towel to create a high humidity environment (>50%). These containers were stored under each of the four temperature environments. Specimens were removed two times per week, desiccant was replaced and they were stored at -80°C until all were collected. Specimen sets were analyzed in triplicate using the PerkinElmer G6PD Neonatal kit.

Results: G6PD activity remained >95% in specimens stored at -20°C for 30 days with or without desiccant. Specimens stored at 4°C retained >95% activity except those stored with desiccant under >50% humidity, which lost 15% of initial activity. All specimens stored at RT lost an average of 20% initial activity by the end of 30 days. Specimens stored at 37°C with desiccant lost 50% initial activity by the end of the study; those stored without desiccant under low and high humidity lost an average of 33% initial activity within the first week. By week three, activity in specimens stored at 37°C under high humidity and without desiccant fell below the NSQAP cutoff.

Conclusion: We determined that if humidity is maintained at <30%, G6PD PT specimens can be stored reliably at -20°C or 4°C for up to 30 days. Storage at RT and 37°C is not recommended since activity declined significantly by the end of 30 days. Humidity exacerbated activity loss and should be mitigated at all temperatures with the use of desiccant.

Presenter: Victor De Jesus, PhD, CDC, 4770 Buford Hwy NE, Atlanta, GA, 30341, Email: vdejesus@cdc.gov

Growth of CDC’s Newborn Screening Quality Assurance Program Since 1978
S. Zobel, J. Mei, V. De Jesus, I. Williams, L. Shockley, S. Brown, C. Nguyen, Centers for Disease Control and Prevention, Atlanta, GA

Newborn screening laboratories rely on external quality assurance (QA) dried blood spot (DBS) materials to ensure their testing methods yield accurate results. This led to the 1978 start of the Centers for Disease Control and Prevention’s Newborn Screening Quality Assurance Program (NSQAP). NSQAP first began producing QA materials for congenital hypothyroidism since the incidence rate was higher than phenylketonuria, and since many newborn screening laboratories were testing for this disease. In 1979, NSQAP shipped its first DBS quality control (QC) materials containing Thyroxine (T4)
and Thyroid-Stimulating Hormone (TSH) to 42 laboratories. The following year, proficiency testing (PT) materials of those same analytes were distributed.

Over the past 36 years, the NSQAP has grown significantly. The program now provides QA materials for 48 analytes and covers all of the core conditions and most of the secondary conditions listed in the U.S. Recommended Uniform Screening Panel. Three times per year, NSQAP provides QA materials to over 600 participants in 77 countries. We offer 12 DBS PT programs and eight DBS QC programs (covering both biochemical and molecular methods) for newborn screening laboratories. In 2015, NSQAP introduced two new programs: PT and QC materials for X-linked adrenoleukodystrophy (X-ALD) and PT materials for glucose-6-phosphate dehydrogenase deficiency (G6PD).

NSQAP recently added an electronic mailbox to accept data report forms, enrollments, requests for information, and shipping inquiries. We now offer automatic tracking of DBS packages to provide up-to-date information for NSQAP participants. The NSQAP statistics as of July 2015 include the following: 616 participating laboratories; 16 gallons of blood processed in 2014, which is the equivalent of about 11 average sized adults; 13,000 DBS packages prepared for the July 2015 shipment; 465,000 QC results received from participants and 2,336 laboratory PT evaluations released in 2014.

NSQAP continues growing and enhancing our program to better serve participants. The program collaborates closely with state, national, and international partners to meet their QA needs for new tests, technologies, and disorders.

**Presenter:** Sherri Zobel, BS, Centers for Disease Control and Prevention, 4770 Buford Highway, MS F19, Atlanta, GA, 30341, Email: szobel@cdc.gov

**P-65**

**Assessing Challenges to Fragile X Syndrome Newborn Screening**

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**Background:** Fragile X syndrome (FXS) is the most common known inherited form of intellectual disability. Early identification is important in order to link affected individuals with appropriate medical and social services. Newborn screening (NBS) is one approach that has been used to facilitate early identification. This presentation offers a description of the current status of FXS as it relates to NBS, the barriers and the strengths and weaknesses of potential solutions to the barriers.

**Methods:** A traditional PubMed search was conducted, as well as a Google Scholar search, using a series of search terms including “fragile X syndrome”; “FMR1”; “newborn screening”; “screening”; and “genetic testing.” Internet search engines (Google, Bing, Yahoo) were used to locate the “gray literature” (e.g. organization/agency websites, unpublished reports). In order to identify other barriers and challenges not evident in the literature, 30-minute semi-structured telephone interviews were conducted with nine key informants, drawn from a range of stakeholder groups - patient advocacy groups, pediatricians, state laboratories, early intervention programs, genetic counselors, medical geneticists, companies who have developed screening tests, and FXS clinicians and researchers. An interview guide was used to elicit policy issues, common barriers, unidentified or under-discussed barriers, potential solutions, key stakeholder groups, and stakeholder interests.
Results: Drawing on the published literature, the gray literature, and key informant interviews, eight primary barriers to NBS for FXS were identified. These barriers were further categorized into five themes: public health burden, treatment, timing, screening/testing methodologies, and translating results. Potential solutions will be presented for each barrier identified. An example of a solution presented under treatment is to conduct a well-designed study of the efficacy of early intervention (EI) for infants versus children. There are several challenges noted to conducting such a study, one of which is the diversity in the nature and quality of EI programs around the country and what metrics would be used.

Conclusion: This study identifies key areas of knowledge important in assessing NBS for FXS. In some key areas, limited information is available and further research is needed to facilitate assessing the possibility of NBS for FXS. This could be through a series of smaller research findings or a larger breakthrough, for example a clinical trial demonstrating an effective targeted treatment. Either could prompt a change in how FXS is assessed relative to NBS. Future research will take the coming together of a large community of clinicians, researchers, public health professionals, educational specialists, behavioral specialists, advocates for the FXS population, and individuals with fragile X syndrome and their families.

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P-66
Use of Newborn Dried Blood Spots for Detection of Prenatal Alcohol Exposure
A. Baldwin, United States Drug Testing Laboratory, Des Plaines, IL

Prenatal alcohol exposure is the leading preventable cause of birth defects in the United States, producing an array of neurological, behavioral and physical abnormalities collectively known as fetal alcohol spectrum disorders (FASD). In the United States, the prevalence of all levels within the continuum of FASD estimated to be as high as 2 to 5% of school children. While no cure exists for FASD, early diagnosis is the key to effective interventions and treatments, particularly in helping children from birth to 3 years of age take advantage of early neuroplasticity and reduce the long-term adverse effects of fetal alcohol exposure. Many challenges exist in documenting prenatal alcohol exposure which leads to under diagnosis of FASD, particularly in neonates who would benefit the most from early interventions.

The primary objective of our studies has been to examine the clinical utility of detecting the alcohol biomarker phosphatidylethanol (PETH) in dried blood spots as a biological screening test for fetal alcohol exposure in newborns. Our laboratory has developed and validated an assay for the extraction and detection of PETH from dried blood spots using liquid chromatography-tandem mass spectrometry (LC-MS/MS). To test the performance of this assay anonymous dried blood spot cards were collected and analyzed for detection of PETH in three different populations with varying degrees of risk for prenatal alcohol exposure: (1) from 250 residual, dried blood spot cards from a Midwestern state health department; (2); from 487 newborns born at the Charleston Area Medical Center (CAMC) Women and Children’s Hospital in West Virginia and (3) from 135 newborns born at the National Social Security Perinatology Unit in Montevideo, Uruguay.
In our analysis of 250 anonymous newborn dried blood spots from a Midwestern state, where reported alcohol consumption is less frequent, PEth was detected in 10 samples (4%). At the CAMC Women and Children’s Hospital in West Virginia, where the population of mothers is of lower socioeconomic status and alcohol consumption during pregnancy is of concern, PEth was detected in 112 samples (23%). In the public health care hospital in Montevideo where alcohol consumption during pregnancy is commonly reported, PEth was detected in 107 samples (79%).

Detection of PEth in dried blood spots is an effective method to screen for prenatal alcohol exposure during late pregnancy and results from these studies indicate that the prevalence rates of prenatal alcohol exposure vary significantly among populations with varying degrees of risk for alcohol consumption during pregnancy. Further studies are underway to fully examine the clinical utility of PEth as a biological screening test for prenatal alcohol exposure.

