The Newborn Screening Translational Research Network: An Update on Efforts to Coordinate Newborn Screening Pilots
A. Brower, J. Adelberg, M. Watson and H. Dessie, American College of Medical Genetics and Genomics, Bethesda, MD

The Newborn Screening Saves Lives Reauthorization Act of 2014 directed federal agencies to continue efforts to improve, advance and assess the state-based newborn screening programs. The legislation authorized the expansion of the National Institute of Health Eunice Kennedy Shriver National Institute of Child Health and Human Development’s Hunter Kelly Newborn Screening (NBS) Research Program to support research that provides information to support the evidence based review of conditions that are candidates for routine newborn screening, and to conduct pilots on conditions recommended for nationwide screening by the Advisory Committee on Heritable Disorders in Newborns and Children to translate the research findings into the public health environment and routine clinical care. To accomplish these charges, the Hunter Kelly NBS Research Program established the Newborn Screening Translational Research Network (NBSTRN). The American College of Medical Genetics and Genomics (ACMG) operates the NBSTRN Coordinating Center (NBSTRN-CC) through a contract with NICHD and facilitates research related to newborn screening research. A key activity of the NBSTRN-CC is to facilitate pilots of both candidate conditions and newly recommended conditions by convening discussions focused on sharing information and data to establish analytical and clinical validation of novel technologies to screen and diagnose as well as therapies. To date the NBSTRN has coordinated pilots of five conditions recommended by the Advisory Committee and twelve conditions that are candidates for screening. We have collected over one million data points describing the natural history of these conditions, and are working to provide access to this data to the community of NBS stakeholders. Our presentation will summarize the lessons learned, and outline a plan for creating a robust system to support an increasing number of pilots, including genomic sequencing in the newborn period. We will highlight the important activities of our partners who work directly with the state-based NBS programs, the parents, families and advocates, the Regional Genetics Service Networks, and the federal agencies that support and guide these efforts.

Presenter: Amy Brower, American College of Medical Genetics and Genomics, Bethesda, MD, Email: abrower@acmg.net
P-002

Creating a Shared Infrastructure to Support the Advancement of Rare Disease Research and Newborn Screening
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The majority of the conditions that are part of, or candidates for, newborn screening in the United States are rare or very rare. This necessitates the sharing of data from basic research designed to advance understanding of the disease process, to translational research to develop technologies to screen and therapies to treat, to public health implementation of a comprehensive newborn screening program to identify newborns at risk. Advances in information technology have enabled the creation of tools to facilitate this sharing, aggregation and analysis of data. The use of standardized vocabularies, interactive computer systems, and robust security measures are key components that enable the creation of a shared infrastructure available to researchers, public health team members, and clinicians. We describe the contributions of the National Institute of Health Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Newborn Screening Translational Research Network (NBSTRN) to this shared infrastructure. The American College of Medical Genetics and Genomics (ACMG) operates the NBSTRN Coordinating Center (NBSTRN-CC) that is working to develop and implement a suite of tools to efforts to translate basic research discoveries to the state-based newborn screening programs. The suite of tools includes a Virtual Repository of Dried Blood Spots, the Region 4 Stork, the Longitudinal Pediatric Data Resource, and Ask ELSA. These tools have been used in over thirty basic, translational, public health and clinical research projects. We will summarize the activities of over 100 researchers and newborn screening programs, and the 26 funding opportunities that are utilizing these resources.

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P-003

Developing Regional Models for Genetic Service
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Introduction: Genetics and genomics is a continually evolving field that helps improve knowledge for patients and providers. Even with the genetic advances, access barriers are still very prevalent. In order to understand how gaps in access can be lessened, the National Coordinating Center for the seven Regional Genetic Service Collaboratives undertook a comprehensive review of the current state of genetic services, including laboratory testing for genetic conditions, to offer potential future models for genetic services.

Methods: In the summer of 2015, NCC established the NCC Regional Support Service Model Workgroup (RSSM WG) and an Advisory Committee (AC) to examine the current system of genetics services and offer a recommendation for a new system to improve access and address barriers. Through an iterative process, the RSSM WG identified areas of need that a genetic service system could address, developed eight potential models, and identified a hybrid regional model that would best address current gaps.

2017 APHL Newborn Screening & Genetic Testing Symposium, New Orleans, LA, September 10-13, 2017
Results: Through data collected from national needs assessments and targeted stakeholder feedback, six priority areas were identified that a genetic service system should address. These priority areas (listed in alphabetical order) were data collection; education and training on genetics for non-genetic providers; efficient practice; formal relationships with state public health, healthcare institutions, and university center; practice support; and promotion of family engagement. Upon reviewing current systems, the WG identified eight potential regional models: Regional Genetic Service Resource Network, Regional Clinical Support Centers, Regional Genetics Education and Technical Assistance Centers, Regional Patient Engagement Centers, Public Health Model, Quality Improvement Model, Regional Clinical Support Network, and Genetic Service Data Centers. Using the six priority areas as the pillars for a new genetic service system, the WG recommended that a hybrid model, called the Genetic Services Support Model, be created that had elements of the Regional Genetic Service Resource Network, Regional Clinical Support Centers, and the Regional Genetics Education and Technical Assistance Centers.

Conclusion: The Genetic Service Support Model acknowledges the strength of a regional infrastructure, which includes public health, providers, and consumers; promotes efficient practice with genetic centers through the use of technical assistance and other activities; and emphasizes an aggressive and targeted support of non-genetics providers. This proposed system should alleviate current barriers to genetic services.

The NCC is funded by U22MC24100, awarded as a cooperative agreement between the Maternal and Child Health Bureau/Health Resources and Services Administration, Genetic Services Branch, and the American College of Medical Genetics and Genomics.

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P-004

Evaluation of Critical Congenital Heart Defects Screening in the Neonatal Intensive Care Unit
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Background and Objectives: Screening for early detection of critical congenital heart defects (CCHD) using pulse oximetry is near universal in the U.S. with many states requiring screening of all infants irrespective of clinical status or setting. Empiric evidence to guide screening in the neonatal intensive care unit (NICU) is quite limited. To evaluate the feasibility and burden of universal CCHD screening our objectives were to assess: 1) early timing options for screening and 2) whether infants with a prenatal CHD diagnosis, echocardiography conducted before screening, and/or those born extremely premature can be excluded from universal CCHD screening in the NICU.

Methodology: Prospective evaluation of a multi-stage modified CCHD screening algorithm and implementation survey conducted in 21 NICUs across five states. Infants born February 1, 2015-September 30, 2015 with a NICU stay of >23 hours were eligible for inclusion (n=4556). The final sample was comprised of 4120 infants with at least one set of screening results inclusive of those with a prenatal CHD diagnosis or echocardiogram before screening (pre-identifying factors) to evaluate universal NICU screening. NJ algorithm thresholds were utilized during data analysis to determine pass/rescreen/fail status for all screens.
Results: Of 4120 infants with complete screens, 92% did not have prenatal CHD diagnosis or echocardiography before screening, 72% were not receiving oxygen at 24-48 hours and 56% were normal birthweight. Thirty-seven infants failed screening (0.9%); none with an unsuspected CCHD. One infant with previously unrecognized CHD was identified due to screening. False positive rates were low for infants not receiving oxygen (0.5%) and those screened after weaning (0.6%), yet higher among infants born extremely preterm (3.8%). Unnecessary echocardiograms due to screening were minimal (0.2%) as was the reported staff burden of implementation.

Conclusion: Given the majority of infants were not on oxygen, not pre-identified for CCHD, and born normal birthweight, systematic screening at 24-48 hours may be of benefit for early detection of CCHD with minimal burden. Challenges arose when screening extremely preterm infants and those receiving supplemental oxygen. Further investigation is needed to optimize a screening algorithm for early identification of CCHD in the NICU.

Presenter: Regina Grazel, MSN, RN, BC, APN-C, Program Director, New Jersey Department of Health, CCHD Screening and Infant Zika Surveillance, New Jersey Chapter, American Academy of Pediatrics and NJ Dept of Health, East Windsor, NJ, Phone: 609.577.5002, Email: rgrazel@njaap.org

P-005

Results from the 2017 Newborn Screening Molecular Testing Survey
J. Rosalez, L. Russell, G. Zarbalian, J. Ojodu and D. Kim, Association of Public Health Laboratories, Silver Spring, MD

Background: Utilization of molecular testing in newborn screening (NBS) across the United States was previously assessed in 2010 by the APHL NBS Molecular Subcommittee in collaboration with the Centers for Disease Control and Prevention (CDC). Since then, the landscape of NBS has changed, with the emergence and increased availability of new molecular testing technologies, and new disorders added to the Recommended Uniform Screening Panel (RUSP). Molecular testing provides utility in NBS to reduce unnecessary referrals, clarify ambiguous results and increase testing specificity. The Molecular Subcommittee works in collaboration with the CDC Molecular Quality Improvement Program to provide technical assistance and quality assurance materials as NBS programs incorporate molecular assays into their testing algorithms.

Methods: In January of 2017 APHL fielded an online survey to 53 NBS program directors to probe their program’s molecular testing capabilities. A univariate analysis of the survey data was performed to assess the current status of molecular testing across state newborn screening programs, molecular testing technologies currently being used, and needs of NBS programs to guide Molecular Subcommittee activities moving forward.

Results: Forty-eight surveys were completed resulting in a response rate of 91% of NBS programs. Forty-seven programs reported utilizing molecular testing with 31 that do so in-house and 16 that have their testing performed by another lab. Real-time PCR was the method most identified (81%) among those NBS programs that utilize molecular assays. When asked about molecular testing and various NBS conditions an overwhelming majority (83%) of programs reported they are currently screening SCID with a molecular assay. This was followed closely with 77% of programs reporting they screen Cystic Fibrosis with a molecular assay. With regards to future plans, 46% of programs reported they plan to screen for MPS 1 with a molecular assay. In addition, 44% plan to screen for Pompe disease and 40% for X-ALD with a molecular assay. Furthermore, a third of NBS programs reported there was a critical need for molecular
QA materials for SCID. This was followed by 40% of programs reporting a critical need for molecular QA materials for Cystic Fibrosis.

**Implications:** The results indicate a vast majority of NBS programs are currently utilizing molecular testing as a part of their routine testing algorithms, which will increase as programs are planning to implement molecular testing for conditions recently added to the RUSP. Therefore, efforts to provide technical assistance should focus on the implementation of testing for these disorders and the technologies being used. It will be imperative to continue monitoring molecular testing progress as programs move through various phases of implementation, and as new conditions are considered for the RUSP.

**Presenter:** Jacob Rosalez, Senior Specialist, Association of Public Health Laboratories, Silver Spring, MD, Phone: 240.485.3830, Email: jacob.rosalez@aphl.org

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**P-006**

**Review of Newborn Screening Dried Blood Spot Cards to Inform an Emergency Supply**

L. Russell, Association of Public Health Laboratories, Silver Spring, MD

**Objectives:** The Association of Public Health Laboratories (APHL), in collaboration with the Centers for Disease Control and Prevention (CDC) maintains an emergency supply of newborn screening (NBS) dried blood spot (DBS) collection cards available to state NBS programs upon request. As a component of the update to the NBS contingency plan, in 2016, the APHL NBS QA/QC Subcommittee initiated a comprehensive review of state NBS DBS cards to inform the update of the supply.

**Methodology:** The Subcommittee conducted the review using the following methods: • Reviewed data elements on all newborn screening dried blood spot cards housed within the APHL Newborn Screening Technical Assistance and Evaluation Program (NewSTEPS) data repository. Data elements currently not included on the emergency supply were tallied in a spreadsheet. • Data analysis was conducted by ranking the spreadsheet by total number of states with each element from highest to lowest. • Any data elements included on 20 or more state DBS cards were considered for inclusion and underwent additional review (n=15). • The top 15 elements were reviewed for variation in data collection. The Subcommittee determined final data definitions using subject matter expertise to determine which definitions would provide necessary information to perform data analysis and follow up. • Once the review for variation in data collection was complete, the Subcommittee was tasked to complete a survey and focus groups on additional elements to include from the perspective of elements necessary for data analysis and follow up.

**Results:** • 15 data elements were included on 20 or more state DBS cards. • The review of variation in data elements showed differences in nomenclature, measurement, detail of information collected, and meaning. Further discussion by the Subcommittee based on subject matter expertise allowed for the selection of data definitions that are applicable to the majority of states and necessary for data analysis and follow up. • The total number of additional elements to be included on the updated emergency supply will be determined upon completion of the Subcommittee survey and focus groups by April 2017. • In spring 2017, APHL will submit a vendor request for proposals and work with the selected vendor to determine layout of the updated emergency cards. The estimated completion date for this project is Fall 2017.

**Conclusions:** In the event of an emergency, the APHL/CDC supply of emergency cards will be acceptable and usable by the majority of NBS programs and will enhance NBS contingency planning as a reliable source of NBS DBS cards.
New Online Course to Help Laboratory Professionals and Healthcare Providers Improve Pre-analytic Processes of Biochemical Genetic Testing
B. Su and K. Breckenridge, Association of Public Health Laboratories, Silver Spring, MD

Introduction. Biochemical genetic tests are associated with a wide range of pre-analytic variables, including test selection and ordering, patient conditions, quality and timing of specimen collection, handling of test requests and specimens by referring laboratories, specimen transport, and communications with healthcare providers, patients, and collaborating laboratories. Studies have shown that the pre-analytic phase might be the most error-prone during the total testing process in many laboratory disciplines including genetic testing. Pre-analytic errors could result in inappropriate use of laboratory services and compromise the quality of test results, leading to increased risk for medical errors, adverse patient outcome, and increased healthcare costs.