**Presenter:** Aileen Baldwin, PhD, MPH, United States Drug Testing Laboratory, 1700 S Mount Prospect Rd, Des Plaines, IL, 60018, Email: aileen.baldwin@usdtl.com

**P-67**

**A Novel ETHE1 Mutation Identified in a First Nation Canadian Patient, Ascertained Following a Positive Newborn Screen for Isovaleric academia**

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**Case Report:** A male child born to consanguineous First Nations parents was referred for a positive newborn screen for isovaleric acidemia (IVA). At 1.5 months of age, the patient was admitted to ICU for poor feeding, necessitating nasogastric feeds. He was subsequently re-admitted at 4 months with tachypnea and lactic acidosis, and passed away 3 days later.

**Results:** Initial investigations revealed: markedly elevated urine ethylmalonic (EMA) and glutaric (GA) acids; elevated urine methylsuccinic (MSA), 2-hydroxyglutaric, malic and fumaric acids, and butyryl- and isovalerylglycines; and mildly elevated plasma C4 and C5 acylcarnitines. Newborn screen dried blood spots were also analyzed at the Mayo Clinic and showed significantly elevated EMA with elevations of GA and MSA. A working diagnosis of glutaric aciduria type II was suspected but not confirmed by the time of death. Subsequent sequencing of the ETHE1 gene revealed a novel homozygous c.423delC frameshift mutation.

**Discussion:** We report a novel ETHE1 mutation in the first case of EE reported in a patient of First Nation ancestry. Furthermore, the false positive newborn screen for IVA in this case, raises the question whether other patients with EE may be ascertained through false positive C5 newborn screens.

**Presenter:** Iveta Sosova, University of Alberta Hospital, 8440-112 Street, Edmonton, AB, T6G 2B7, Canada, Email: iveta.sosova@albertahealthservices.ca
Newborn Screening for Cystic Fibrosis in Nuevo León, México
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Cystic fibrosis (CF) is an autosomal recessive hereditary disorder, characterized for pancreatic insufficiency, progressive damage to the respiratory system which leads to failure to thrive. Early diagnosis and prompt intervention limits the disease progression. In September 2011, CF was included in the expanded Neonatal screening (NS) in Nuevo León México.

Objective: To known the CF incidence in the Northeastern Mexico population and to give a timely multidisciplinary management.

Methods: Quantification of immunoreactive trypsinogen (IRT) in blood spot samples was made by immunofluorometric assay (Perkin Elmer Neonatal Kit). Results above the cut off value in first sample were repeated in the same one and if proved again high, a second sample was requested.

Results: From September 2011 to April 2015 a total of 105,500 babies were screened; 214 (0.2%) were abnormal and only 176 (82.2%) second samples were recovered, from which, 159 (90.3%) were normal and 17 (9.7%) continue above normal levels. The latter were referred to the CF clinic for a sweat electrolytes test (SET). We diagnosed 12 cases with abnormal SET and referred to molecular test for CFTR mutations.

Discussion and conclusion: The incidence of CF found was 1/10000 alive-newborn, similar to the one reported in Caucasian population. Early detection allowed babys received timely specialized evaluation in the CF clinic with a positive impact on morbidity and mortality avoiding interventions that would delay treatment. This is the second NS program for CF in Mexicans and can be used as a reference for incidence at least in Northeast population.

Presenter: Maria del Rosario Torres Sepulveda, QCB, Universidad Autonoma De Nuevo Leon, 1, Monterrey, NL, Mexico, Email: qcbrtorres@live.com.mx

Feasibility Study Using the Six-Plex PerkinElmer LSD Reagents Utilising Tandem Mass Spectrometry (MSMS) for Newborn Screening and Diagnostic Testing for Six Lysosomal Storage Diseases (LSD)
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Introduction: Lysosomal storage disorders (LSD’s) are a group of more than 40 disorders caused by the specific deficiency of enzymes (& co-factors) within the lysosome. This leads to the accumulation of substrates, lipids, sphingolipids and mucopolysaccharides resulting in organelle and cellular dysfunction and the pathology in the LSD’s. The estimated incidence has been determined to be as high as 1 in 7,000 in the Australian population (1), even higher in certain ethic groups. Early identification and treatment (e.g. enzyme replacement, substrate depletion & BMT therapies) reduces the pathology and greatly enhances the quality of life and life expectancy. Therefore newborn screening for LSD’s provides the ability to identify prior to the onset of disease and the commencement of targeted treatment when
asymptomatic. We present the results on the use of the Perkinelmer 6-plex LSD reagents for the determination of 6 LSD enzyme activities from a single dried blood-spot (3.2mm) determined by tandem mass spectrometry (MSMS).

**Method:** The 6 LSD enzymes were galactocerebrosidase b-galactosidase (GALC; Krabbe disease), acid a-galactosidase A (GLA; Fabry disease), acid sphingomyelinase (ASM; Niemann Pick A/B disease), a-iduronidase (IDUA; mucopolysaccharidosis type I) and b-glucocerebrosidase (ABG; Gaucher disease). De-identified newborn dried blood-spots (DBS) were sourced from the South Australian population (~1,000) and DBS from confirmed positive LSD cases (N=75) obtained from the National Referral Laboratory (NRL) that included GALC (5), GLA (14) & carriers (28), ASM A/B (1), IDUA (6), GAA (20) and ABG (20). The PE LSD reagents were obtained from Perkinelmer (Waltham, MA, USA) for research use only. Individual LSD enzyme activities were determined by stable isotope dilution technique by MRM after a single organic solvent extraction and determination by FIA on an API5000 MSMS. MSMS setting were, IS voltage 5000, DP 95, CE 55 & CXP 15.

**Results & Discussion:** All six LSD analytical assay performance were acceptable with CV% on repeat DBS QC analysis of <12%. The statistical analysis of the normal newborn population gave the 1st centile in IU/hr/L whole blood for each enzyme as ABG 2.2, IDUA 1.3, GAA 4.1, ASM 0.8, GALC 1.8 & GLA 1.3, these were used to assess the activities of the known positive LSD DBS. A comparison between LSD activities measured in leucocytes using the standard 4-MU fluorogenic method and dried blood-spots using the 6-Plex PE MSMS showed excellent correlation. All LSD cases had activities below the 1st centile of the respective normal population, GLA showed some overlap, due to the non-enzymatic in-source fragmentation of the substrate, this was specific to the API5000 and not seen on the API3200 or 4000 instruments. Each assay showed >3 orders of magnitude in dynamic range with excellent lower end sensitivity.

**Conclusion:** The simplicity and accuracy of the PE 6-plex MSMS LSD reagents makes it amenable for use in newborn screening. Our study clearly showed the superior performance characteristics of large dynamic range and lower end assay sensitivity that was able to distinguish between unaffected and confirmed true positive LSD cases. This assay is currently being evaluated for both newborn screening and to replacing the existing 4-MU leucocyte based assays for the diagnosis of LSD.

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**P-70**

**Newborn Screening in Slovakia after Starting Tandem Mass Spectrometry: New Epidemiological Data in Slovak Newborns Population**

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**Background:** Newborn screening (NS) in Slovakia expanded in 2013 from 4 to 13 treatable inborn errors of metabolism (IEM). MS/MS technology was introduced in laboratory of Newborn Screening Centre Slovak Republic at the University Children Hospital Banská Bystrica, which is the Central laboratory for NS in Slovakia. Aminoacids and acylcarnitines profiles with special software uncovered more metabolic
diseases, than it was established in pilot study in 2012. Different screening prevalence in Roma ethnic, than Caucasian ethnic in Slovakia was detected.

**Methodology:** Derivatised Chormsystems kit was used for analysis of 145,609 dry blood spots, in time period January 2013 – August 2015. Agilent 6420 Triple Quadrupole LC/MS system was used as laboratory equipment with special evaluation software for 39 amino acids and acylcarnitines with appropriate ratios and cut off’s for aminoacidopathy, mitochondrial diseases of fatty acid oxidation and organic acidemias.

**Results:** 145,609 newborns were analysed in period 2013 – 08/2015. For IEM, analysed with MS/MS technology, 138 positive cases has been confirmed, with screen prevalence 1 : 1055 for neonatal population. The prevalence for Caucasian newborns is 1 : 1735, for Slovakian Roma population 1 : 334. Registration of ethnicity in NS in Slovakia is important part of epidemiological data in NS. Monitoring ethnicities allows us to find out one of the biggest frequency IEM in Roma ethnic in Europe. MCADD and SCADD are the most often, notably SCADD with almost 100% coverage in Roma newborns.

**Conclusions:** NS in Slovakia is successful preventive program with 30 year history. 1.9 million newborns were screened from 1985 to 08/2015. MS/MS technology allowed screen for aminoacidopathy, fatty acid oxidation diseases and organic acidemias in NS in Slovakia. Preliminary results in Slovakia point to high frequency metabolic diseases, notably for Roma newborns. These results are preliminary, needs to be more data.

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**P-72**

**The Philippine Expanded Newborn Screening-The First Year of Implementation**

M.M.L. Alcausin and C. Padilla, University of the Philippines-Manila, Manila, Philippines

The Philippine Newborn Screening (NBS) Program started in 1996 as a pilot study involving 24 hospitals in the national capital region with the objectives of 1) determining the incidence in the Filipino population of five conditions: congenital hypothyroidism, congenital adrenal hyperplasia, phenylketonuria, galactosemia and homocystinuria and 2) to come up with recommendations for a national newborn screening program. Homocystinuria was removed from the panel after one year of the study because no case was picked up and was replaced with glucose-6-phosphate dehydrogenase deficiency. Screening for these five disorders was recommended to the Department of Health and finally, in 2004, the Newborn Screening Act or the Republic Act 9288 was enacted to institutionalize NBS in the country.

Eighteen years later, in December 24, 2014, after successful integration into the public health system and inclusion in 2012 of maple syrup urine disease in the panel of tests, the Philippine NBS program started offering a second option from the six-test panel, the expanded NBS, testing for more than 20 disorders including hemoglobinopathies, disorders of metabolism of amino acids, organic acids and fatty acid oxidation, biotinidase disorders and cystic fibrosis. The inclusion of these conditions in the
expanded panel was based on occurrence of conditions in babies of Filipino descent in the California State NBS.

This is a descriptive study with the objective of determining the incidence of disorders in the expanded panel in the population during the first year of implementation of the expanded NBS.

Preliminary data shows, after screening 15,080 by the end of July, predominance of hemoglobinopathies in the screened population. There were 15 confirmed cases of alpha thalassemia disease, 11 alpha thalassemia carrier, two sickle cell trait, 41 Hemoglobin E trait, eight Hemoglobin D trait and one Hemoglobin C trait. One case of Multiple Carboxylase Deficiency was confirmed.

The Philippine NBS program just recently started offering expanded NBS. This is not mandatory and is offered as a second option to the basic six-panel test because of cost issues. Despite this limitation, the program has been able to pick up confirmed cases, mostly hemoglobinopathies, and the number is expected to increase as health providers and the public become more aware of the availability of this service.

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Newborn Screening Incidental Findings: Variability in State Practices
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**Background:** The rapid expansion of newborn screening (NBS) has created an increasing number of incidental findings (IF) detected through the course of routine screening, or findings that arise outside the original purpose for which the test or procedure was conducted. Incidental findings can be anticipatable (a finding that is known to be associated with a test or procedure) or unanticipatable (a finding that could not have been anticipated given the current state of scientific knowledge). In the case of NBS, incidental findings are results that are not the target of screening within a given program, but are found as a result of screening and the resulting diagnostic workup for that target. These findings may or may not have immediate clinical impact for the infant. The objective of this project was to understand how newborn screening incidental findings are managed across the U.S.

**Methods:** A literature review and presentation to NBS stakeholders was conducted to define and understand the concept of “incidental findings” as it relates to newborn screening. The insight gained was used to develop a cross-sectional, self-reported online survey design that was distributed to 50 states and 3 territories.

**Results:** Of the 26 newborn screening programs who completed the survey, all 26 report Sickle Cell Trait; 21 of 26 programs ensure follow-up of incidental findings for Sickle Cell Trait and 16 programs do so for CF Carrier; 17 programs reported following CRMS until diagnosis is determined and 23 follow VLCADD variants of unknown significance. A majority of NBS programs store incidental findings with other NBS positive results. Nineteen programs have a policy addressing length of storage for abnormal
results, and 17 of 19 programs store incidental findings and abnormal results the same length of time. Finally, 20 programs can ensure follow-up for incidental findings detection.

**Conclusions:** How NBS programs manage incidental findings varies across the U.S. Reporting and follow-up varies depending on the finding and the program. Some programs can ensure infants receive follow-up care for incidental findings detection. The results from this survey will ultimately be used to develop national guidelines for incidental findings management in the United States.

1 Anticipate and Communicate: Ethical Management of Incidental and Secondary Findings in the Clinical, Research, and Direct-to-Consumer Contexts. [http://bioethics.gov/node/3183](http://bioethics.gov/node/3183)

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**The State of Newborn Screening Systems**

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**Background:** Newborn screening public health systems in the U.S. are guided by national recommendations; however, implementation and program development decisions occur at the state level. States vary in their program policies, number of disorders screened, fees, and other elements of program activity. The NewSTEPs Data Repository maintains information on US NBS programs in the form of State Profiles. Examination of individual state profiles, as well as comparison across states of publicly available categories within the profiles can inform us of the state of newborn screening systems.

**Objective:** To describe the landscape of state newborn screening programs in 2016.

**Methods:** NewSTEPs queried information within State Profiles and sought confirmation regarding the information provided by sharing reports with each state newborn screening program prior to public presentation. Data presented includes 50 states, the District of Columbia and Puerto Rico.

**Results:** NewSTEPs State Profiles capture data within the following categories: Policies, Disorders Screened (core, secondary, other), Addition of disorders to the NBS panel, Fees, Program Structure, Information Technology, Advisory Committee, among others.

As of August 2015, 52 newborn screening programs comprised of 36 newborn screening laboratories served newborn populations ranging in size from 6,100 births per year to over 500,000 (median 52,200). Regional laboratories serve 13 states, while all states have their own short-term follow-up programs.

Consent is implied for screening in most states, with a majority of states allowing parents to opt-out of NBS. Funding of NBS activities is secured from different sources, however most programs (n=47/51) have an NBS fee (median $71, range $15 – 157).

Storing residual dried blood spots ranges from 1 month to more than 27 years, with a median of 1 year. New disorders are added to state NBS panels via a variety of mechanisms (legislation n=22, commissioner/board of health n=18, other n=2, unknown n=9).

Weekend operating status is varied within the United States, as is the composition of Advisory Committees. The process to add additional disorders to the RUSP is captured, with states varying in the time between addition of a disorder to the RUSP to implementation of screening in their states.
**Conclusion:** Newborn Screening programs within the United States do not operate in a uniform fashion; regardless all states do screen for a minimum panel of disorders. The differences between programs can be understood by examining a variety of categories. The patterns that emerge as well as the differences identified can serve demonstrate the dynamic state of Newborn Screening and related policies in the United States in 2016. Maintaining a centralized repository of uniform data elements and producing reports annually can allow programs to benefit from practices and lessons learned in other states.

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P-75 – Withdrawn

P-76 – Withdrawn

P-77 – Withdrawn

P-78 – Withdrawn

P-79

**Severe Combined Immunodeficiency (SCID) Newborn Screening Implementation: Grantee Experience**

R. Salsbury¹, S. Singh¹, Y. Kellar-Guenther², J. Ojodu¹, M. Sontag²; ¹Association of Public Health Laboratories, Silver Spring, MD, ²Colorado School of Public Health, Aurora, CO

Severe Combined Immunodeficiency (SCID) newborn screening was added to the Recommended Uniform Screening Panel (RUSP) in May 2010. The implementation of SCID screening at the state level has been steady and yet 20 states continue to face barriers to universal screening of SCID as of April 1, 2015. Current challenges faced by states newborn screening programs in implementing SCID screening include (1) integration of new technology within the lab, (2) laboratory staffing to conduct tests, (3) clinical follow-up capacity and resources, (4) funding for personnel, equipment, education, and (5) legislative or statutory approval.