Method: CDC published a guideline “Good Laboratory Practices for Biochemical Genetic Testing and Newborn Screening for Inherited Metabolic Disorders” in 2012 to help newborn screening and biochemical genetic testing (BGT) laboratories in their quality improvement activities. Since 2013, CDC has been collaborating with the Association of Public Health Laboratories to advance the use and impact of these good laboratory practice recommendations. Two discussion groups were held on the use and impact of the recommendations and findings documented a need for training to supplement the guideline. To meet that need, a new online training course titled “Good Laboratory Practice Recommendations for Biochemical Genetic Testing: Pre-analytic Phase” has been developed under the APHL-CDC cooperative agreement to help laboratory professionals and healthcare providers improve pre-analytic practices for BGT.

Results: The multimedia online course consists of 3 lessons on quality assurance for test requisitions, specimen collection and submission, laboratory-clinician communications, and pre-analytic quality assessment. Case scenarios are included to illustrate how the practice recommendations can be applied to improve pre-analytic quality and patient outcomes. After completing this course, participants will be able to recognize the role of each stakeholder group in the biochemical genetic testing pre-analytic processes, choose the pre-analytic procedures that are consistent with regulatory requirements and good laboratory practices, select indicators for pre-analytic quality assessments, and explain the communication needs of each stakeholder group. Continuing education credits are available from this course, including 1.5 hours of the ASCLS P.A.C.E. credit and 1.5 contact hours for Florida Laboratory Licensees.

Conclusions: This course is expected to go live in late 2017 and will be accessible from CDC Laboratory Training. Participation and continuing education credits will be free of charge.

Presenter: Bertina Su, MPH, Senior Specialist, Association of Public Health Laboratories, Silver Spring, MD, Phone: 240.485.2729, Email: bertina.su@aphl.org
The State of Newborn Screening Systems in the United States
C. Yusuf¹, J. Miller¹, T. Wood¹, S. Singh¹, Y. Kellar-Guenther², R. Sheller¹, G. Zarbalian¹, M. Sontag²;
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Background: Newborn screening public health systems in the US are guided by national recommendations; however, implementation and program development decisions occur at the state/territorial level. The NewSTEPs Data Repository collects and maintains information on US NBS programs and serves as a national resource for this information including the number of disorders screened, the status of electronic data exchange, NBS program operating hours, and other elements of program activity.

Objective: To describe the landscape of US newborn screening programs in 2017. Methods: US NBS programs update their own data within the NewSTEPs Data Repository and NewSTEPs queried information including: Disorders Screened (core, secondary, other), Program Structure, Health Information Technology activities, among other categories. Data presented includes 50 states, the District of Columbia, Guam and Puerto Rico.

Results: As of March 2017, 53 newborn screening programs (each associated with 1 of 36 NBS laboratories) served newborn populations ranging in size from 3,366 births per year to over 500,000 (median 53,122), with 14 of those 53 programs requiring 2 screens. Regional laboratories serve 13 states, while each state has its own short-term follow-up program. NBS programs in the US screen for between 28 – 34 disorders on the Recommended Uniform Screening Panel (RUSP) as well as a variety of other disorders. As of March 27, 2017, 45 NBS programs report universal screening for Severe Combined Immunodeficiency (SCID); 6 NBS programs universally screen for Pompe; 4 NBS programs universally screen for Mucopolysaccharidosis I (MPS I); and 4 NBS programs universally screen for X-linked Adrenoleukodystrophy (X-ALD). Funding of NBS activities is secured from different sources, however most programs (n=47/52) have an NBS fee (median $92, range $30 – 163). NBS program operating hours vary; 35 NBS laboratories are open Monday – Saturday and 9 NBS laboratories are open 7 days a week. Few NBS programs currently utilize electronic exchange of NBS data: of the 16 NBS programs interviewed, 6 NBS programs receive Health Level 7 (HL7) orders and 9 NBS programs send HL7 results out. The NBS Health Information Technology (HIT) workgroup is actively updating the HL7 implementation guidelines for electronic ordering and results reporting and will encourage its use among all NBS programs.

Conclusion: US NBS programs are resourceful and responsive to a dynamic landscape. This is illustrated by the expansion of state panels, longer operating hours and the utilization of HIT to improve timeliness of NBS.

Presenter: Careema Yusuf, MPH, Manager, NewSTEPs, Association of Public Health Laboratories, Silver Spring, MD, Email: careema.yusuf@aphl.org
Definitions for Medical Intervention and Diagnosis of Cases Identified through Newborn Screening

C. Yusuf1, M. Sontag2, J. Miller3, S. Singh1, Y. Kellar-Guenther3, J. Ojodu1; 1Association of Public Health Laboratories, Silver Spring, MD, 2Colorado School of Public Health, Aurora, CO

**Background:** The newborn screening (NBS) system is charged with ensuring newborns with an out-of-range result on their screen receive appropriate follow-up and confirmatory testing. Most programs define short-term follow-up as the duration between analysis of the positive newborn screen to when an infant receives a diagnosis (or when a diagnosis is ruled out). Receipt of a diagnosis is often preceded by the care of the infant changing (i.e. a clinical action was rendered based on follow-up on the newborn screening results before a diagnosis is made).

**Objective:** To describe medical intervention and diagnosis of confirmed cases identified through newborn screening.

**Methods:** NewSTEPs in collaboration with clinicians who treat infants with NBS disorders, developed definitions for timing of diagnosis and medical intervention – by disorder. This was accomplished via a series of teleconference calls and emails over an 18 month period. The definitions were shared with NBS programs that are providing case data to the NewSTEPs Data Repository for uniformity of data collection efforts. Definitions for the three most recent disorders added to the RUSP (Pompe, Mucopolysaccharidosis I (MPS I) and X-linked Adrenoleukodystrophy (X-ALD) are currently being worked on. Case level data related to time from birth to diagnosis, collected in the NewSTEPs data repository were analyzed. NBS program who had entered more than 75 cases for the years 2012 through 2016 were included.

**Results:** Date of medical intervention and date of diagnosis are available for the following NBS disorders on the core Recommended Uniform Screening Panel (RUSP): Cystic fibrosis, Endocrine disorders, hemoglobinopathies, metabolic disorders, and classic Galactosemia. As of March 2017, there 17 NBS programs who reported >75 confirmed cases for the years 2012 in the NewSTEPs data repository. Median time from birth to diagnosis ranged from 7 – 63 days for all disorders. Among the core RUSP disorders, 3-Hydroxy-3-methylglutaric aciduria had the shortest time from birth to diagnosis (median = 8 days) and Trifunctional protein deficiency had the longest time from birth to diagnosis (median = 203 days).

**Conclusion:** Time to diagnosis varies across NBS programs and disorders. Many factors contribute to this variation. Time from birth to medical intervention may serve as a better measure of infants getting timely and appropriate care. Definitions for time to diagnosis and time to medical intervention allow for data collection that is standardized, leaving minimal space for variability and provide a more consistent description of outcomes of NBS across the United States.

**Presenter:** Careema Yusuf, MPH, Manager, NewSTEPs, Association of Public Health Laboratories, Silver Spring, MD, Email: careema.yusuf@aphl.org

P-010

Electronic Test Order and Results for Newborn Screening in Florida

K. Kocevar1, J. Butler2, E. Gonzalez Loumiet2; 1Florida Department of Health, Tallahassee, FL, 2UberOps, Tallahassee, FL

The Florida Department of Health along with their IT vendor has successfully implemented the Newborn Screening Electronic Lab Order and Lab Result connection with two of the largest hospital systems in the 2017 APHL Newborn Screening & Genetic Testing Symposium, New Orleans, LA, September 10-13, 2017
state. Learn about this innovative process and what it has done to improve the timeliness of the newborn screening test process in the state that helps save lives. How they can implement electronic orders and results to increase the turnaround time for newborn screening test results, which leads to better healthcare by addressing detected medical issues from the onset. Cost savings as well common pitfalls when implementing a new IT project will be discussed. Why is this session timely? Electronic ordering and resulting within newborn screening and the blood card has been an ongoing challenge within the newborn screening discipline. New technologies and new tests are continually compounding the problem. This session will help labs navigate this complex environment.

Presenter: Christine Urban, Project Manager, UberOps/Florida Public Health Laboratory, Tallahassee, FL, Phone: 970.769.0951, Email: christine.urban@uberops.com

P-011

Comprehensive Detection of CFTR Variants using Anchored Multiplex PCR and Next-Generation Sequencing

M. Hardison¹, K. Moore², P. Roberts², L. Griffin², R. Walters², B. Culver²; ¹Baby Genes, Inc., Golden, CO, ²ArcherDX, Inc., Boulder, CO

Introduction: Cystic Fibrosis (CF) is an autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. CF is characterized by the build-up of thick mucus resulting in chronic lung infections and airway inflammation. Carrier identification and newborn screening have significant implications in the overall prognosis of CF patients. Underlying CFTR mutations were recently shown to vary significantly across ethnic groups. However, current CFTR genotyping assays detect mutations highly prevalent in white individuals, yet fail to detect mutations that are more prevalent in nonwhite individuals. Furthermore, these assays also fail to detect large deletions, such as the CFTRdele2, 3(21kb), which is prevalent in Central and Eastern European populations and confers a severe CF phenotype. Here, we present a method based on Anchored Multiplex PCR (AMP™) and next-generation sequencing (NGS) for comprehensive, pan-ethnic detection of CFTR variants, including common base substitutions and large deletions.

Methods: AMP is a library preparation method for NGS that uses unidirectional gene-specific primers (GSPs) and molecular barcoded adaptors ligated to random start sites for open-ended amplification. This enables NGS-based identification of both known and unknown mutations across a panel of target regions. Furthermore, anchored GSPs amplify large genomic regions from both ends independently, permitting sequencing of both wildtype and variant alleles from the same GSPs.

Results: Using a set of 150 blinded specimens from the Coriell Institute for Medical Research, we show that AMP-based NGS detects known CFTR variants with 100% accuracy. In addition, we detected the CFTRdele2, 3(21kb) in a pre-validated DNA sample obtained from the Coriell Institute for Medical Research. Pan-ethnic screening of 1,585 clinical samples identified 34 unique mutations, several of which were identified in multiple individuals. 73% (25/34) of these unique mutations and 60% (74/123) of total mutations detected are not currently included in the ACMG-recommended 23-mutation panel for CF carrier screening. Furthermore, this screen revealed ethnic differences in clinically significant CFTR variants and a pan-ethnic carrier rate of approximately 7%.

Conclusions: We demonstrate that AMP-based NGS enables comprehensive detection of both known and novel variants in the CFTR gene, with the ability to detect large deletions. Identification of novel variants is critical for global carrier and newborn screening, as CF driver mutations have not been fully characterized across all ethnicities. Our findings suggest that the pan-ethnic carrier rate of CF may be
higher than originally predicted. As the reagents for AMP-based NGS are lyophilized and thus do not require refrigeration, this may be a practical method for CF screening in global communities.

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P-012 - Withdrawn

P-013

Benefits of Fast, Affordable, and Comprehensive Next-Generation Sequencing for Newborn Screening, Confirmatory, and Carrier Testing
S. Deis, M. Hardison, M. Nemcek and K. Dollerschell, Baby Genes, Golden, CO

Baby Genes, Inc. constantly strives to provide fast, affordable, and definitive newborn screening (NBS). To meet this need, the Archer® VariantPlexTM assay is used to create target-enriched libraries for next-generation sequencing (NGS). The NBS targeted panel covers 213,160 base positions including 1,143 exons and 4,049 established disease-causing mutations in 99 genes to test for 71 conditions. The conditions on the panel are inclusive of all core and secondary conditions on the Recommended Uniform Screening Panel (RUSP). Furthermore, testing results are available within an average turnaround time of 80 hours; thereby, providing results within a few days after birth. On average, base coverage is =6x for 99.93% of the total bases and 99.96% of clinically significant bases with an average depth at each base position of 270x. Due to high base coverage, the same NGS panel is applicable for gene-specific confirmatory testing and a carrier test covering 106 genes linked to 171 conditions. As of January 2017, Baby Genes performed 278 NBS, 397 confirmatory, and 2266 carrier tests and identified 1177, 815, and 10123 variants, respectively. In NBS testing 9% of variants identified were pathogenic, 3% were likely pathogenic and 35% were variants of unknown significance (VOUS). In confirmatory testing, where a condition is already suspected in the gene(s) of interest, 37% of variants identified were pathogenic, 10% were likely pathogenic and 18% were VOUS. For the more extensive carrier screen 12% of variants identified were pathogenic, 3% were likely pathogenic, and 47% were VOUS. Thus, the genetic testing at Baby Genes consistently provides clinically relevant information from high-quality sequencing data. There was also a correlation between frequency of disease findings and occurrence in the population. Pathogenic and likely pathogenic variants were identified from the NBS test most frequently for biotinidase deficiency (5.4% of patients), cystic fibrosis (5.0%), glucose-6 phosphate dehydrogenase deficiency (4.0%), and medium chain acyl-CoA dehydrogenase deficiency (4.0%). In confirmatory testing, pathogenic and likely pathogenic variants were identified most frequently for phenylketonuria (16%), congenital adrenal hyperplasia (6.8%), medium chain acyl-CoA dehydrogenase deficiency (6.5%), and cystic fibrosis (4.5%). The high frequency of patients with pathogenic variants associated with cystic fibrosis, phenylketonuria, congenital adrenal hyperplasia, and medium chain acyl-CoA dehydrogenase deficiency is consistent with cystic fibrosis occurrence in one out of every 3,000 Caucasian births and the other three diseases, in addition to cystic fibrosis in non-Caucasian populations, occurrence in one out of every ~15,000 births. The frequency was enriched in the tested population as it is mainly newborns with a suspected NBS condition due to heredity or symptoms displayed.
**P-014**

**A Fluorimetric Enzyme Assay Panel for High Throughput Screening of Pompe, MPS I and MPS II on a Single Cartridge**

R. Ng, C. Graham, R. Singh and V. Pamula, Baebies, Inc., Durham, NC

Pompe disease and Mucopolysaccharidosis Type I (MPS I) are two lysosomal storage disorders (LSDs) recently added to the U.S. Department of Health and Human Services Recommended Uniform Screening Panel (RUSP) for newborn screening (NBS). Mucopolysaccharidosis Type II (MPS II) is another LSD that satisfies all criteria laid out by the Secretary’s Advisory Committee on Heritable Disorders in Newborns and Children for inclusion in the RUSP, with the exception that no population screening data is available. To enable customized NBS for MPS II or other LSDs in the future, we modified our existing digital microfluidic (DMF) cartridge to perform fluorimetric assays for acid-alpha glucosidase (GAA), acid alpha-L-iduronidase (IDUA) and iduronate-2-sulfatase (IDS), which are deficient in Pompe, MPS I and MPS II disorders, respectively. The automated assays are performed from a single 3.2 mm dried blood spot (DBS) punch using an established and validated digital microfluidic system that is FDA cleared and has been used by the Missouri State Public Health Laboratory (MSPHL) for over four years to screen for Pompe, Fabry, Gaucher and MPS I disorders. We will present preliminary feasibility data on this high throughput assay panel including results showing differentiation between two hundred presumed normal and 26 affected MPS II DBS. Each DMF cartridge contains 48 sample reservoirs and can run up to five assays in less than 4 hours using 75 nanoliter droplets. The modified LSD DMF platform presented here will enable NBS laboratories to flexibly and easily add screening for Pompe, MPS I and MPS II.

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**P-015**

**Rapid Digital Microfluidic Enzyme Assay Analysis for Pompe Disease Can Shorten the Referral Time and Lead to Faster Treatment Initiation**

S. Norton, R. Singh and V. Pamula, Baebies, Inc., Durham, NC

Newborn screening for lysosomal storage disorders (LSDs) is increasingly becoming widespread; both Pompe disease and Mucopolysaccharidosis Type I (MPS I) were recently added to the Recommended Uniform Screening Panel (RUSP) and state public health laboratories are actively pursuing LSD screening. There is general agreement that children affected by LSDs would benefit significantly from newborn screening (NBS), since the damage caused by the accumulation of storage products can be irreversible. It is also imperative that NBS results be reported as quickly as possible to referral centers if a screen positive is indicated; this is especially true with diseases such as Pompe which have infantile onset and late onset types. Recent evidence from Taiwan suggests that the timeliness of confirmatory diagnosis and start of treatment is critically important to patient outcomes. Yang et al (J. Pediatrics; 2016; 169:174-80) report better biological, physical and developmental outcomes for infantile onset Pompe patients whose average age of enzyme replacement therapy treatment initiation was 11.9 days compared to those that received initial treatment just 10 days later (average 21.6 days).
throughput NBS for Pompe and other lysosomal storage disease enzyme testing from dried blood spot (DBS) samples can be performed using two different technologies – tandem mass spectrometry (MS/MS) or the FDA-cleared SEEKER digital microfluidic fluorometry (DMF) system. Both platforms use special reagent kits that are readily available, but the workflows for the two platforms differ greatly. The latest 6-plex LSD flow injection MS/MS protocol by Elliot et al. (Pompe, Fabry, Gaucher, MPS I, Krabbe and Niemann-Pick-A/B; Data in Brief 8; 2016: 915-24) requires a total workflow time of approximately 40 hours while the digital microfluidic platform provides sample to answer results in under 4 hours. NBS testing/referral algorithms vary by program, but in most programs screen positive samples (deemed high risk for disease) require repeat testing from the same DBS sample. With the SEEKER digital microfluidic platform, it is possible to perform both initial and repeat testing and report results on the same day; repeat testing with MS/MS will take several days. For infants affected with Pompe disease, delays as short as a few days can have striking effects on patient outcomes. Same day testing with the SEEKER platform enables public health laboratories to flag a screen positive sample in a single day, thus allowing high risk samples to be referred as early as possible.

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P-016

The Use of Multivariate Tools to improve Second-tier Screening Algorithms for Congenital Adrenal Hyperplasia (CAH)
G. Sinclair, British Columbia Children’s Hospital, Vancouver, BC, Canada

**Background:** The British Columbia newborn screening program implemented a second-tier steroid profiling assay for the screening of Congenital Adrenal Hyperplasia (CAH) in 2011. The screening algorithm utilized variable age and birth weight related cutoffs for the first-tier 17OH progesterone immunoassay followed by a five steroid panel (17OH progesterone, cortisol, androstenedione, 11 deoxycortisol, and 21 deoxycortisol) as the second-tier. The screening algorithm was based on previously published work (Janzen et al 2007) and included the ratio (17OHP+21deoxycortisol)/cortisol as the primary screening marker on the second-tier. Although initial performance of this algorithm was acceptable (30% PPV), we noted a drift to lower performance over time with only a 10% PPV in 2015. All false positive cases arose from premature/low birth weight infants with very low cortisol levels.

**Methods:** Utilizing the principal component analysis (PCA) tools available on MetaboAnalyst (www.metabolomicscentre.ca) we evaluated steroid profiles from 13,000 newborn samples to identify the features with the best discrimination between true positive, false positive, and true negative cases.

**Results:** The multivariate analysis identified reduced 11 deoxycortisol as the best discriminator between true and false positive cases and the ratio of 17OH progesterone/11 deoxycortisol as the most specific marker for true CAH deficiency. Based on historical data, a combination of 17OH progesterone > 20 nM and a ratio of 17OH progesterone/11 deoxycortisol > 9 would have 100% sensitivity and a PPV of 79%. We are currently validating this algorithm with prospective data.

**Conclusions:** The use of the direct precursor to product ratio (17OH progesterone/11 deoxycortisol) has significantly better performance for the identification of CAH cases as compared to our previous algorithm utilizing cortisol as the denominator given non-specific low cortisol levels in some premature or low birthweight infants.

Targeted Sequencing of the DMD Gene Enables Rapid Detection and Characterization of Mutations Linked to Duchenne Muscular Dystrophy

L. Akin, R. Hrdlickova, M. Toloue, D. Fox and J. Nehyba, Bioo Scientific, Austin, TX

Duchenne muscular dystrophy (DMD) is the most common fatal genetic disorder diagnosed in childhood, with approximately 20,000 new cases diagnosed each year. Caused by mutations in the DMD gene which encodes the protein dystrophin, patients display progressive muscle weakness, loss of ambulation, trouble breathing, and increased susceptibility to injury over the course of their lifetime. Current strategies for the detection of DMD begin with standard biochemical assays of serum creatine kinase levels in newborns, and positive results prompt immunohistochemistry tests of muscle tissue and MPLA analysis. However, the specific detection of mutations contributing to DMD requires highly resolved genetic screening before any reliable disease characterization can be made. Targeted DNA sequencing offers an affordable, rapid, and robust strategy for the detection of germline mutations and provides genotypic characterization with little extraneous testing. Additionally, mutation analysis can indicate the presence of copy number variants (CNVs) by comparing control amplicon performance to experimental amplicon performance. This is performed with a combination of field-accepted open-source tools for alignment and small mutation detection, and proprietary software for sequence grooming, CNV detection, and reporting. The NEXTflex® Duchenne Muscular Dystrophy Amplicon Panel detects mutations across all canonical coding exons of the DMD locus (~25.7 kb of genomic sequence) using custom software analysis. Using this sequencing technology, we successfully characterized mutations across the DMD gene in 15 separate samples in a single sequencing run, modeling the applicability of targeted sequencing in rapid genetic analysis. Our results also show that amplicon panels can be modified to include coverage of additional genes or regions of interest without modification of sample input or protocol. Future expansion of this kit will include coverage of numerous creatine kinase-associated genes, complementing current biochemical assays. Highly specific sequence coverage of genes responsible for both dystrophy and elevated creatine kinase levels, coupled with analytical software, could accelerate the characterization of mutations linked to this harmful disease by joining separate analytical steps into a single procedure.

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**P-018**

Targeted DNA Sequencing and Customized Analysis of Genes Associated with Lysosomal Storage Diseases
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Corresponding with an increase in the availability and implementation of newborn genetic tests in recent years, significant efforts have been made to expand the coverage and detection of mutations linked to various diseases present during infancy and early childhood. Screening of mutations linked to diseases such as cystic fibrosis (CF), phenylketonuria (PKU), congenital adrenal hyperplasia (CAH), and various hemoglobin-related disorders have long-established positions in the research setting, while others have only recently gained major attention. One such class of genes, those associated with lysosomal storage diseases (LSDs), have quickly become major sequencing targets in newborn genetic screening efforts due to their responsibility for numerous conditions associated with the toxic build-up of lysosomal byproducts or perturbed lysosomal function. Here we detail the impressive coverage and analytical capabilities of a new amplicon panel that covers 11 genes associated with different lysosomal storage diseases commonly seen in newborns, coupled with custom-designed software to enable rapid, high-resolution mutation analysis. The NEXTflex® Lysosomal Storage Diseases Amplicon Panel detects 99% of mutations across all canonical coding exons of the following 11 genes related to various lysosomal storage diseases: GALNS, SUMF1, GALC, IDS, GLB1, IDUA, ARSB, GLA, SMPD1, GUSB, and GAA. Targeted DNA sequencing using this customizable amplicon technology offers an affordable, rapid, and robust strategy for the detection of mutations by providing genotypic characterization without the need for extraneous biochemical testing. Mutation analysis using a combination of field-accepted open-source tools for alignment and small mutation detection in conjunction with proprietary software for sequence grooming, CNV detection, and reporting can indicate the presence of copy number variants (CNVs) by comparing amplicon performance between control and experimental samples. Using this strategy, we successfully characterized mutations found in the DNA of multiple clinical samples, modeling the applicability of targeted sequencing in rapid genetic analysis. Our results also displayed the ability of this amplicon panel to detect large deletions through the design of primer sets flanking both ends of a large deletion site (~10 kb) within the GALC gene coding region, without the need for enzymatic modifications or MPLA analysis. This amplicon panel offers a concise and time-efficient method for the detection of mutations in genes related to various lysosomal storage diseases without the need for preliminary biochemical assays, providing support for their continued development.

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**P-019**

Preliminary Genomic Findings from the BabySeq Project
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**Objectives:** The BabySeq Project is a randomized clinical trial assessing medical, behavioral and economic outcomes after providing genomic sequencing (GS) information from whole exome sequence
(WES) analysis of DNA collected after birth to a baby's parents and clinicians. While enrollment is still open, we summarize our initial genomic findings.

**Methods:** Well and sick infants are randomized to either family history (FH) and newborn screen (NBS) informed standard care or FH and NBS informed standard care plus GS. The analytic pipeline focuses on genes with strong evidence to be associated with pediatric onset disorders. Pathogenic or likely pathogenic variants are reported if penetrance is estimated to be moderate or high. Result disclosure is performed in-person by a genetic counselor and study physician.

**Results:** Results are reported to parents in 3 categories: Monogenic disease risk, carrier status for recessive traits and pharmacogenomic (PGx) risk. Of the first 83 newborns sequenced, 71 (85.5%) were enrolled from well baby nurseries and 12 (14.5%) from ICUs. For carrier status, 169 variants were reported (range: 0 - 6 variants/subject, average: 2 variants/subject). No variants were reported in 8 subjects (9.6%). Monogenic disease risk was reported in 8 subjects (5.6%) well subjects (partial biotinidase deficiency (BTD), supravalvar aortic stenosis (ELN), dilated cardiomyopathy (VCL and TTN)) and 1 sick subject (KBG syndrome (ANKRD11)). In addition, an indication based analysis in an infant with hypoplastic left heart syndrome revealed a VUS in NKx2-5. The recessive conditions for which carrier status was most commonly reported included: biotinidase deficiency (BTD variant in 7/83), Connexin 26 related non-syndromic hearing loss (GJB2 variant in 7/83), thrombocytopenia absent radius syndrome (RBMB8A variant in 7/83), Cystic Fibrosis (CFTR variant in 4/83), Joubert’s syndrome (variant in multiple genes: 4/83), Stargardt macular degeneration (ABCA4 variants in 4/83), Smith-Lemli-Opitz syndrome (DHCR7 variants in 3/83), familial adenomatous polyposis (MUTYH variants in 3/83) and Neimann-Pick disease type C (NPC1 variants in 3/83). The most common pathogenic variant reported was p.As446His in BTD (6/83). PGx risk variants were reported to 4 subjects (2 TPMT and 2 DPYD). Independent parental follow-up testing of a subject with a recessive CTNS variant determined that the couple was at 25% risk for having a child with cystinosis.

**Conclusions:** GS of newborns selectively filtered for pathogenic and likely pathogenic variants in genes strongly associated with highly penetrant pediatric onset disorders, identified genetic variants for monogenic disease risk in 6% of enrolled newborns. On average, subjects were carriers for 2 pediatric onset disorders, most commonly for variants in BTD, GJB2 and RBMB8A.

**Presenter:** Richard Parad, MD, MPH, Director of Neonatal Genomic Medicine Program; Attending Neonatologist, Brigham and Women’s Hospital, Boston, MA, Phone: 617.732.7371, Email: rparad@partners.org

**P-020**

**Post-analytic QI: A Hospital’s Approach to Optimization of Abnormal Newborn Screening (NBS) Reporting on Inpatients**

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**Problem:** NBS is a complex process that can be broken down into pre-analytic, analytic and post-analytic phases. Through process mapping of the pre-analytic phase in our large urban birth hospital, we previously identified involvement of six hospital services and 29 steps to collect and transport each NBS specimen. With regard to the post-analytic phase, we found that while the majority of abnormal results were on newborns who had been discharged (with NBS program reporting results to an outpatient primary care provider), 25% of abnormals were for the 10% of babies who remained inpatients. The reporting of results to inpatient care providers was mapped, and each step evaluated for improvement
opportunity with a goal of avoiding missing, incorrectly interpreting, or not acting upon abnormal results.