In 2015 APHL was funded by the Health Resources Services Administration (HRSA) to perform SCID implementation in the United States. APHL has funded 12 programs (11 states and the Immune Deficiency Foundation) to support SCID laboratory screening, follow-up, clinical network development, and educational resource development activities. This poster will outline the states' starting points, progress, and expected outcomes as a result of being grantees. The poster will also share technical assistance resource that have been made available through this funding.

**Presenter:** Ruthanne Salsbury, Association of Public Health Laboratories, 8515 Georgia Avenue, Silver Spring, MD, 20910, Email: ruthanne.salsbury@aphl.org
Impact of a Marketing Campaign to Raise Awareness of a Continuing Education Activity
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Objective: To evaluate the impact of the marketing campaign to raise awareness of the availability of the free online continuing education (CE) activity for the 2012 CDC guideline “Good Laboratory Practices for Biochemical Genetic Testing and Newborn Screening for Inherited Metabolic Disorders” at the 2014 APHL Newborn Screening and Genetic Testing Symposium (NBSGTS) Program and in two issues of the Genetics in Medicine journal.

Methodology: The marketing campaign for the continuing education activity consisted of placing advertisements to two separate audiences: a) one targeted to the attendees of the 2014 APHL NBSGTS and b) one targeted to subscription holders of the Genetics in Medicine journal. To measure the impact of the targeted advertisements, APHL and CDC staff developed and fielded two online surveys. Survey 1 was sent to the APHL NBSGTS participants, reminding them of the various ways that the CE activity was marketed at the Symposium. Survey 2 was sent via a Society for Inherited Metabolic Disorders listserv, calling attention to the advertisement that was in the October and November 2014 issues of the Genetics in Medicine (GIM) journal. Respondents were asked to how they learned about the CE activity and how it impacted their testing procedures and quality management protocols. Information was also gathered from the CE hosting program to obtain a baseline of registrants and document activity during the marketing initiative.

Results: There were 71 responses to the survey marketed to the NBSGTS attendees and 39 responses to the survey marketed to the GIM readers. The analysis of the data will include summarizing the number and types of participants and how the respondents shared information gained from the CE activity and who they shared it with.

Conclusion: The data is currently being analyzed. A summary report will be developed and used to populate the conclusion section of the poster.

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Results from the 2014 APHL Newborn Screening Timeliness Survey
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Background: In order to effectively detect potentially disabling conditions in newborns, newborn screening (NBS) must occur in a timely manner. In 2014 the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) has recommended the following timeframes related to NBS: initial NBS specimens should be collected at 24 to 48 hours of life; NBS specimens should be received at the laboratory within 24 hours of collection; newborn screen results for time-critical conditions should be available within five days of life; and all NBS results should be available within five days of collection.

Method: In July of 2014 APHL fielded an online survey to 53 NBS program directors to probe possible factors that may impact a NBS program’s ability to meet the recommended time frames.
Results: The two factors for recommendation 1 that garnered the highest levels of impact by NBS programs were premature births (54.9%) and compliance with specimen collection from premature/sick infants guidelines (43.1%). The two factors for recommendation 2 that garnered the highest levels of impact by NBS programs were geographic distance from birthing facility to NBS laboratory (64.8%) and batching of specimens by birthing facilities (47.1%). The two factors for recommendation 3 that garnered the highest levels of impact by NBS programs were specimen receipt falls outside of the recommended time frame (67.5%) and operating hours of courier system (45.9%). The two factors for recommendation 4 that garnered the highest levels of impact by NBS programs were delays in the processes that lead up to release of NBS results (78.4%) and nature of the test itself (54.9%).

Implications: Newborn Screening is a system involving education, screening, follow-up, diagnostic confirmation, management, program evaluation and continuous quality improvement. There are MANY partners in a successful NBS program. Results of this survey reveal that the impacting factors may be within the domain of specific programs in the NBS system (e.g., batching specimens by birthing facilities), but impact a NBS program’s overall ability to meet the ACHDNC recommendations for timeliness. The issues of timeliness are not solely part of one specific program in the NBS system, but instead are uniquely part of all the programs that make the NBS system. While ensuring that birthing facilities do not batch specimens is an important step to take to address timeliness, it is also of importance to address issues with courier services, geographic distance between the birthing facilities and the NBS laboratory, testing procedures, and follow-up. Continuous quality improvement and communication between the multiple programs in the NBS system are an essential component to meet the ACHDNC recommendations for timeliness.

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Homogeneous Single-point PCR Detection of TREC and Beta-Actin DNA in Dried Blood Spot Samples
K. Mattila, P. Ollikka, H-M. Raussi, M. Sjoroos, P. Kerokoski, A. Ylikoski, PerkinElmer, Turku, Finland

Simple methods are needed to perform genetic tests. Many of the homogeneous PCR assays require either DNA extraction from dried blood spot (DBS) samples or other pre-treatment steps of the DBS samples to perform the analysis. Our aim was to study whether multiplexed detection of T-cell receptor excision circles (TREC) and beta-actin DNA is feasible from DBS samples with minimal sample pre-treatment.

A 1.5 mm disk sample was punched into the reaction well and elution incubation was performed. This was followed by the addition of the PCR mix. The reactions were sealed and moved to a thermal cycler. After cycling, the amount of TREC and beta-actin amplicons was measured through the seal without removing the disk. The methodology utilizes dual-label time-resolved fluorescence resonance energy transfer as detection chemistry. Each experiment contained negative (no template) and positive control reactions.

Our findings suggest, as will be presented in the poster, that DBS is a suitable matrix for an extremely simple method to perform multiplexed single-point PCR measurement. The analysis of known and unknown DBS reference materials showed that TREC and beta-actin amplification products may be
successfully and correctly detected. To respond to the requirements of screening laboratories, we present that DBS may be used as a sample matrix in PCR with very simple pre-processing of the sample disks.

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**P-83**

**Results of Prototype Automated Creatine Kinase Muscle Isozyme Immunoassay for Potential Duchenne Muscular Dystrophy Identification**

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**Introduction:** Duchenne muscular dystrophy (DMD) causes progressive muscle degeneration and premature death affecting 1 in 3600–6000 live male births. DMD is caused by a dysfunctional dystrophin gene located in the X-chromosome. In DMD cases, the progressive breakdown of skeletal muscle cells releases intracellular creatine kinase muscle isozyme (CK-MM) to the circulation, making it possible to screen for DMD with this biomarker.

**Objective:** Our objective was to evaluate the analytical performance of a prototype automated immunoassay for measuring CK-MM from dried blood spots (DBS), which is currently under development for the GSP® high throughput analyzer system from PerkinElmer.

**Methods:** Using a panel of DBS samples prepared from human whole blood, some spiked with purified human CK-MM, we characterized the precision, analytical limits and measuring range of the prototype assay under development. We also studied the stability of CK-MM in DBS samples and measured a panel of 259 de-identified neonatal samples and 10 de-identified DMD affected DBS samples.

**Results:** The median CK-MM concentrations of the neonatal samples and DMD affected samples were 130 ng/mL (range: 29 to 804 ng/mL) and 5560 ng/mL (range: 1220 to 9920 ng/mL), respectively. Thus the highest unaffected sample was lower than the lowest DMD affected sample. The assay could detect CK-MM down to the <10 ng/mL level, so none of the neonatal samples tested were too low to measure. Three of the DMD affected samples were above the highest calibrator of the assay (8000 ng/mL) and thus technically above the measuring range. The precision of the assay was acceptable within the measuring range. Humidity and temperature had a significant effect on the stability of CK-MM in DBS samples, but acceptable long-term stability could be achieved by freezing the samples at -20°C.

**Conclusions:** The results suggest that the GSP® CK-MM assay under development has a sufficient measuring range to span the relevant range of affected and unaffected neonatal samples. While results above the measuring range are not analytically precise, the elevation would be indicative of a sample with a CK-MM concentration outside levels found in healthy individuals. There was a complete separation between the distributions of the normal and DMD affected samples in this study. For long-term studies, DBS samples should be stored frozen to ensure the stability of CK-MM.