**Methodology:** We developed an in-hospital NBS QI team, created process maps for pre- and post-analytic NBS phases and focused on improvement of in-hospital results reporting and documentation. **Results:** Our NBS QI team identified a significant problem with results reporting in the failure of documentation of abnormal NBS results for inpatient records. In the absence of direct downloading of NBS results from the NBS lab to our hospital lab system and EHR, results are typically reported via phone call and fax, and followed by a paper mailing – and may not make it into the EHR for weeks. We found our clinical staff had almost no documentation of the course of action taken in response to reported abnormal results, and paper reports were not in the EHR, making handoffs unreliable. Our large, busy NICU (over 60 beds) has frequent staff turnover (shifts, rotations), leading to multiple handoffs and rarely an accessible memory of events from the caretakers present at the time of an abnormal report. We dissected the process, and determined that designating a standard QI team to accept abnormal NBS reports, and defining a process for documentation, could improve EHR documentation of result reporting and recommendations. We implemented a new protocol in which the NBS QI team acted as an intermediary between the NBS lab and the ICU clinician. The NBS lab directed abnormal results to the QI team member on call via email and/or page. The QI team member uploaded the results as a .pdf file directly into the EHR and documented in an EHR event note reporting details. The EHR required a sign off by the patient’s attending physician. In addition to these steps, the QI team emails and directly contacts the attending physician. **Conclusions:** A post-analytic QI intervention was implemented to improve in-hospital NBS result reporting. All abnormal results communicated from the NBS lab to our QI team are documented in the EHR and made available for review to the appropriate clinical team member. Though this particular protocol is tailored to our hospital, the process we followed to create the protocol can translate easily to other birth centers.

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**P-021**

**Screening Performance of the FDA-Approved TREC Assay in California**

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**Background:** On May 28, 2015, the California Newborn Screening Program changed the severe combined immunodeficiency (SCID) assay from a LDT (Laboratory Developed Test) real-time PCR assay to the FDA-approved assay, the EnLiteTM Neonatal TREC kit, which is an in-situ end-point/single-point qPCR assay used to quantify T-cell receptor excision circles (TRECs). The analytical performance of the original and new assay is compared, including the precision, accuracy and clinical validity. **Methods:** 4,900 newborn specimens were run using both assays to establish TREC cutoffs for the new TREC assay. Clinical validation of the new assay was performed using 350 dried blood specimens (DBS) of the whole blood sent to flow cytometry. The monthly and average rate for positive screens and incomplete screens, for which second DBS were requested, for the new assay were determined using 696,175 specimens collected from June 2015 through October 2016.

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Results: Both assays showed imprecision rates of ~40% at the level of 25 copies/µL and had skewed TREC distributions. The new FDA-approved assay produced a lower TREC median (140 vs 185 copies/µL) and lower TREC readings in the lower percentiles (≠2.5) of the distribution. The new assay measured 21 specimens with TREC values between 0-25 copies/µL, while the LDT identified 15 specimens in this range. The coefficient of determination (R²) between TREC results of the confirmatory DBS and absolute CD3 T-cell counts for both assays was 0.5. Compared to the LDT, we had higher follow-up rates with the new assay during the study period.

Discussion: Both methodologies were able to detect the targeted disorders. The FDA approved methodology was able to be performed at our 5 regional laboratories. This assay also used less blood and was easier to perform. The new assay also produced a higher rate of follow-up with a TREC cutoff of 22 copies/µL.

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P-022

Copy Number Variants Limit Exomic Sequencing Approaches to Primary Newborn Screening: A Case Study of Isovaleric Acidemia
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Introduction: The NBSeq project is part of the NSIGHT consortium funded by a grant from NICHD/NHGRI to investigate the possible utility of next-generation sequencing (NGS) in the newborn. This project aims to learn whether NGS could replace current newborn screening (NBS) by tandem mass spectrometry (MS/MS) for inborn errors of amino acid and fat metabolism and to explore how addition of NGS could improve NBS.

Methods: Archived NBS dried blood spots (DBS) for all the true positive cases of inborn errors of metabolism detected by MS/MS in California between 2007 and 2013 plus DBS for selected false positive cases were retrieved from the California Biobank Program. Genomic DNA prepared from two 3 mm punches and exon capture and sequencing produced exomes comparable to those from fresh whole blood. Bioinformatic pipelines were developed in consideration of the demands of whole population screening. Variant calls from the exome analysis were compared with the diagnoses from the metabolic center follow-up. A small number of discrepant cases were selected for whole genome analysis to evaluate possible additional information.

Results: In the first 184 samples sequenced and analyzed, there were two cases of isovaleric acidemia, caused by variants in the IVD gene, encoding isovaleryl-CoA dehydrogenase. Both were missed by the initial exome analysis pipeline, though both had IVD exons with low coverage. The whole genome analysis of these cases provided convincing evidence of a resolution. One case had a homozygous deletion of the first three exons of IVD. The other had a more complex structural variation that was not fully characterized, which included a heterozygous deletion of most of the gene combined with a heterozygous, possibly mosaic, deletion of exon 12.

Conclusion: Whole genome sequencing can resolve cases not correctly diagnosed purely by an automated exome analysis pipeline. Further improvements for the bioinformatic analysis of copy number variation in exomes are under development and evaluation.
California Adrenoleukodystrophy Screening Update
L. Feuchtbaum, P. Neogi, K. Dhillon, P. Roworth, R. Koupaei and R. Olney, California Department of Public Health, Richmond, CA

**Background:** ALD is a progressive neurological disease that usually does not present clinically before age three, though clinically significant symptoms can appear at any point in the lifespan in males; milder symptoms can impact female carriers later in life. ALD screening in California began on September 21, 2016. All babies born on or after February 16, 2016 are also being screened, retroactively, in accordance with California Senate Bill 1095. This report describes the flow of patients through each tier of screening and the types of mutations identified.

**Methods:** Using the same dried blood spots collected for routine newborn screening, ALD screening includes a 3-tiered approach. Tier-1 uses FIA-MS/MS to measure C26:0 lysophosphatidylcholine (C26:0-LPC) with a cut-off of >0.42. Tier-2 provides a second measurement of C26:0 LPC using LC-MS/MS with a cut-off of >0.15. Tier-3 consists of DNA sequencing of the ABCD1 gene to identify mutations. Greenwood Genetics (South Carolina) is conducting the molecular testing. All Tier-2 positive cases due to elevated C26:0-LPC are referred to one of 15 metabolic clinics for final confirmatory testing and long term follow-up, after the molecular testing results are available.

**Results:** As of February 2, 2017, 337,800 California newborns had Tier 1 screening (including 137,068 retroactive). For this group, 2.4% (n= 8,025) had a Tier-1 positive test (1/42); all of were reflexed to Tier-2 screening. The overall positive rate after Tier 2 was 0.015% and all cases (n=51) were sent for molecular testing. Forty eight molecular reports have been received to date for 19 boys and 29 girls. Among the boys, 3 had a known ABCD1 gene mutation, 13 had a Variant of Unknown Clinical Significance (VOUS), 2 had no mutations identified and 1 had a polymorphism. Counting the ALD mutation and VOUS cases, the rate of ALD among boys was 1/20,046. Among the girls, 1 had a known ALD mutation, 15 had VOUS findings, 10 had a polymorphism, 2 had no mutations identified and 1 had a polymorphism/VOUS combination. Counting the ALD mutation and VOUS cases, the female carrier rate is 1/20,046. One Zellweger case was identified for a female case with a polymorphism.

**Discussion:** These data represent five months of screening in California (updated numbers will be provided). The most common molecular finding was VOUS among boys and girls. Long-term follow-up of these newborns will be required to eventually find out if these mutations cause clinical disease.

**Presenter:** Lisa Feuchtbaum, Chief, Program Development & Evaluation, Genetic Disease Screening Program, California Department of Public Health, Richmond, CA, Phone: 510.412.1455, Email: Lisa.Feuchtbaum@cdph.ca.gov
**Newborn Screening Long Term Follow-Up by Primary Care Providers Using REDCap: A feasibility study using Primary Congenital Hypothyroidism**

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**Background:** The Primary Congenital Hypothyroidism (PCH) Project was a HRSA-funded pilot study that involved a cross-sectional survey among primary care providers (PCP) to assess the case management and care coordination patterns of children with PCH. It included two-year follow-up of selected PCPs and their patients to assess the feasibility of collecting clinical long-term follow-up (LTFU) data from PCPs using the REDCap electronic data collection platform. Hereby we are reporting findings on the experience of providers and the overall feasibility of the PCP-centered data collection model using data from a survey conducted among participating PCPs after LTFU data-collection had ended.

**Methods:** Two years of patient data was collected from January 1, 2014-December 31, 2015. For each consented patient, PCPs completed a one-time data enrollment form, a follow-up visit form for every patient visit and a laboratory results form when new lab results were available. We provided $200 per patient annually to PCPs as compensation. At study completion, PCPs were invited to complete a 43-question online survey to evaluate their experience.

**Findings:** Of the 19 physicians who enrolled in the PCH Project in 2014, 17 completed the survey. Seventy percent reported improved knowledge/confidence in confirming a PCH diagnosis and initiating treatment as a result of study participation; 82% said it was “easy” to obtain patient informed consent; all reported a minimal burden to collect data; 53% preferred receiving a reminder from study staff to enter data into REDCap; 82% performed data-entry themselves and only one said the electronic report forms were moderately difficult to use; 38% said they would have participated in the study without compensation. Visits to an endocrinologist varied from every three months to once a year, mostly to discuss medication dosage and lab results. About half of the PCPs said that they would consider providing follow up data in the future, regardless of the schedule for entering data to REDCap.

**Discussion:** For a disorder that is relatively easy to manage, such as PCH, the pilot study demonstrated that PCPs are able to collect LTFU data using a REDCap tool. However, our findings were based on a very small self-selected sample of PCPs that were provided a yearly cash incentive to participate. The feasibility of using this approach for ongoing LTFU of PCH, or other disorders diagnosed through Newborn Screening, needs further investigation.

**Presenter:** Lisa Feuchtbaum, Chief, Program Development & Evaluation, Genetic Disease Screening Program, California Department of Public Health, Richmond, CA, Phone: 510.412.1455, Email: Lisa.Feuchtbaum@cdph.ca.gov

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**Tetrahydrobiopterin Deficiencies in California: Findings from a Seven Year Study**

M. Sartippour, R. Mathur, Y. Shchechin, S. DeSousa, J. Dhaliwal, H. Azizi, S. Narwal and C. Aznar, California Department of Public Health, Richmond, CA

**Background:** Phenylalanine hydroxylase (PAH) is a hepatic enzyme which metabolizes phenylalanine to tyrosine. The deficiency of PAH leads to classical Phenylketonuria (PKU). Atypical forms of PKU are due to the deficiency of tetrahydrobiopterin (BH4) which is a PAH cofactor. The frequency of PKU due to BH4 is very low in California.

**Discussion:** For a disorder that is relatively easy to manage, such as PKU, the pilot study demonstrated that PCPs are able to collect LTFU data using a REDCap tool. However, our findings were based on a very small self-selected sample of PCPs that were provided a yearly cash incentive to participate. The feasibility of using this approach for ongoing LTFU of PCH, or other disorders diagnosed through Newborn Screening, needs further investigation.
deficiency is believed to be 1% to 3% of total PKU cases. BH4 deficiency is a severe but treatable disease and early detection in newborns is essential to avoid irreversible brain damage.

**Design:** Annually approximately 500,000 newborns are tested for PKU using tandem mass spectrometry. Cases of atypical PKU are referred to the Genetic Disease Laboratory Branch of the Genetic Disease Screening Program for BH4 deficiency testing. The laboratory diagnosis of BH4 deficiency is based on a positive newborn screening for PKU, characteristic profiles of urinary or dried blood spot pterins (biopterin and neopterin), and the measurement of DHPR activity in dried blood spots. Our laboratory tests for the three most common causes of pterin metabolite deficiencies: 1) GTP cyclohydrolase I (GTPCH); 2) 6-Pyruvoyl-tetrahydropterin synthase (PTPS); and 3) Dihydropteridine reductase (DHPR) deficiencies. Method: We use urine and dried blood spot specimens to test PTPS, GTPCH, and DHPR. The biopterin and neopterin are separated by reverse-phase HPLC and quantified by a fluorescence detector. Dihydropteridine reductase activity is measured by spectrophotometer by conversion of ferric cytochrome C to ferrous cytochrome C at 530nm.

**Results:** Out of nearly 3.4 million screened newborns in California from 2010 through 2016, a total of 149 confirmed PKU cases (4.34 per 100,000 newborns) were detected. From 211 cases suspected for BH4 deficiency during the same period, we identified six cases of PTPS deficiencies and six cases of DHPR deficiencies. No GTPCH cases were found.

**Conclusion:** Our study shows similar relative frequencies for PTPS and DHPR deficiencies, of ~3% among those studied for BH4 deficiency. Our findings are in agreement with the study performed by Khatami et al. with BH4 deficiency. A few other studies reported different percentages of PTPS and DHPR deficiencies in newborns diagnosed with BH4 deficiency, which could be due to difference in ethnicity and genetic background of the studied populations.
false positive rate was higher among DS newborns than non-DS newborns (0.14% vs. 0.05%). Through individual case review, we identified 5 more individuals with DS who tested negative in initial screen but were confirmed as PCH cases later, mostly within one month after birth.* All DS newborns with PCH false negative screening had other medical conditions in addition to DS.

**Conclusions:** Screening for PCH among newborns with DS is challenging due to higher risk for both false positive and false negative results, especially the latter. The presence of other medical conditions might play a role in delayed TSH elevation. These findings support existing American Academy of Pediatrics recommendations for rescreening newborns with DS and provide additional evidence for the need to rescreen those with other health issues at birth, to improve early detection of PCH.