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Developing Improvements to a Non-derivatized Mass Spectrometry Method
D. Shah¹, L. Goncalves¹, J. Campbell¹, T. Lehtonen², T. Nikula², J-M. Brozinski², M. Kuracina¹, M. Schermer¹; PerkinElmer, ¹Waltham, MA, ²Turku, Finland

The PerkinElmer® NeoBaseTM (NB1) is a newborn screening kit that uses non-derivatized MSMS method to measure blood levels of amino acids, free carnitines and acyl carnitines in newborns. Introduced in 2006, abnormal levels determined by the kit may be indicative of inborn errors of metabolism. The next generation of NeoBase kit, NeoBaseTM 2 (NB2), is being developed to improve analyte detection, laboratory productivity and instrument compatibility.

In this development, assay functionality is increased by including additional small molecule markers of inborn errors of metabolism to the current NB1 panel. The NB2 kit is proposed to measure all NB1 analytes plus four new amino acids – arginine (Arg), ornithine (Orn), glutamine (Gln) and argininosuccinic acid (ASA); five new acyl carnitines - C18:2OH, C20, C22, C24 and C26; four new lysophospholipids C26:0 LPC, C24:0 LPC, C22:0 LPC and C20:0 LPC; and two purine nucleosides adenosine (ADO) and deoxyadenosine (dADO). The literature suggests that abnormal levels of one or more of these markers may be associated with OTCD (ornithine transcarbamylase deficiency) diseases, ASA-LD (argininosuccinic acid lyase deficiency), X-ALD (X-linked adrenoleukodystrophy) and ADAD (ADA-SCID, Adenosine Deaminase Deficiency).

Compared to some other methods the NB1 kit has somewhat lower recovery of succinylacetone and longer sample preparation time. Based on preliminary development work, succinylacetone's recovery can be enhanced considerably while sample preparation time can be reduced significantly to 90 minutes from 165 minutes. The concentration of the marker analytes spiked into the low and high control dried blood spots (DBS) are being updated to provide levels more representative of the concentrations with clinical relevance. The development goals of the NB2 project are based upon nine years of usability from the NB1 globally sold product. The NB2 project aims to incorporate new markers, improved performance and enhanced functionality.

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Improvement in the Extraction Efficiency of Succinylacetone
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Succinylacetone (SA) is a primary marker for Tyrosinemia type 1 disease. The NeoBaseTM (NB1) kit has produced lower recovery of the SA analyte with CDC proficiency samples compared to other assays. Lower SA recovery has not led to any clinical performance issues but it commonly raises questions from users. More significantly, the added incubation time required for the conversion of SA impacts laboratory efficiency.
SA, in its endogenous form, is mainly bound to blood proteins and peptides. To enable the quantitation of SA, hydrazine (or a hydrazine analogue) in the presence of an acid is used. It facilitates the unbinding of the molecule from the blood matrix and creates a stable, free SA hydrazone, which is detected in the mass spectrometer. Therefore, the extraction efficiency of SA is a combination of extent to which the bound SA is unbound and converted to SA hydrazone in addition to the factors which typically influence the recovery, such as solubility in the extraction solution and the final ionization efficiency.

In this study, hydrazine and its analogue dansylhydrazine were tested to identify which works better in improving succinyl acetone extraction efficiency. A disadvantage of increasing the concentration of hydrazine or dansylhydrazine is ion suppression; therefore optimizing the concentration is crucial. The effects of hydrazine in concentrations of 0, 0.6 (control – NB1), 2, 3, and 5 mM and dansylhydrazine – 3mM were evaluated on SA recovery. It was observed that, the recovery of SA increases significantly with increasing hydrazine concentration from 0.6 mM to 3 mM. Higher concentrations also drive the reaction to completion in shorter time while there is a minimal gain in increasing hydrazine from 3mM to 5 mM. From these experiments 2mM of hydrazine concentration was determined to be optimum in terms of SA recovery and ion suppression of the other analytes, and with a shorter incubation time.

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**P-86**

**Systematic Stability Test of Markers of Inborn Errors of Metabolism (IEM) in Dried Blood Spots**

D. Shah†, L. Goncalves†, J. DiPerna‡, A. Grushecky‡, M. Schermer†; †PerkinElmer, Waltham, MA, ‡PerkinElmer Genetics, Bridgeville, PA

Clinical validation of a new newborn screening assay traditionally requires the use of archived positive DBS samples. With established assays, positives are detected only rarely, and with new assays positive samples are only found in diagnostic labs. Archived samples may be months to many years old. The use of such samples in validations depends upon their analyte levels being stable throughout the storage period.

Some studies of DBS sample stability have been published, but the sample sets surveyed have been limited in scope and the number of samples tested at each time point has been limited. With the generous support of the California Biobank, we collected 120 archived (frozen) putative normal newborn DBS samples with each of the 11 following storage times: 0, 0.1, 0.5, 1, 2, 3, 4, 5, 7, 10 and 15 years. Time point 0 was tested at 2 days after sample collection and time point 0.1 years was tested 35 to 40 days after sample collection. All the samples were collected within 31 days of birth, with infant weight between 2500g or higher (full term). The archived samples had been stored frozen at -20°C.

These samples were assayed with an in-development non-derivatized MSMS amino acid / acylcarnitine (AAAC) assay on a new platform under evaluation, using a Waters® Xevo® TQD instrument over a period of one week. The freshest samples were assayed first. We measured 56 analytes including 13 amino acids, 36 acylcarnitines, one ketone, four lysophospholipids and two purine nucleosides. When measured concentrations of each analyte with mean and median are plotted against the age of the sample, no significant trend of degradation was seen in any of the analytes. While not fully conclusive in
evaluating the analytes that are at very low endogenous levels, this finding supports the use of frozen, archived DBS samples for long term stability testing of the above biomarkers and for validation studies of Inborn Errors of Metabolism (IEM) assays.

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**Throughput Optimization of Two NBS Assays on a Single MS Analysis Platform**

J. Cournoyer, A. Potier, J. Trometer, D. Shah and M. Schermer, PerkinElmer, Waltham, MA

Budget and space restrictions combined with an ever growing panel of required tests forces newborn screening laboratories to work at maximum efficiency. To help, mass spectrometry (MS)-based assays offer the advantage of multiplexing; generating a multitude of results from a single dried blood spot (DBS) assay. Furthermore, flexible software allows MS-based platforms to automatically run samples that originate from different assays, aiding further in maximizing the test result output within a 24 hour period. Considering these advantages, there is an emerging need to develop multiplexed MS-based assays that utilize aligned instrument configurations. The data presented here shows how two such aligned MS-based assays can be used in tandem to maximize the test result output of single MS instrument.

Two assays, the commercially available metabolite assay measuring 43 analytes and an enzyme activity assay (in development) measuring 6 enzymes, were run on a single MS instrument over a simulated 6-day work week starting on Monday with samples arriving at the lab at 11AM. The metabolite assay requires a 4-hour extraction workup and the enzyme activity assay requires overnight incubation and a 1.5 hour post-incubation sample workup. Samples generated from the metabolite assay were run overnight on the MS system and the enzyme activity samples were run during the day. Both assays use different flow and wash solvents and different inlet and MS methods but the software was configured to easily switch between the two set-ups with only a small amount of time (0.5 hour) required for daily instrument maintenance. The 6-day test generated results for ~1200 DBS samples representing a throughput of approximately 60k samples per year. Both assays showed as good precision and accuracy as when run on a single assay-dedicated instrument. In summary, the study shows that these two instrument-aligned MS-based assays can be run on a single MS instrument with good accuracy and precision.

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Long-term Stability of ABG, ASM, GAA, GALC, GLA and IDUA in Frozen DBS

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Validation of a new enzyme activity assay traditionally requires the use of low-activity archived DBS samples. With established assays, low-activity sample are detected only rarely, and with new assays these low-activity samples are only found in diagnostic labs. Archived samples may be months to many years old. The use of such samples in validations depends upon their enzyme activity levels being stable throughout the storage period.

Some studies of DBS sample stability have been published, but the sample sets surveyed have been narrow in scope and the number of samples tested at each time point has been limited. With the generous support of the California Biobank, we collected 120 archived (frozen) putative normal newborn DBS samples with each of the 11 following storage times: 0, 0.1, 0.5, 1, 2, 3, 4, 5, 7, 10 and 15 years. Time point 0 was tested at 2 days after sample collection and time point 0.1 years was tested 35 to 40 days after sample collection. All the samples were collected within 31 days of birth, with infant weight of 2500g or higher (full term). The archived samples had been stored frozen at -20°C.

These samples were assayed with an in-development MSMS-based enzyme activity assay over a period of one week. We measured the activity of 6 enzymes: ABG, ASM, GAA, GALC, GLA and IDUA. When median and mean averages of measured activities of each enzyme are plotted against the age of the sample, the degradation in activity was in the range of 3-10% for all of the enzymes. While not fully conclusive in evaluating the enzyme activities at very low levels, this finding supports the use of frozen, archived DBS samples for assay validation studies that measure the activity of these enzymes.

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Contrived Enzyme-specific Deficiency in Dried Blood Spots

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A routine practice amongst laboratories today is the use of multi-level control dried blood spots (DBS) during assay development, validation and for quality control purposes. These DBS can be made by spiking in known amounts of analytes into endogenous blood when one wants to measure biomarkers. Alternatively, if monitoring blood enzyme activities is needed, DBS samples can be made from dilutions of white blood cells (WBC) by mixing different ratios of whole blood, buffy coat, and plasma with leukocyte-depleted blood. One disadvantage to the latter procedure is that diluting WBC is not enzyme-specific; dilution will cause all enzymes to decrease approximately the same amount relative to their initial activities. This work addresses an unmet quality control need: how to make dried blood spots that are deficient in only one specific enzyme. More specifically, controls were made to monitor six lysosomal enzyme (ABG, ASM, GAA, GALC, GLA and IDUA) activities in DBS.
This new approach in making enzyme-specific deficient DBS begins by processing leukocyte-filtered blood as described by the Centers for Disease Control (CDC). Next, controlled mixtures of recombinant human enzymes were spiked into different aliquots of blood and mixed accordingly to reach specific activities. The desired target activities for any one set of controls was to have five of the six enzymes at or above the average for DBS samples while one enzyme was below the CDC low control range. All six sets of controls were successfully made and when tested against the CDC control DBS showed acceptable correlation and precision.

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P-90

Using an Amino Acid and Acylcarnitines Metabolic Profile as a Potential Tool for Premature Baby Assessment
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In two relatively recent clinical trials, we studied more than 1100 patients producing nearly 4000 DBS samples that were analyzed by MS/MS for amino acids and acylcarnitines. In the first study, the influence of two concentrations of amino acid solutions in TPN, “protein dose”, was examined whereas the second study differences in profiles based on gestational and sample age were obtained. No infant was diagnosed with an inborn error of metabolism in either study. However, a significant number of alerts (7.2%) were reported. The median values of acylcarnitines and amino acids were examined based on the sample collection protein and carnitine dose, day of collection and gestational age. The data processed using excel and further examined using Spotfire. The data confirms what is known that interpretation should be made in light of sample collection day ranging from day 1-42. It also shows, that gestational age, and exposure to parenteral nutrition has a significant impact on medians and false result rates. It can be concluded that the interpretation of results for premature infants who are commonly receiving parenteral nutrition requires a different approach than that for term newborns collected on day 2-3.

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A Novel Five-Plex Real-Time PCR Assay that Detects SMN1, SMN2, TREC, KREC, and RPP30
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A five-plex real-time PCR assay was developed to detect five different loci using DNA isolated from a single 3.2mm punch of a dried blood spot (DBS). The PCR assay detects the absence of exon seven in the SMN1 gene while simultaneously detecting and possibly quantifying the copy number of the SMN2 gene. This is achieved by using dual labeled Locked Nucleic Acid (LNA) Taqman® probes for both the SMN1 and SMN2 genes. The LNA Taqman® probes for SMN1 and SMN2 differ in sequence by a single
nucleotide polymorphism (SNP). Using elevated annealing temperatures and different fluorescent labels on the LNA Taqman® probes, the PCR amplification for SMN1 and SMN2 can be specifically determined in the real-time PCR assay.

The amplification of the house-keeping gene, RPP30, is included in the assay as a positive control of DNA purification and can be used to help determine the copy number of SMN2 by a ratio analysis. To further demonstrate the utility of a multiplex assay, PCR primers and standard dual labeled Taqman® probes for T-cell receptor excision circles (TREC) and for K-deleting recombination excision circles (KREC) were included. The five-plex real-time PCR assay performance was demonstrated on +2000 DNA samples extracted from 3.2mm punches of putative normal DBS, as well as DNA from several characterized reference samples and controls. The results from this study with a five-plex real-time PCR assay demonstrates the potential of future molecular DBS assays.

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**Evaluation of an Enzymatic Method for Screening of Biotinidase Deficiency from Newborns**

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Biotinidase is an enzyme releasing biotin (also known as vitamin B7 or H) from dietary proteins. Biotinidase deficiency can lead to a decrease in biotin availability and if untreated, it is associated with the neurologic and cutaneous consequences. Biotin supplementation can prevent the development of symptoms of biotinidase deficiency, which makes the early diagnosis very important.

Newborn screening of biotinidase activity from dried blood spots can identify affected patients shortly after the birth. The new Neonatal Biotinidase of Labsystems Diagnostics is a modification of the quantitative fluorometric method published in 1992 by Pitkänen and Tuuminen. It is based on monitoring of fluorescent signal of the 6-Aminoquinoline (6-AQ), product formed during the reaction as a result of the biotinidase activity.

For the clinical evaluation of the Neonatal Biotinidase kit, we tested 483 negative samples, collected from healthy newborn babies, and 23 positive samples from babies with biotinidase deficiency. All samples were verified by a hospital laboratory in Switzerland and stored at -20°C prior analysis. In addition, we evaluated several different types of filter papers for collecting the samples from the pricked heel of a baby after birth. The filter papers are not removed before measurement and hence any floating traces of paper can disturb the measurement.

The cut off of the assay was set to be at 100U (where Unit=nmol/min/dL of 6-AQ released due to enzyme activity). The detection limit of the assay was shown to be 13U, and the linear range of the test was up to 400U. The test was shown to be stable and the reproducibility was good. All the positive samples from newborns with verified total or partial biotinidase deficiency gave a positive result. Four of the negative samples gave a result below the cut off. With this material, the sensitivity of the test was 100% and the specificity 99%.
Reference:
Pitkänen L and Tuuminen T. A quantitative fluorometric micromethod used for the neonatal screening of biotinidase deficiency in Finland. Screening, 1992 1, 185-194.

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New NeoMass™ AAAC Kit for Expanded Newborn Screening of Multiple Metabolic Disorders by LC–MS/MS
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Abnormal blood levels of amino acids, free carnitine and acylcarnitines may indicate an inborn error of metabolism or genetic metabolic deficiency. Amino acids are markers of amino acidopathies, whereas free carnitine and acylcarnitines are markers of the oxidative disorders of the fatty acids (FAO) and organic aciduria (AO). Labsystems Diagnostics’ recently developed NeoMass™ AAAC kit provides the expanded screening of 14 amino acids, free carnitine and 12 acylcarnitines from dried blood spots (DBS) using stable isotope labeled internal standards (ISTD), DBS controls and a rapid extraction method prior to LC–MS/MS analysis. In addition, the screening of succinylacetone (SUAC) and argininosuccinic acid (ASA) are provided as separate extraction protocol without derivatization.

NeoMass™ AAAC includes three DBS controls (low, medium and high levels) and a simple four step protocol for the screening of the analytes without derivatization; punching, 20 min extraction on 96 well plates with ISTDs for each analyte, plate transfer and injection into LC–MS/MS. NeoMass™ AAAC screening is performed by selective multiple reaction monitoring (MRM) mode and have been assessed by different LC–MS/MS instrumentation systems e.g. Shimadzu LCMS-8040 instrument. Analysis is fast (< 2 min) and sensitive, since only 5 µl injection volume required.

Kit performance and clinical evaluation were performed using Quality control samples of the Quality Control Program, Centers for Disease Control and Prevention (CDC, Atlanta, Georgia, USA) and confirmed newborn samples provided from the University Children’s Hospital (Zurich, Switzerland). For clinical evaluation, over 500 DBS samples were analyzed having high specificity and sensitivity. Inter-assay and intra-assay precision was shown to be excellent with approx. CV ≤ 15%. NeoMass™ AAAC provides efficient and reliable high throughput screening of potential metabolic disorders of newborns.