**Presenter:** Hao Tang, California Department of Public Health, Richmond, CA, Email: hao.tang@cdph.ca.gov

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**P-027**

**Effect of Regulations and Education on Timeliness in Newborn Screening (NBS) in California - NICU vs. Regular Nursery**

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**Background:** Newborn Screening (NBS) specimen collection and transit timeliness came under scrutiny as a result of a Milwaukee article published in 2013. California is involved in a three year project with NewSTEPS 360 to achieve collection times between 12 and 48 hours for 95% of births and to receive 85% of all specimens in our regional laboratories (labs) within two days.

**Methods:** The California Area Service Centers (ASCs) monitor newborn screen collection and transit quality improvement via data acquired from the Genetic Disease Screening Program’s Screening Information System (SIS). Data is shared with nurseries, NICUs and midwives by phone, email, and during site visits. The quality improvement tools include monthly and quarterly Hospital Evaluation Performance Reports that track newborn screen collection times and transit time for regular nurseries and NICUs. The ASCs support the facilities in reviewing this data through on-site visits, conference calls with stakeholders, and quarterly newsletters focused on timeliness goals to improve specimen collection and transit processes. In addition, NBS regulations were implemented in June, 2016 that decreased collection time from 6 days or before discharge, to 12 to 48 hours. For this study we analyzed SIS timeliness data for the period from October 1, 2015-December 31, 2016.

**Results:** During the study period, in the regular nurseries, NBS specimen collection time improved from 90.5% to 96.96% of specimens collected between 12 and 48 hours of life. In the NICUs, 85.44% of specimens were collected between 12 and 48 hours of age. The percent of specimens arriving in the labs within 2 days increased from 73.32% to 78.27% in the regular nursery and for the NICU population, 80.64% of the specimens arrived at the lab within two days.

**Discussion:** The original goal of specimen time collection in the Regular Nursery has been met and exceeded, while the NICU goal of 95% has been difficult to reach. Changing collection time practices will involve careful clinical evaluation and additional reviews of practices. The transit goal of 85% for all specimens has also been difficult to achieve. Courier pickup of newborn screens has increased from 5 to 6 days for some facilities and the courier is not available for 6 day pick up in some areas. Educational efforts have reinforced changes in NBS state regulations, especially in the NICU population. In the
future, we expect to continue streamlining the specimen collection and transit time processes to make additional improvements.

**Presenter:** Robin Thomas, BSN, MPA, Nurse Consultant III, Newborn Screening Program, California Department of Public Health, Richmond, CA, Email: robin.thomas@cdph.ca.gov

**P-028**

**Isotopically Labeled Argininosuccinic Acid - A New Reference Standard for Argininosuccinic Aciduria**
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The urea cycle disorder, Argininosuccinic aciduria (ASA) occurs in 1/70,000 babies in the USA and is included on the newborn screening panel in all 50 states as a core condition in the RUSP (Recommended Uniform Screening Panel). In the neonatal onset form of ASA, ammonia accumulates in the blood and, without treatment within the first week, results in lethargy, refusal to feed, respiratory alkalosis and death. High throughput MS/MS screening of the DBS (Dried Blood Spot) uses citrulline, as a surrogate marker for ASA. However, citrulline is also a marker for Citrullinemia I, Citrullinemia II, and Pyruvate Carboxylase Deficiency causing challenges in deconvoluting the screening result. The use of argininosuccinic acid as a primary marker for ASA resolves this problem. In this presentation, we report the validation of a new method for the direct identification of ASA from standard DBS (Dried Blood Spot) preparation and analysis by tandem MS/MS analysis. A new stable isotopically labeled argininosuccinic acid reference allows confident quantitative measurement of the argininosuccinic acid level from Dried Blood Spots. Data will be presented from known and blinded samples.

**Presenter:** Adrienne Manning, Division Director, Newborn Screening, Connecticut Department of Public Health Laboratory, Rocky Hill, CT, Email: adrienne.manning@ct.gov

**P-029**

**Simultaneous Quantitation of Amino Acids, Acylcarnitines, Succinylacetone, Creatine, and Guanidinoacetate**
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**Background:** Guanidinoacetate methyltransferase (GAMT) deficiency is an autosomal recessive genetic disorder which results in global developmental delay and intellectual disability. There is evidence that early treatment prevents intellectual disability and seizures. GAMT deficiency is a potential addition to the U.S. Recommended Uniform Screening Panel (RUSP), thus suitable newborn screening methods and quality control materials are essential.

**Methods:** Dried blood spot (DBS) materials were prepared with enriched creatine (CRE) and guanidinoacetic acid (GAA) as well as selected amino acids (AA), acylcarnitines (AC), and succinylacetone (SUAC). Punches (3.2 mm) of these DBS were extracted using a working internal standard solution (WISS) comprised of 80:20 acetonitrile/water containing 0.1% formic acid, 3 mmol/L hydrazine hydrate, and stable isotope-labeled standards for AA, AC, SUAC, GAA, and CRE. Extracts were analyzed using flow injection-tandem mass spectrometry (FIA-MS/MS) on a Waters Xevo TQD system.

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**Results:** Three methods of extraction were compared: one method analyzing only AA, AC, and SUAC (AAAC non-derivatized); one method analyzing only GAA and CRE (GAMT derivatized); and one new method analyzing AA, AC, SUAC, GAA, and CRE (AAAC-GAMT non-derivatized). Group means for selected analytes of the new, combined AAAC-GAMT non-derivatized assay showed similar apparent recovery when compared to existing AAAC and GAMT assays. No statistically significant differences were found. Precision, linearity, limit of blank (LoB), and limit of detection (LoD) were all determined following CLSI EP17.

**Conclusions:** The non-derivatized AAAC-GAMT method performance characteristics shown provide preliminary evidence of the method’s suitability for high-throughput GAMT deficiency neonatal screening. Small differences (< 15%) in group means were observed for both AAAC and GAMT analytes between the assays. Our results indicated that the recoveries of all the assayed biomarkers were comparable to the results obtained from the two stand-alone methods. The described method and quality control DBS are expected to assist newborn screening for GAMT deficiency efforts.

**Presenter:** Carter Asef, BS, ORISE Research Fellow, Biochemical Mass Spectrometry Laboratory, Centers for Disease Control and Prevention, Atlanta, GA, Phone: 770.488.7297, Email: xhy9@cdc.gov

**P-030**

**Assessment of Analyte Recoverability, Analyte Stability, and Method Performance Using Dried Blood Spots Proficiency Testing Materials for Hormones and Total Galactose**

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**Background:** The Newborn Screening Quality Assurance Program (NSQAP) creates and distributes dried blood spot (DBS) quality assurance materials that resemble newborn specimens. These specimens are used for quality control (QC), validation of new screening tests, and proficiency testing (PT). Using participant summary data from PT events, analyte recoverability by method, method performance, and stability during transit were assessed.

**Methods:** PT DBS materials were prepared from single-donor, hematocrit-adjusted whole blood, enriched with varying levels of thyroxine (T4), thyroid-stimulating hormone (TSH), 17 alpha-hydroxyprogesterone (17-OHP), and total galactose (TGal), packaged with desiccant, and distributed at ambient temperature to newborn screening laboratories worldwide. Participating laboratories tested the PT specimens, reported analytical results, and methods used. Select PT specimens were duplicated within panels and between panels during 2015 and 2016. Analyte stability, method reproducibility, and method performance over time were assessed using PT data from 79 T4 participants; 308 TSH participants; 241 17-OHP participants; and 150 TGal participants, by various methods of analysis.

**Results:** Summary statistics (mean, SD, paired t-test) were produced for each PT specimen. No significant differences in analyte recovery between methods was observed for each analyte and PT specimen. However considerable variability was observed within methods. No difference in analyte stability was observed in materials sent as close as 10 miles (Decatur, GA, < 1 day in transit) or as far as 9300 miles (Sydney, Australia, 14 days in transit).

**Conclusions:** PT is used to assess individual laboratory performance and can also evaluate method performance, analyte recoverability, and stability. Data collected from NSQAP participants showed that T4, TSH, 17-OHP, and TGal in DBS were stable during transit around the globe under ambient conditions. Hundreds of participating laboratories reported results using different methods. Methods gave comparable recoverability for each analyte while PT data indicate variability exists within methods.

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Continued monitoring of summary quality assurance data provides a means to document method performance and analyte stability over time.

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**P-031**

**Creation of Quality Assurance Sample Repository for Identification of Congenital Adrenal Hyperplasia Causing Mutations in the CYP21A2 Gene**

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**Objective:** To collect DNA, dried blood spots (DBS) and lymphocytes that cover a spectrum of CYP21A2 mutations from congenital adrenal hyperplasia (CAH) affected families. Confirm mutation status with a Centers for Disease Control and Prevention (CDC) developed long-range amplification-based method and Sanger sequencing.

**Background:** CAH is caused by a group of autosomal recessive disorders of cortisol biosynthesis. The most common form is caused by a deficiency in the production of the enzyme 21a-hydroxylase and occurs at 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. This assay has been well documented to have a high false positive rate and false negative rate. Studies are ongoing in Minnesota to test the utility of a second tier genotyping assay that would allow the 17OHP cutoff level to be lowered, thereby increasing the test’s sensitivity, while maintaining specificity with a second tier assay to detect CYP21A2 mutations. Pending the results of this study, a second tier molecular assay may be adopted for routine screening requiring quality assurance materials.

**Methods:** Blood samples were collected from CAH patients and families from participating pediatric centers and DBS were created by spotting liquid blood on to filter paper. DNA was also extracted from 250 µL of blood using DNA Mini kit. White blood cells were cryopreserved for future cell line transformation. A CDC developed long-range amplification-based method was used to identify or confirm the presence of the CYP21A2 gene, any recombinant 30kb deletion, or gene conversion alleles which were detected using the capillary electrophoresis instrument or agarose gel electrophoresis. In addition, each sample had complete gene Sanger sequencing to detect or confirm mutations in CYP21A2, deletion breakpoints in any 30kb deletion and gene conversions alleles.

**Results and Conclusions:** Analysis of the gene sequencing results of the 97 families with 109 CAH affected Minnesota children identified the 12 common mutations found in diagnostic panels, large 30kb deletions, large gene conversions, ten rare and one novel CYP21A2 mutations. The CDC’s CAH mutation repository currently contains 25 unique genotypes, which are represented in 75 distinct allele combinations within the study population. These CYP21A2 alleles had the following clinical manifestations: 16 Salt Wasting, 2 Simple Virilizing, 5 Non-Classical, 1 Salt Wasting/Simple Virilizing, and 1 Non-Classical/Simple Virilizing. All samples are banked as DBS, purified DNA and cryopreserved white blood cells. The CDC’s CYP21A2 mutations repository will be used for quality assurance in newborn screening laboratories that perform second tier molecular assays for CAH screening.
CDC’s Cystic Fibrosis Mutation Repository Expanded to Include EBV Transformed B-Lymphocytes
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CDC’s Cystic Fibrosis DNA Proficiency Testing (CF DNA PT) program is responsible for providing proficiency testing and quality assurance materials to newborn screening programs performing second tier CFTR mutation detection. The program was implemented in 2007 based on a 2004 MMWR publication that recommended CF be included in newborn screening. The initial CF DNA repository consisted of patient and carrier blood samples collected by CF Care Centers in Maryland, Ohio, and Wisconsin that were spotted onto filter paper to create dried blood spots (DBS). Between 2007 and 2010 the CF DNA repository collected 72 samples representing 44 variants (23 ACMG and 21 non-ACMG variants) with 51 unique genotype combinations. These samples were an excellent source of quality assurance materials, however they were limited by the quantity of blood collected. With the increasing number of CF DNA PT participants (33 domestic and 48 international labs in quarter 1 2017), the number and complexity of methods being utilized (single-plex, multiplex, gene sequencing), and the increasing number of variants screened, it has become essential to expand the repository both in quantity and variety of CFTR variants. Since 2011, the California Department of Public Health and the Sequoia Foundation have collaborated with CDC to replenish (N=132 samples) and increase the number of CFTR variants (N=62 variants) in the CF DNA repository as well as expand the repository to include cryopreserved white blood cells that can be immortalized for the creation of future quality assurance materials, negating the need to continuously collect blood from CF patients. Cryopreserved white blood cells from patient and carrier samples in the CF DNA repository were transformed using Epstein Barr Virus to create immortalized cell lines. So far, 24 cryopreserved CF DNA samples have been transformed in-house using Phytohemagluttinin, a mitogen that causes T cells to rapidly transform into blast cells. To assure the quality of these cell lines, they have been tested for mycoplasma contamination using a 16S rRNA PCR detection method, seven microsatellites and the Amelogenin sex marker to confirm the cell lines matched the original blood sample, and genotyped with two commercial methods to confirm the presence of the CFTR mutations. All genotyping results were confirmed with Sanger Sequencing data on DNA extracted from whole blood. Future steps will be to use these cell lines to make laboratory created DBS which will provide a sustainable source of rare quality assurance materials for newborn screening laboratories.

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Newborn Screening Molecular Assessment Program: A Tool for Molecular Testing Quality Improvement  
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The Newborn Screening Molecular Assessment Program (MAP) is a site visit program developed by CDC in partnership with APHL’s Newborn Screening and Genetics Program. Its purpose is to provide assistance to NBS laboratories as they prepare for new molecular screening assays or to perform a quality assessment of their current molecular testing. MAP teams are composed of an APHL representative and molecular biologists from CDC and public health laboratories with experience in mutation detection, quantitative PCR, and DNA sequencing. The goal of the site visit is to assist programs in determining how best to meet their unique current and future needs with their available resources, all the while assuring the highest quality of testing. During the MAP site visit, all phases of the molecular testing process are evaluated including standard operating procedures and quality control documentation, molecular assay validation, laboratory set-up, testing capacity, and results reporting.

Molecular testing has rapidly expanded in newborn screening laboratories both as a second tier test to enhance a primary biochemical assay or provide supplemental information to clinicians and as a primary test. In addition, five newborn screening laboratories to date have incorporated gene sequencing as a second tier assay for Cystic Fibrosis, Hemoglobinopathies, Pompe, Krabbe, VLCADD, and X-ALD. Several additional laboratories are also anticipating adding gene sequencing in the next few years. To aid public health laboratories as they expand into this area, the MAP site visit offers gene sequencing specific guidance for sequencing implementation and validation as part of the site visit to help support programs in their planning for and implementation of gene sequencing. As of March 2017, MAP has performed 19 newborn screening laboratory site visits. Some of the common challenges identified when implementing molecular assays include modifying workspace for unidirectional workflow, assay validation guidelines, defining molecular-specific quality assurance processes and harmonization of standard operating procedures. Some of the outcomes from the MAP visits include inter-program collaborations for quality control materials and processes, laboratory expansion and reorganization to accommodate molecular workflow, molecular specific staff development recommendations, and valuable feedback to CDC for quality assurance and training needs. Site visits are non-regulatory and provided at no cost. Programs that have previously participated in a MAP visit are encouraged to request additional visits for follow-up of current testing practices or preparation for new molecular tests. In the era of molecular testing, MAP is a unique tool that newborn screening programs can utilize for continual quality improvement during the rapid changes in the molecular arena.

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**P-034**

**Dried Blood Spot Quality Assurance for Mutations in the GALT gene Associated with Classic and Duarte Galactosemia**  
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Classic Galactosemia, an autosomal recessive disorder, has a prevalence of approximately 1/50,000 live births in the United States (U.S.) and results from mutations in the galactose-1-phosphate uridylyltransferase (GALT) gene. If an infant with Galactosemia continues to consume milk, the affected infant may experience symptoms including vomiting, diarrhea, and jaundice, which can quickly lead to hepatomegaly, E. coli sepsis, and death. If the infant is identified quickly and switched to a galactose-restricted diet, such as soy formula, these potentially lethal symptoms can be avoided. All newborn screening programs in the U.S. have been screening for galactosemia since 2004, using an indirect activity assay for galactose-1-phosphate uridylyltransferase. In addition, some programs quantify total galactose, either in all samples or in those samples with a low GALT activity. Second tier molecular methods are currently used to detect some of the more common mutations associated with Galactosemia as well as the Duarte variant, which causes decreased GALT enzyme activity. GALT mutation analysis is considered “Just in Time” information that supplements the biochemical newborn screening results to assist physicians in direct timely treatment of the infant. The number of variants screened differs by newborn screening program and can include Q188R (c.563A>G), S135L (c.404C>T), N314D (Duarte - c.940A>G), K285N (c.855G>T), L195P (c.584T>C), F171S (c.512T>C), Y209C (c.626A>G), T138M (c.413C>T), IVS2-2A>G (c.253-2A>G) and a 5kb deletion (g.(?_4752)_(?_9014)). The GALT mutation repository at CDC’s Newborn Screening Quality Assurance Program (NSQAP) contains 7 variants in 10 genotype combinations, most of which were a generous gift from Dr. J. Fridovich-Keil at Emory University. Because it is challenging to obtain whole blood from patients with rare mutations, CDC has developed a process to make laboratory-created dried blood spots (DBS). This involves mixing transformed lymphoblasts containing specific GALT mutations, serum and leuko-depleted blood. In keeping with the CF DNA mutation detection program, the lab created DBS have been designed to mimic a sample in the lower range of normal with 1.5 x 107 cells per mL. All DBS have been tested at CDC using fluorescent probe hybridization (Taqman) assays, amplification refractory mutation system (ARMS) assays, gap-PCR 5 Kb deletion detection assay and Sanger sequencing assays with 100% concordance. These samples have also been sent to NBS laboratories to evaluate their performance in the field.

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**P-035**

**Characterizing CDC’S Cystic Fibrosis DNA Repository Specimens Using a High-throughput Single-plex Genotyping Assay**  
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**Background:** There are a variety of molecular assays utilized by newborn screening laboratories to characterize the CFTR gene. TaqMan, or allelic discrimination genotyping assays, query a single variant and provide a homozygous mutant, heterozygous or normal genotype. This assay has been used successfully by newborn screening laboratories for small numbers of variants, however with increasing...
numbers, the throughput decreases significantly in 96 or 384 well formats. The OpenArray Genotyping system allows simultaneously genotyping as many as 3072 genotyping assays in a single specialized plate, which is sufficient throughput for most newborn laboratories. One caveat is the input DNA volume of 2.5µL (50ng/µL stock), which is greater than the DNA yield extracted from DBS. Thus, we sought to optimize this method with low concentrated DNA extracted from a subset of the CF DNA repository DBS samples using this method.

**Methods:** DNA was extracted from a 3mm DBS punch from selected samples in the CF DNA repository using commercial DNA Purification and Elution Solutions. Due to the low DNA concentration and yield in DNA extracted from DBS, CFTR target regions for the variants tested were pre-amplified using a preamp pool that matches the custom genotyping assay. The pre-amplified samples were diluted, combined with genotyping master mix and placed on a specialized system for loading plates. The plates were sealed, loaded onto the instrument and run. Raw data files were imported into the Genotyper software for inspection and analysis of individual assays. The final software analysis was then run through a macro-enabled Excel worksheet which can produce final genotypes, taking into account multiple assays for regions such as F508del (c.1521_1523delCTT) and the IVS8 poly T (c.1210-12[5_9]) regions.

**Results and Conclusions:** OpenArray custom CFTR assays were run on 47 samples representing 46 unique genotype combinations. Since several assays had to be considered in order to make the final genotype call for 4 of the variants, the macro-enabled worksheet was critical in assisting with streamlining data analysis. After analysis, all final genotypes were found to be concordant with previous characterization results from Sanger or Cystic Fibrosis 139-variant Next-Generation sequencing assay. Each plate contained 60 single-plex assays and could genotype 48 samples in a single run taking 3.5 hours, excluding the pre-amplification. Since the calling algorithm used by the Genotyper software allows the use of control and reference panels, this negates the necessity to run positive controls for each genotyping assay with each run. This technology and macro-enabled Excel worksheet can also be customized for genotyping more or less variants based on individual laboratory needs.

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P-036

**Dried Blood Spot Quality Control Materials and a Multiplexed Assay for X-linked Adrenoleukodystrophy and Metachromatic Leukodystrophy Newborn Screening**

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**Background:** Metachromatic leukodystrophy (MLD) and X-linked adrenoleukodystrophy (X-ALD) are genetic neurodegenerative diseases which result in the accumulation of sulfatides or very long chain lysophosphatidylcholines (LPCs), respectively, in blood. Thus, sulfatides and LPCs may be useful biomarkers for MLD and X-ALD, but a multiplexed assay to measure sulfatides and LPCs and quality control materials containing both lipids is not available. We developed a mass spectrometry-based method to measure sulfatides and LPCs and created dried blood spot (DBS) quality control materials which contain these analytes.

**Methods:** Blood was adjusted to 50% hematocrit and lysed by a freeze-thaw cycle. Sulfatides and LPCs were added dropwise while stirring, followed by spotting (100 µL) onto Whatman 903 filter paper cards. The cards were dried overnight and stored with desiccant at -20°C. Punches (3.2 mm) of the DBS were extracted in an organic solvent mixture containing 1-hexacosanoyl-D4-2-hydroxy-sn-glycero-3-
phosphocholine (D4-C26 LPC) and N-omega-CD3-Octadecanoyl-sulfatide (D3-C18 sulfatide) followed by LC-ESI-MS/MS analysis in negative ion mode.

**Results and conclusions:** The LC-ESI-MS/MS method quantified sulfatides and LPCs from the quality control material, and may be a valuable tool to screen for X-ALD and MLD. The use of quality control materials enriched with LPCs and sulfatides may facilitate the implementation of a multiplexed screening technique for these disorders.

**Presenter:** Brandon M. Kenwood, Centers for Disease Control and Prevention, Atlanta, GA, Phone: 404.498.1146, Email: mnv9@cdc.gov

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**P-037**

**Development of Sequencing and TaqMan Assays to Characterize the ACADM Gene in MCADD Patient Samples**

D. Koontz, C. Cuthbert and S. Cordovado, Centers for Disease Control and Prevention, Atlanta, GA

**Objective:** To develop a Sanger sequencing assay for the ACADM gene associated with Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCADD) and a TaqMan assay for the common c.985A>G variant for use in the characterization of samples from patients diagnosed with MCADD.

**Background:** Development of second-tier molecular testing by newborn screening laboratories is becoming increasingly common as a means by which to decrease the false positive rate inherent in some primary assays or to provide just in time supplemental information for time critical conditions. The primary test for MCADD is a tandem mass spectrometry analysis of acylcarnitine levels that looks specifically for the accumulation of octanoylcarnitine (C8). Newborn screening laboratories that employ second tier molecular methods for MCADD positive samples currently perform genotyping assays to look for common ACADM variants specific to their population. CDC's Newborn Screening Quality Assurance Program (NSQAP) is exploring the feasibility of creating an ACADM mutation DBS repository through a collaboration with the California Department of Health and the Sequoia Foundation.

**Methods:** DNA was extracted from whole blood using a silica column extraction and from 3mm DBS punches using both a commercially available elution solution and an in-house prepared lysis buffer. A Sanger sequencing method that covered the 5'-flanking regulatory regions and 12 exons with flanking intronic regions of the ACADM gene was developed at CDC and tested on MCADD positive samples from Coriell. The primer selections for amplification of the gene regions were based on BLAST results of all published primers that yielded the least amount of variation spanning the area of the primers. PCR amplifications were optimized for use with the same master mix and cycling conditions for all amplicons. PCR primers were tagged with an M13 tail to allow for the use of universal M13 primers for cycle sequencing. Pre-designed commercial TaqMan assays were used for allelic discrimination.

**Results/Conclusion:** Sanger sequencing of the ACADM gene and TaqMan assays for allelic discrimination performed reliably with DNA extracted from DBS using a commercially available elution solution or an in-house lysis buffer, both of which are quick and inexpensive. Thus, these methods are suitable for use in the characterization of DBS samples from patients with MCADD. These methods will support the creation of an MCADD mutation repository at CDC.

**Presenter:** Deborah Koontz, Centers for Disease Control and Prevention, Atlanta, GA, Email: duk5@cdc.gov
Proficiency Testing for T-Cell Receptor Excision Circles (TREC) Assay to Detect Severe Combined Immunodeficiency (SCID) in Newborn Screening – Method Performance from 2015 – 2017

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Population based newborn screening to detect severe combined immune deficiency (SCID) began in two states in the US in 2009. SCID was added to the US HHS Recommended Uniform Screening Panel (RUSP) in 2010, and screening is now conducted in 45 newborn screening programs covering 88% of US newborns annually. The screening test is based on quantification of T-cell Receptor Excision Circle (TREC), an 80Kb DNA segment excised from the germline genome during the somatic recombination that forms the intact gene for T-cell receptors in the mature T lymphocytes.

CDC began providing proficiency testing (PT) for the TREC assays in 2010. Current participants include 38 US and 20 international laboratories. At the onset of the PT program, all participating labs used laboratory developed tests based on real-time PCR technology, either with or without a separate DNA extraction step. Since 2015, an increasing number of labs started using a commercially-available test kit, which is based on a single-point PCR approach. An additional method using the new digital PCR platform was reported by one participant.

CDC prepares dried blood spots (DBS) PT specimens 1) from cord blood with TREC and total leukocyte levels in the expected range for full-term newborns without T-cell deficiencies; 2) DBS that simulate SCID made from lymphocyte-depleted blood from older adult donors, with TREC levels below the expected range for newborns; and 3) DBS made from leukocyte-depleted blood that give inconclusive results due to lack of both TREC and genomic DNA. Participants were asked to report the TREC results as ‘No follow-up required’ or ‘Follow-up required’. When the TREC results indicated follow-up required, the labs were further asked to report whether the reference gene (RG) level in the specimen was within or outside of the standard reference range. The RG is commonly used to determine whether the abnormal TREC screening result is consistent with a SCID profile (abnormal TREC with normal RG), or inconclusive (both TREC and RG below expected level; possibly due to prematurity of donor, or PCR inhibition). Over 2000 DBS PT specimens (including 454 SCID-like, 404 leukocyte-depleted, and 1212 normal samples) have been tested by the participating labs during the 2015-2017 period. We analyzed the reported PT results for general trends in performance characteristics of different assays for both TREC and reference genes. Their effect on the interpretation of SCID screening results using PT specimens will be discussed in the presentation.

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Computation and Analysis of Multiple of the Median Based on Cycle of Quantification Values (Cq or Ct) in the TREC and Other Real-time PCR Assays

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With the successful implementation of the T cell receptor excision circle (TREC) test for Severe Combined Immunodeficiency syndrome, real-time PCR has become a familiar technology to many newborn screening (NBS) labs. Other assays based on the same platform (e.g., SMA) are likely to follow. One remaining challenge with the TREC assay was the lack of consensus in how to define the TREC level in a sample. The nonconformity makes comparing sample results or cutoff values between labs difficult. Many labs use the value of copies per microliter of blood, based on a standard curve. However, the absence of a universally accepted calibrator introduces persistent problem of uniformity. Use of plasmids (circular or linearized) may lead to 6-10 fold differences between labs. Moreover, any standard curve in the TREC assay necessarily includes values close to limit of quantification, which often introduces large variance. Significant difference of variance along the range of the curve challenges the validity of the linear regression model, and causes an inconsistent slope. Other labs use the cycle of quantification value (Cq or Ct). Cq is read off the amplification curve of each sample and represents the number of PCR cycles it takes for the product fluorescence to reach a certain threshold. Threshold chosen by PCR software varies from run to run and introduces more inconsistency. Fixed threshold chosen by the investigator offers more consistency, but the optimal threshold differs with instrument models and varies from lab to lab. Moreover, unlike a standard curve, Cq lacks a mechanism to normalize lot to lot difference in reagents, or day to day variations in assay performance. The third option is to use the multiple of the median (MoM), which is a measure of how far an individual test result deviates from the median. MoM can be determined by the ratio “Individual sample level/Population median level”. Since both the individual sample and the median have been subjected equally to the inherent factors that affect the assay, MoM is naturally normalized. The most attractive application of MoM is by Cq values, because that would avoid the limitations associated with a standard curve. However, it is important to note that Cq values are not normally distributed in a population. Since each cycle in PCR amplification represents a 2-fold difference in copy number, Cq is an exponential value with a base of 2. Dividing an exponent by another yields the root, and not the ratio as required in MoM. Using division of Cq, as practiced by a few labs, results in a very small numerical difference between 1%-100% of the median. We have derived a formula to convert Cq values of sample and median into a MoM value that is equal to the result based on copy numbers. MoM = 2^((CqS-CqM)) where CqS is the Cq for the sample, and CqM is the population median Cq. The mathematical derivation, and application of the MoM equation to data from a state population will be discussed.