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The State of Newborn Screening for Hereditary Tyrosinemia Type 1 (HT-1) in the United States

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Introduction: Hereditary Tyrosinemia Type 1 (HT-1) is a rare autosomal recessive disease that can cause liver failure and death within the first years of life if not detected early. Newborn screening (NBS) is necessary for early asymptomatic diagnosis and ensuring an improved HT-1 patient prognosis. While elevated tyrosine can be a marker for HT-1, it is not specific for the disease and can be falsely normal in early infancy. Succinylacetone (SUAC) is a specific diagnostic marker for HT-1 that represents a significant improvement over screening via tyrosine levels. However, across the US, NBS programs continue to use both tyrosine and SUAC assays. We are interested in understanding the shift amongst NBS programs from tyrosine to SUAC as an HT-1 assay, noting how this shift can enable accurate timely diagnosis and potentially reduce symptomatic HT-1 presentation.

Method: We conducted online research, augmented by telephone and email correspondence with state newborn screening programs, during the 2nd quarter of 2015 to develop a comprehensive picture of the current state of HT-1 newborn screening throughout the United States.

Results: Of 51 NBS programs, 40 currently test for SUAC as a primary marker for HT-1, including Virginia and Indiana, which have implemented SUAC since 2013. Two states use SUAC as a secondary marker. The other nine states use tyrosine alone as an assay for HT-1 NBS. Further adoption of SUAC testing is on the horizon, with North Carolina currently piloting the assay.

Conclusion: While progress has been made toward uniform NBS via SUAC for HT-1, the use of the SUAC assay is still not consistently implemented in the United States. Broader implementation of SUAC testing is contingent upon the priority a given state assigns to funding the upgrade, piloting, validating, and optimization of the assays. Additionally, positive HT-1 patient specimens are required for successful validation of the assays, a challenge faced by at least one state. Piloting and validation of SUAC testing could be expedited through collaboration and sharing of information between all states. Only when all 51 NBS programs are testing for SUAC as a primary marker in newborn screening can we be confident in effectively eliminating the symptomatic presentation of HT-1.

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ELSI Advantage- A Technology Solution for Ethical, Legal, and Social Issues in Newborn Screening

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Background: The American College of Medical Genetics and Genomics operates the Newborn Screening Translational Research Network (NBSTRN) Coordinating Center for the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The NBSTRN is a resource for newborn screening-related research, focusing on creating content and infrastructure to establish an analytical, clinical and research framework. Our work focuses on the creation of information technology (IT) solutions that support the development of new technologies, treatments, and clinical information in newborn screening.
**Methods:** In partnership with 5AM Solutions, a software solutions company specializing in healthcare and life sciences, we built an interactive web resource to educate and support the NBS Community on the ethical and legal implications of genetic screening. This informatics tool supports the NBS researcher in the planning and implementation of their research projects. The NBSTRN interviewed clinical and research professionals that had an advanced knowledge and expertise of the most important ethical, legal, and social issues (ELSI) to help develop the most useful and relevant tool for the newborn screening community.

**Results:** The NBSTRN team and 5AM Solutions developed the ELSI Advantage, a collection of tools and information that can be used to support research related to NBS. ELSI Advantage informs researchers about ethical, legal and social issues that may arise in the planning and implementation of their research. Two of it’s tools are highlighted below:

The Custom Consent Form Builder allows researchers to initiate the creation of informed consent forms that are specific to their research project. It takes the user step-by-step through the common elements of an informed consent form and allows the entry of project-specific information. The custom consent form can be exported and used to consult with the researcher’s institution and IRB to further refine specific consent requirements.

“ELSA” is an interactive avatar that serves as an “Ethical Legal and Social Assistant” that helps answer ELSI-related queries. As an avatar, “she” can answer questions related to Informed Consent, IRB’s, Return of Results and State-specific NBS information. When ELSA is unable to answer a question the user is directed to contact the NBSTRN team or the NBSTRN workgroup members directly. This resource allows users to receive feedback from various stakeholders in a single query.

**Conclusions:** The NBSTRN resources are available for researchers conducting efforts related to NBS. The use of NBSTRN resources allows research dollars to be devoted to screening additional births rather than to the development of IT and informatics. The NBSTRN establishes a valuable resource to support additional states to implement NBS for new diseases and to collect longitudinal information about NBS identified individuals.

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**Improve Newborn Screening Pre-Analytical Timeliness with Sample-Tracking Software**

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Newborn screening lab staff don’t know when a baby is born. That means they never know how many samples are in transit to their lab and where they originate. They may not know when a sample is delayed or lost. Can informatics solve this problem? Absolutely. It is possible to track every sample from the moment it is collected to the time that laboratory results are reported and made available to physicians and parents. New software called Track-Kit gives each stakeholder their own secure portal, including medical facilities, midwives, laboratory staff, physicians, and most importantly, parents. If a sample doesn’t arrive at the lab within the specified time, staff is notified and can see where it originated and where it was last touched and by whom, to find it quickly and avoid further delays. Also important is the much-needed direct communication channel Track-Kit gives NBS programs so that they can provide information bulletins to each stakeholder, such as new collection procedures to medical facilities. Parents can access location and language-specific resources via their portal. Everyone is kept
informed as appropriate and is alerted of any problems so prompt action can be taken to stay on track and optimize timeliness.

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**Treatable Causes of Intellectual Disability: An Indian Newborn Screening Experience**

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India has 2.04 million developmentally disabled children aged between 0 and 6 years. Early identification of genetic causes of intellectual disability will enable early intervention. Since 2007 our lab has been doing newborn screening using Tandem Mass spectrometry to detect various inborn errors of metabolism.

We have been able to screen 18 of the 81 treatable causes for intellectual disability. (C.D.M. van Karnebeek et al Mol.Genet.Metab. 2012).

We aim to present a summary of our etiological yield of these treatable disorders.

Since September 2007 to January 2015 using LC-MS/MS over 55,196 newborns have been screened by our lab. Around 53.2% were healthy newborns and 46.8% were either older infants or symptomatic cases. Aminoacidopathies account for 34.5% of the disorders (Tyrosinemia (n=41), MSUD (n=115), PKU (n=59)), Organic acidemias (3MCC, 3MBG, HMG, BKT (n=28), IVA (n=35), MMA or PA (n=219), GA I (n=49)) 53.2%, Argininemia 2.09%, and the rest due to Biotinidase deficiency (n=68 by qualitative enzyme assay).

Our results are pointers to the need for developing guidelines for effective management and treatment of these disorders as well as increasing the widespread use of newborn screening by TMS for Indian children to prevent intellectual disability.

**Abbreviations**

MSUD - Maple syrup urine disease  
PKU - Phenylketonuria  
3MCC - 3 Methyl Crotonyl Coa Carboxylase  
HMG - 3 hydroxy-3-methylglutaryl-CoA lyase  
2MBG - 2-methylbutyryl-CoA dehydrogenase  
BKT - Ketothiolase  
IVA - Isovaleric academia  
MMA - Methylmalonic academia  
PA - Propionic academia  
GA I - Glutaric academia
**Benefits of a Comprehensive NextGen Sequencing (NGS) Panel for Supplemental Newborn Screening and Confirmatory Testing: Surprise Findings beyond the Primary Diagnosis!**

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Baby Genes, Inc. has performed NGS of 90 genes corresponding to 57 of the 60 current or proposed RUSP conditions in a single assay on 283 clinical samples to date. Indications for testing were (a) supplemental NBS specimens (n = 95) and (b) reflex diagnostic specimens (n = 188). Diagnostic yield according to these categories is roughly 2-14% (a) and 14-22% (b). Most of the molecular variants were classified as “benign” or “likely benign” and not reported to the family or referring physician. The next most common category was a variant of unknown significance (VOUS) representing 84% of all reported findings. The remaining variants fell into the categories of “likely pathogenic” (11%) or “pathogenic” (5%).

Average turnaround time after specimen arrival in the lab was 62 hours but never exceeded 96 hours. NGS can be both faster and cheaper than Sanger sequencing of single genes. If results are ambiguous, parental samples are requested in order to provide a definitive diagnosis, as well as to facilitate genetic counseling. Parental data may shed light on issues such as de-novo mutation, uniparental disomy (UPD), whole gene deletion or unsuspected consanguinity. The Baby Genes panel provided answers beyond what was expected in these examples:

**Case 1 -** Homozygosity for a rare likely pathogenic mutation in IVD [c.1042G>A (p.Gly348Ser) Chr15:40708546] was found as the presumptive cause for this Hispanic teenager’s isovaleric aciduria. However, multiple other homozygous variants in the same region of chromosome 15 were identified (13 of 13 in IVD at 15q15.1 & 19 of 19 in DUOX2 at 15q21.1), raising the suspicion of consanguinity or UPD. Analysis of more distally located genes (31 Mb away) on the NGS panel (ETFA at 15q24.2-q24.3 & FAHat 15q25.1) revealed heterozygous loci as well as evidence for low-grade mosaicism.