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Enhancing the Efficiency of the GALK Deletion and Sequencing Assays to Detect Infantile Krabbe in Newborn Screening
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Globoid Cell Leukodystrophy (Krabbe disease) is a rare inherited disorder that affects the central nervous system. Early detection for the most aggressive form, infantile Krabbe, is critical to avoiding serious consequences leading to premature death. Babies affected with Krabbe are deficient in GALK, an enzyme needed for production of healthy myelin. The absence of GALK leads to the rapid destruction of both the cells that make myelin and myelin itself. The most common pathogenic variant in the GALK gene seen in patients with infantile Krabbe disease is a g.30kb deletion impacting exons 11-17. This deletion accounts for 35% to 45% of the pathogenic alleles depending on the population. A second, g.7.4kb deletion has been also reported and the remaining pathogenic variants are rare thus gene sequencing is required. New York was the first state mandated to screen for Krabbe in 2006 and as such pioneered the methods for biochemical and molecular screening of the disease. The algorithm involves a MS/MS primary test to identify babies with decreased GALK enzyme activity followed by molecular testing of the g.30 and g.7.4 Kb deletions and complete gene Sanger sequencing. As Krabbe screening is adopted by more NBS programs, this study sought to find opportunities to simplify and enhance the efficiency of the molecular assays using a multi-purpose multiplex DNA polymerase. The gap-PCR deletion assays currently used to detect the g.30 and g.7.4 kb deletions are performed in separate amplification reactions. By employing a multiplex DNA polymerase, it allows for the amplification of both deletions and the normal alleles in a single tube assay. This multiplexed single tube reaction is then detectable by electrophoresis on a 3% agarose gel with all fragments having a separation of at least 36bp. The GALK gene sequencing assay requires the amplification of 18 fragments in order to sequence the promoter and all coding regions. Several of the PCR setups in the current assay have unique MgCl2 and primer concentrations to obtain robust amplification. By employing the same multiplex DNA polymerase, it enabled harmonization of reaction conditions for all amplicons required. These new conditions eliminate the need for unique PCR setups, thereby simplifying the process.

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Background: In 2006 the Newborn Screening Quality Assurance Program (NSQAP) began proficiency testing (PT) program for second-tier liquid chromatography tandem mass spectrometry (LC-MS/MS) methods used to screen for congenital adrenal hyperplasia (CAH). NSQAP has been distributing blind-coded dried blood spot (DBS) PT panels containing five hormones associated with CAH: 17-hydroxypregesterone (17OHP), 4-androstenedione (4AD), cortisol (CORT), 11-deoxycortisol (11D), and 21-deoxycortisol (21D). The PT panels were sent from 2006 through 2016. Participants were asked to...
report the steroid concentration for each analyte and clinical assessment based the ratio of (17OHP + 4AD)/CORT). Data from the testing of the PT panels was used to assess method performance and analyte recovery over time.

**Methods:** Enriched DBS representing the following clinical CAH profiles were distributed over a ten-year period: true negative, challenge, false positive and true positive specimens. The steroid concentrations were enriched so that only the true positive specimens had a (17OHP + 4AD)/CORT ratio greater than 1.0. The specimens were made using charcoal stripped serum to minimize endogenous hormone concentration. Participating laboratories reported the concentration of each analyte (ng/mL serum) and the ratio based on (17OHP + 4AD)/CORT. The reported values were compared to the expected analyte concentrations to determine the analyte percent agreement (% agreement = reported concentration/expected concentration × 100). The expected concentrations were determined by summing the base pool endogenous analyte concentration and the enrichment concentration.

**Results:** From 2015 to 2016, the average percent agreement across all enriched PT specimens was: 17OHP, 107.2%; 4AD, 94.4%; CORT, 106.7%; 11D, 96.0%; and 21D, 88.1%. The average percent agreement from PT specimens sent between 2006 and 2016, were also compared. While the percent agreement for 21D improved from 2015 to 2016, this analyte had a low response in the LC-MS/MS method.

**Conclusions:** Agreements improved from 2006 to 2016 for all steroids, shown in poster. This improvement reflects the efforts of laboratories to optimize their LC-MS/MS methods. NSQAP was able to document this improvement through the use of PT materials over time. Continuous quality assessment is essential for the long-term evaluation of laboratory and method performance.

**Presenter:** Gyliann Pena, Chemist, Centers for Disease Control and Prevention, Atlanta, GA, Phone: 770.488.7924, Email: klx9@cdc.gov

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**P-042**

**Development and Inter-laboratory Evaluation of Dried Blood Spot (DBS) Reference Materials for Assays to Detect Spinal Muscular Atrophy (SMA) by Newborn Bloodspot Screening (NBS)**

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Spinal Muscular Atrophy (SMA), the leading genetic cause of infant death, results from the absence of a functional SMN1 gene. Over 90% of the SMN1 mutations that cause SMA occur in exon 7, either by deletion or by conversion to the paralog SMN2 gene. Therefore, newborn screening assays to identify SMA genetic risk from dried blood spots (DBS) generally focus on this region. Detecting the absence of SMN1 requires highly specific assays to minimize cross-reactivity with the nearly identical SMN2. Specificity is especially important because SMN2 is a normal constituent of the human genome, present in variable copy numbers ranging from zero to five or greater. To ensure that the presence of SMN2 does not obscure the absence of SMN1, quality control (QC) for SMA-NBS assays should include DBS that are SMN1 null and that contain multiple copies per genome of the relevant SMN2 sequence in exon 7. This genotype may be found in cultured EBV-immortalized B-cell lines established from older SMA patients. Such cell lines also contain the various genomic reference genes used by different laboratories. However, they lack the non-genomic TREC sequence, which is normally detectable in DBS from newborns unless they are affected by T-cell deficiencies such as SCID. To prepare DBS reference

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materials that emulate specimens from newborns with SMA but not with a concurrent T-cell deficiency, we used both an SMA B-cell line and synthetic dsDNA polynucleotide blocks that contain the TREC signal joint sequence. We started with a base pool made from leukocyte-depleted adult blood which was washed, reconstituted with human serum to 50% hematocrit, and spotted onto collection cards at 75 microliters per spot. The base pool DBS were tested by real-time PCR to ensure the absence of detectable SMN1, RPP30, and TREC. Spiked pools were then prepared by adding the SMA B-cells and the TREC polynucleotide blocks to the base pool. DBS made from the spiked pools were evaluated by real-time qPCR and droplet digital PCR to confirm the absence of SMN1, the presence of SMN2 and RPP30, and a total TREC copy number in the expected range for typical newborns. We also performed real-time PCR assays directly on DBS punches as well as extracted DNA, obtaining consistent results by both processing methods. These prototype SMA-like DBS, along with SCID-like DBS and control DBS made from normal cord blood, were evaluated by three public health newborn screening laboratories experienced in SMA and SCID testing. Categorical and analytical results were compiled for review by the collaborating laboratories. Participation in this technical study group will be opened to additional newborn screening laboratories as SMA-like DBS reference materials are validated and preparation is scaled up.

**Presenter:** Golriz Yazdanpanah, MS, Centers for Disease Control and Prevention, CDC Foundation, Atlanta, GA, Email: czu1@cdc.gov

**P-043**

**NSQAP Laboratory Support for Newborn Bloodspot Screening to Detect Lysosomal Storage Disorders**


**Background:** Since 2006, the Newborn Screening and Molecular Biology Branch (NSMBB) at the U.S. Centers for Disease Control and Prevention (CDC) has provided laboratory support for detecting lysosomal storage disorders (LSDs) by newborn bloodspot screening (NBS). This support included the distribution of donated reagents for measuring six lysosomal enzyme activities by MS/MS assays, and providing quality control (QC) and proficiency testing (PT) dried bloodspots (DBS) to participating laboratories. The production and distribution of these reagents was concluded in 2016. However, the QC and PT reference DBS remain available to NBS programs and, beginning in 2018, will include certified values for two assay methods in current use by NBS laboratories. 

**Methods:** QC DBS sets were made from mixtures containing 100%, 50%, 5%, and 0% pooled umbilical cord blood (UCB) diluted volumetrically into a base pool made from double-leukodepleted RBC and heat-inactivated charcoal-striped serum. Condition-specific DBS for PT were prepared by adding immortalized cultured cells obtained from LSD patients into a base pool. These QC and PT DBS were assayed at CDC for six lysosomal enzyme activities by MS/MS using two methods: one with separate reactions at optimal pH for each enzyme (since 2006) and the other with a single multiplexed reaction for all enzymes (since 2017). Four of these enzymes were also assayed at CDC using digital microfluidic fluorescence (DMF). The QC and PT DBS were also analyzed by multiple public health NBS laboratories. Results from all assays were reported in micromoles per liter per hour.

**Results:** QC DBS analysis for the 6 enzymes assayed by MS/MS showed different ranges of activity using the separate-reaction and multiplex-reaction systems. Both systems showed linear regression R2 values near 1.0 when observed values were compared to the expected 0, 5, 50, and 100% enzyme activity. The DMF platform showed different ranges of activity compared to either of the two MS/MS assays, and also
showed R2 values near 1.0 when compared to expected percentage of enzyme activity. QC DBS analysis at public health NBS laboratories showed that activity levels of both pooled UCB and the 50% dilution were in the normal range for all lysosomal enzymes, the base pool was in the deficient range for all enzymes, and the 5% UCB dilution was deficient or borderline for all enzymes. PT DBS analysis at NBS laboratories showed that all 3 analytical systems correctly identified Pompe Disease (acid alpha-glucosidase deficiency, N = 100) and MPS I (alpha-L-iduronidase deficiency, N = 39) in all deficient specimens.

Conclusions: The QC DBS from NSMBB are suitable for use as external QC materials for LSD assays using any of the three analytical systems described here. The PT DBS showed the expected condition-specific enzyme deficiencies for Pompe Disease and for MPS-I in all public health NBS laboratories using any of the three systems. CDC will continue the refinement, certification, and expansion of DBS reference materials for NBS assays to detect LSDs.

Presenter: Christopher Haynes, PhD, Centers for Disease Control and Prevention, Atlanta, GA, Email: cph7@cdc.gov

P-044

Evaluation of Dried Bloodspot Reference Materials for Quality Control of Assays to Detect Lysosomal Storage Disorders by Digital Microfluidic Fluorescence
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Newborn bloodspot screening for one or more lysosomal storage disorders (NBS-LSD) is currently performed by multiple NBS programs in the United States and abroad. Two LSD (Pompe Disease and Mucopolysaccharidosis I) have been included on the US HHS Recommended Uniform Screening Panel. Among the different tests used for NBS-LSD is the Digital Microfluidics Fluorescence (DMF) method, which was recently authorized for use in newborn screening by the US FDA. The Missouri State Public Health Laboratory (MSPHL) has extensive experience using DMF for NBS-LSD, and other public health laboratories may consider this platform when they add NBS-LSD to their panel of tests. The availability of external dried blood spot quality control materials (DBS-QC) with assay-specific certified values is an important component of analytical quality control (QC) in NBS laboratories. Because these assays measure enzyme activity, the quantitative results are dependent on the particular analytical method. Since 2006, the Newborn Screening and Molecular Biology Branch at the Centers for Disease Control and Prevention (NSMBB/CDC) has produced DBS-QC for NBS-LSD. To ensure the validity of these materials for all analytical methods in current use, NSMBB/CDC has conducted long-term analyses of the DBS-QC using DMF to measure four lysosomal enzyme activities. In addition, NSMBB/CDC has collaborated with MSPHL to ensure that these DBS-QC materials are appropriate for use as external QC in high-throughput public health laboratories and relevant to the identification of the designated lysosomal storage disorders in newborns. The same materials have also been subjected to extended analysis by an alternative NBS-LSD assay that uses mass spectrometry to measure enzyme activity. Beginning in 2017, NSMBB/CDC will issue certified enzyme activity values obtained by both assay methods for these DBS-QC.

Presenter: Paul Dantonio, MS, Research Biologist, Centers for Disease Control and Prevention, Atlanta, GA, Email: pdantonio@cdc.gov

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A Compact and Efficient Tissue Culture System for Production of Quality Control Materials for New Molecular and Cellular Assays used in Newborn Screening

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Objective: Recently developed assays in newborn screening (NBS) increasingly rely on genetic or cellular analysis, which require Quality Control (QC) materials that are cell-based with specific mutations or phenotypic profiles. Such QC dried blood spots (DBS) are often produced by NBS labs for laboratory developed tests. Immortalized cell lines established from patient lymphocytes, or genetically modified, can mimic patient samples. Our objective was to develop an efficient yet compact tissue culture system that can produce sufficient cellular QC materials required for first tier NBS assays.