**Case 2 -** Homozygosity for a rare VOUS in MMADHC [c.372+53_372+54insTT (intronic) Chr2:150435889] was found as a questionable cause for this Filipino newborn’s elevated C3 and normal C3/C2 result from the state lab. Furthermore, 13 of 13 additional MMADHC variants were homozygous, consistent with a suspected heterozygous deletion of the entire gene at 2q23.2. Biochemical work-up showed no abnormal metabolites during a healthy state. Evaluation of maternal vitamin B12 status is pending.

**Case 3 -** Homozygosity for a rare pathogenic mutation in PCBD1 [c.313T>C (p.Ter105Gln) Chr10:72643709] was found as the cause for this Bangladeshi newborn’s phenylketonuria due to deficient tetrahydrobiopterin synthesis. In addition, 23 of 23 other variants spanning 3 genes and 52 Mb on chromosome 10 (PCBD1 at 10q22.1, MAT1A at 10q23.1, ACADSB at 10q26.13) were documented as homozygous, suggestive of UPD or consanguinity. Further probing into the family history confirmed...
that the parents were 1st cousins. This baby's phenotype is more severe than typical PCBD1 cases but similar to a child reported from Iran (PMID 241339260).

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The LC-MS/MS Assay of Leukocyte Acid α-Glucosidase Activity Reliably Differentiates Early-onset and Late-onset Pompe Disease

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Background: Glycogen storage disease type II, or Pompe disease (PD) is caused by a deficiency of lysosomal acid α-glucosidase (GAA) and glycogen accumulation in tissues, particularly in muscles. The classic infantile-onset Pompe disease (IOPD) presents in early infancy with hypertrophic cardiomyopathy, generalized muscle weakness and failure to thrive. Patients with IOPD usually die within the first year of life from respiratory failure if not treated with enzyme replacement therapy (ERT). The late-onset Pompe disease (LOPD) is characterized by muscle weakness and respiratory insufficiency with disease onset from childhood to adulthood. Pilot newborn screening (NBS) study of Pompe disease in Taiwan has shown that identification of IOPD and initiation of ERT in neonatal period can reverse the glycogen storage and pathological findings. The importance of diagnosis of IOPD vs LOPD cannot be overstated. Up to date, the fibroblast GAA activity is the most accurate function assessment to differentiate IOPD and LOPD. However, this requires skin biopsy and cell culture. In this study, the leukocyte GAA activities measured in normal and affected patients by a LC-MS/MS method were presented and compared to the fibroblast GAA activities.

Method: De-identified normal and abnormal blood samples were from Mount Sinai Genetic Testing Laboratory. Positive patients were identified from the New York State Pompe NBS program and patients who were consented to our study. All carriers and patients were confirmed by the GAA mutations. The “LOPD” infants identified from the NBS program were defined by both low enzyme activity and two GAA mutations and were predicted to be late-onset. IOPD and LOPD fibroblasts were purchased from Coriel Repository. Normal fibroblasts were from Coriel, ERNDIM and in house de-identified cell lines. Leukocytes were extracted from blood samples with commercial RBC lysing buffer, and the cells were lysed by sonication. Fibroblasts were harvested, and cell lysates were obtained also by sonication. The GAA activity was measured by our 6-plex LC-MS/MS method described previously.

Results: The analytical range (defined as the ratio of product/internal standard mass spectrometry response of the normal control cell lysate divided by response of the no-lysate control) for the GAA assay is extraordinarily high at 1100-1200. This leads to an GAA assay with exceptional high resolution. The leukocytes GAA normal range was 254.7 ± 115.6 nmol/h/mg (n=260). The GAA activities of IOPD (n=2), LOPD (n=18) and carriers (n=42) were 2.8 ± 0.4, 11.2 ± 5.2 and 109.3 ± 67.6 nmol/h/mg, respectively, or 1.1 ± 0.1%, 4.4 ±2.0% and 42.9 ± 26.6 % of the normal mean. The fibroblast GAA normal
range was 735.9 ± 541.7 nmol/h/mg (n=21). The GAA activities of IOPD (n=3) and LOPD (n=3) were 1.2 ± 0.4 and 25.9 ± 21.7 nmol/h/mg, respectively, or 0.2 ± 0.1% and 3.5 ±3.0% of the normal mean. Although, fibroblasts GAA revealed bigger differences between IOPD and LOPD; the leukocytes GAA assay equally separates the two groups.

**Conclusion:** These data demonstrated that the leukocyte LC-MS/MS GAA assay reliably detected patients with Pompe disease; moreover, the IOPD and LOPD patients can be further differentiated. This is probably attributed to the high analytical range of our GAA assay. As the majority of LOPD were asymptomatic in this study, further testing of symptomatic LOPD patients are warranted to better define our affected range and clinical cut-off.

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**Comparison of Tandem Mass Spectrometry and Digital Microfluidics Fluorimetry for Newborn Screening of Lysosomal Storage Diseases: Results of Large Scale Pilot Studies**

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The new PerkinElmer/University of Washington 6-plex newborn screening method for 6 lysosomal storage diseases (Pompe, Fabry, Gaucher, Niemann-Pick-A/B, MPS-I, and Krabbe, or any subset) is being launched in 2016. In the WA state NBS lab we have piloted the 6-plex method out to 34,000 newborns, and we expect to reach ~45,000 newborns at the time of the APHL-2016 conference. Screen cutoffs for each of the 6 assays were obtained by analysis of a set of dried blood spots from patients confirmed to have the relevant lysosomal storage disease. All samples below the cutoff were submitted to molecular analysis to obtain genotype information. NY state newborn screening lab has run the assay out to ~260,000 newborns for Pompe disease. Using the same cutoff values in WA and NY, we obtain virtually identical screen positive rates showing consistency in the method in the different labs. The MO state newborn screening lab has published data on 175,000 newborns using digital microfluidics fluorimetry with 4MU substrates. Cutoffs were established as above (by measuring the enzyme activities in a set of dried blood spots from disease-confirmed patients). All 3 states (WA, NY, and MO) have data for Pompe disease, and a comparison shows that the 6-plex mass spectrometry method has about a ~2-fold lower hit rate (referral rate) than digital microfluidics for Pompe disease. Although the hit rate could be lowered by lowering the cutoff, this would presumably not be prudent as this would bring the cutoff into the range observed for patients confirmed to have Pompe disease (false negative problem).

WA and MO have carried out large scale pilot studies of other lysosomal storage disease (MPS-I, Fabry, and Gaucher), and thus a comparison of the hit rates can be made. This again shows that the 6-plex mass spectrometry method leads to a substantially lower hit rate than digital microfluidics fluorimetry.

Space and personnel requirements for the two methods are similar. Cost comparisons are difficult to make in general because each state newborn screening lab has specific needs in terms of the number of lysosomal storage diseases to screen as well as other factors, but in many states, costs for the two technologies are known to be similar. In small states costs may be further reduced because the same mass spectrometer can be used for amino acid/acyl carnitine analysis and lysosomal storage diseases if the number of samples is relatively low.
More detailed information can be found in a series of on-line videos including a live demo of the 6-plex mass spectrometry method, space and cost comparisons, and comparison of large scale pilot studies:

https://www.youtube.com/watch?v=D6_LBEYsLqA
https://www.youtube.com/watch?v=YwLMxJHKFbs
https://www.youtube.com/watch?v=zRL4JbWLvos
https://www.youtube.com/watch?v=hD5sMplY41Q
https://www.youtube.com/watch?v=HZPCARAvmW4
https://www.youtube.com/watch?v=dqRwpu-Ovew
https://www.youtube.com/watch?v=EsAL_YD5STU
https://www.youtube.com/watch?v=n71baranYn0
https://www.youtube.com/watch?v=oP5b_bCg5rg

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