Methods: Preparation of condition-specific DBS requires the leukocytes in normal blood to be completely replaced with culture cells to avoid genotype from donor being detected. Approximately 40-80 million cells are needed for one milliliter (mL) of blood, which makes 12 DBS. To obtain enough cells, we established continuous cultures of cells in suspension using disposable 1-liter spinner flasks. The basic equipment consisted of a CO2 tissue culture incubator and a hermetically sealed magnetic stirrer capable of operating at 40-80 rpm. We used an inexpensive peristaltic pump and sterile silicon tubing to facilitate cell harvest and transfer of fresh medium. An automatic cell counter was used to monitor cell growth and viability. Handling of cultures were performed inside a biosafety cabinet using sterile techniques. Cell lines for Spinal Muscular Atrophy and six Lysosomal Storage Disorders were obtained from Coriell Institute, and a collaborator provided a transduced cell line containing an inserted TREC sequence used for Severe Combined Immune Deficiency screening. Cultures were initiated from cryopreserved cells at 2-5x105 cells/ml of medium and were eventually transferred to spinner flasks. Fresh medium was added every 2-3 days to maintain appropriate cell density with increasing volume. When the flask reached its maximum volume, the culture was allowed to grow to maximum cell density while sustaining a high viability. At that point, the majority of the culture was harvested and the cells were cryopreserved. Media was then replenished to the remaining cells and the culture continued, with a harvest at every 7-14 days, until enough cells had been obtained (usually 2-3x109 cells).

Results: All cell lines proliferated well in the spinner flask cultures. They reached maximal density of 1-3x106 cells/ml, often two to three times higher than previously attained with stationary cultures. When tested by NBS assays currently in use, DBS prepared with these culture cells emulated samples from newborns with respective disorders.

Conclusion: We have demonstrated a compact tissue culture system sufficient for medium to larger scale production of reference materials in NBS. The equipment cost and space required are modest, which should allow NBS labs to prepare their QC materials locally.

Presenter: Sophia Winchester, ORISE Fellow, Centers for Disease Control and Prevention, Atlanta, GA, Email: yhh2@cdc.gov
Achieving Timely Specimen Collection, Delivery, and Results Reporting Contributes to Timely Medical Intervention

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Background: Newborn screening (NBS) is a time sensitive process in which delays in reporting can lead to serious consequences for affected infants. NBS programs have been engaged in ongoing efforts to improve timely specimen collection, delivery, and results reporting to decrease the risk of these consequences. However, a knowledge gap exists on which NBS system factors have the greatest impact on timeliness, and whether achieving timely specimen collection, delivery, and results reporting contributes significantly to timely medical intervention. Objective: To retrospectively assess the association between on-time specimen collection and on-time specimen delivery with factors in the NBS system and the timely reporting of results, and retrospectively assess if infants with a confirmed diagnosis with a timely reported NBS result were more likely to receive timely medical intervention.

Methods: NewSTEPs conducted a retrospective analysis utilizing timeliness measures collected in the NewSTEPs data repository. Quality indicator and case data measuring time from birth to specimen collection, specimen collection to receipt at the laboratory, specimen receipt to reporting results, birth to reporting results, and birth to medical intervention, entered for the years 2012 through 2015, were analyzed. Specimens that were collected before 48 hours were categorized as on-time collection, and specimens delivered within 24 hours of collection were considered as on-time delivery.

Results: 4,281 infants with a confirmed diagnosis between 2012 and 2015 were reported to the NewSTEPs Repository. Specimens collected on time and received on time had earlier result reporting (Median=6 days, IQR=4 to 7, p < 0.001) than those only received on time (Median=6.5 days, IQR=5 to 9), those only collected on time (Median=7 days, IQR=6 to 9), and those neither collected nor received on time (Median=9, IQR=7 to 12). Infants with a confirmed diagnosis of a time critical disorder and screen results reported within five days of birth received medical intervention significantly earlier (Median=5 days, IQR=4 to 10, p < 0.001) compared to those with results reported after five days of birth (Median=8 days, IQR=6 to 14). In 2015, four of six (67%) NBS laboratories open seven days a week were more likely to report timely test results compared to one of 13 (7.7%) laboratories open six days a week and zero of 12 laboratories open five days a week (0%) (?2=14.3, p=0.002).

Conclusion: Retrospective data demonstrates that programs achieving on-time collection and on-time delivery will report results earlier. Further, programs achieving timely reporting of results contributes significantly to timely medical intervention for infants with a confirmed diagnosis. To continue to improve the system and NBS timeliness, ongoing CQI activities should focus on expanding laboratory operating hours and improving courier services.

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P-047 - Withdrawn
The Current Landscape of Newborn Screening for Lysosomal Storage Disorders: Critical Similarities and Differences between Platforms
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Interest in newborn screening (NBS) for lysosomal storage disorders (LSDs) has gradually gained momentum over the past decade, particularly since the recent additions of Pompe disease and Mucopolysaccharidosis Type I (MPS I) to the Recommended Uniform Screening Panel (RUSP). Two platforms are currently available for high throughput LSD screening from dried blood spot specimens – tandem mass spectrometry (MS/MS) and digital microfluidic fluorometry (DMF), which received FDA clearance for LSD testing early this year. Several NBS programs are poised to add LSD screening and are faced with the important task of deciding which of these two testing options to pursue, and are judiciously reviewing the cost and workflow of each platform. Both platforms offer specific reagent kits, use synthetic substrates, and have the ability to screen for multiple LSDs in a single run. The flexible nature of the digital microfluidic platform enables optimization of both pH and buffer composition because each reaction is run in an isolated droplet. Workflow and equipment complexity vary considerably between the two platforms; DMF being much simpler to set up and use than MS/MS. With regard to the analytical range of each platform, we caution that this metric has limited relevance to the ability of each method to discriminate normal from affected samples because it does not take into account the multiple sources of variability in the screening process (biological variability, DBS sample quality, gestational age at sampling, pseudodeficiency alleles, etc) that exists between NB samples. Indeed, rich output data sets from the prospective LSD screening programs are now available, and do not show significant differences between either platform in terms of the positive predictive value. We suggest that consultation with active and pilot LSD screening programs (IL, MO, NY, NC, MI and others) should be sought for unbiased feedback on their LSD screening experience. State public health laboratories are best advised to focus their attention on emerging clinical results from prospective screening programs and on the cost and workflow considerations of each platform and its suitability to their NBS laboratory needs.

Presenter: David Millington, PhD, Emeritus Professor, Duke University Medicine, Chapel Hill, NC. Phone: 919.448.8221, Email: dmilli@duke.edu

Spinal Muscular Atrophy: Screening in Georgia
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Spinal Muscular Atrophy (SMA) is a progressive motor neuron disease with progressive symmetrical muscular weakness, poor weight gain and ultimately early death from respiratory failure. SMA is common and lethal, with an estimated incidence of 1 in 10,000. Fortunately, an effective treatment, Spinraza (nusinersen), has been developed by Biogen and approved by FDA this past year. Studies have shown that this type of treatment, given intrathecally, must be given as early as possible, before significant loss of motor neurons. This therapeutic opportunity can only be realized with the development of newborn screening (NBS) for SMA to identify affected children soon after birth. To
provide more data on the feasibility and utility of NBS for SMA, we propose a multi-institution project in Georgia among Emory University, the Georgia Public Health laboratory and Northside Hospital, Georgia’s largest birthing hospital with approximately 18,000 deliveries a year. We will screen children born at Northside Hospital for a two-year period, and use our existing short term follow up program to retrieve screen positive children. Data will be published and shared with the Secretary’s Advisory committee. The goal of initiating pilot NBS programs for SMA is to obtain sufficient data that SMA becomes part of the Recommended Uniform Screening Panel (RUSP), and every newborn, in every state, gets screened for SMA. The CDC has developed an efficient and accurate PCR method to detect the absence of exon 7 of the SMN1 gene, the most common genetic mechanism. This method can be multiplexed with the PCR assay currently in use by many NBS labs to detect severe combined immune deficiency (SCID). The short term follow up program at Emory University will retrieve screen-positive children, coordinate diagnostic testing, and refer to pediatric neurology to ensure early and prompt treatment of infants with confirmed diagnosis of SMA.

**Presenter:** Angela Wittenauer, RN, BSN, Short-term Follow-Up Coordinator, Emory University, Atlanta, GA, Phone: 404.778.8489, Email: alwitte@emory.edu

**P-050**

**Pompe, MPSI and ALD Pilot Screening in Georgia**  
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Pompe Disease (PD) and Mucopolysaccharidosis Type I (MPSI) were added to the RUSP in 2015. Adrenoleukodystrophy (ALD) was added in 2016. In order to assist states in the implementation of screening for these conditions, the Eunice Kennedy Shriver National Institute for Child Health and Human Development (NICHD) has issued contracts with several states to fund pilot implementation studies. The goals of these contracts are to support the implementation and proof of concept of the technologies available to screen for these disorders in a newborn screening (NBS) lab setting. Emory University in partnership with the Georgia Department of Public Health, has been awarded a contract for each of these disorders. The contracts with NICHD include screening 50,000 newborns for each condition. For PD and MPSI we are using a two tiered system in which the initial enzyme activities are analyzed using a tandem mass spectrometry (MS/MS) assay. The results are evaluated using the CLIR tools from Mayo. If any sample flags as abnormal, a second spot is analyzed using a six-plex MS/MS enzyme assay, and again the results are evaluated with the CLIR tools. To date we have analyzed approximately 10,000 specimens, and there have been neither false nor true positive cases. The pilot studies are scheduled to be completed by June 30, and updated findings will be reported. The assay for ALD is currently being validated. It consists of quantitating the C26 lysophosphatidylcholines (LPC) by F1/MS/MS, and confirming any positive screens with a second tier LC/MS/MS assay. These data will also be evaluated using the CLIR tools. We will also report on the lessons learned from this project, including setting up work plans among multiple entities, resolving issues across several IT systems, and the success of the CLIR tools in reducing the number of false positive screens, and other findings as we progress through these projects.

**Presenter:** Angela Wittenauer, RN, BSN, Short-term Follow-Up Coordinator, Emory University, Atlanta, GA, Phone: 404.778.8489, Email: alwitte@emory.edu

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P-051

Tackling the Duarte Galactosemia Problem
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Duarte galactosemia (DG) is one of the most common metabolic disorders identified by newborn screening in the US; it results from partial deficiency of the enzyme galactose-1P uridylyltransferase (GALT). Newborns with DG display a compromised ability to metabolize galactose, a sugar abundant in milk, and manifest elevated galactose metabolites in blood and urine while drinking milk. Reports about long-term developmental outcomes in DG are mixed and healthcare providers disagree about whether intervention is warranted. Specifically, some healthcare providers advise dietary restriction of milk for their patients with DG, at least for the first year of life, while others do not. Those who recommend intervention argue that the galactose metabolites that otherwise accumulate might be deleterious. Those who do not recommend intervention argue that the documented benefits of breastfeeding outweigh any possible negative consequences of the accumulated metabolites. The ongoing disagreement leaves parents in a quandary and infants at potential risk. We are conducting a 3-year multi-state study to clarify: (1) whether children with DG, ages 6-12 years old, are at increased risk for developmental problems, and if so, (2) whether patient exposure to milk in early childhood shows an association with developmental outcomes. To conduct this study we are recruiting nearly 300 children, half with DG (cases) representing a spectrum of milk exposures, and half unaffected controls. To assess child developmental outcomes we are using validated tests of cognitive skills (especially memory, executive function, and auditory processing), communication (speech and language), physical development (including motor skills, coordination, and occurrence of tremors), and social-emotional development, all administered by trained professionals. We are collecting information about child diet and possible covariates using a parent/guardian response survey. We will analyze the final data set using stepwise linear regression with generalized estimating equations (GEE) to determine significant predictors of outcome while controlling for relevant covariates (e.g. child age, gender, SES, sib relationships). The results will provide a foundation of shared knowledge enabling healthcare providers and the families they serve to make evidence-based decisions about what to feed their baby with DG. Depending on the outcome of the study, there also may be implications for newborn screening.

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P-052

New Conditions, Same Issues: Surveying the Educational Landscape of New Conditions to the RUSP
N. Bonhomme and A. Mulford, Baby's First Test, Genetic Alliance, Washington, DC

Between March 2015 and February 2016, the Secretary of Health and Human Services added Pompe Disease, X-linked Adrenoleukodystrophy (X-ALD), and Mucopolysaccharoidosis type 1 (MPS I) to the Recommended Uniform Screening Panel (RUSP). As more states expand their newborn screening panels to accommodate these conditions, it is incumbent upon public health programs to meet healthcare provider and family needs around educational resources and support, beyond the technical assistance required by newborn screening labs. To support this endeavor, through a Health Resources and Services
Administration (HRSA) award to the Association of Public Health Laboratories (APHL), Genetic Alliance, through Baby’s First Test, is partnering with APHL to lead efforts in identifying opportunities around education for these new RUSP conditions. Through this project, Genetic Alliance aims to create an enhanced collection of educational resources that support both healthcare providers and families through all stages of newborn screening, diagnosis, follow-up, and beyond for these conditions. It is critical in this work to recognize families as experts on the conditions that affect their families. To capture families’ firsthand experiences and to learn what strategies in education work best for these conditions, Baby’s First Test recruited 4 advocates and one clinician to each of the three condition-specific work group. Each work group will identify key gaps in family and healthcare provider education. This will lead to the development of a framework in which existing materials will be aggregated and reviewed. For areas that lack appropriate materials there will be the opportunity to determine the best approach to fill those gaps. This poster will 1) provide an overview of the work groups’ progress, 2) what we have learned from their fruitful discussions, 3) similarities and differences in the family experiences both within the work groups and across them, 4) identify unifying themes that have emerged, and 5) note points of divergence and/or condition-specific complexities. The poster will also list next steps and tasks for the work groups and how those products will be disseminated to state programs and educators.

**Presenter:** Natasha Bonhomme, Baby's First Test, Genetic Alliance, Washington, DC, Email: nbonhomme@geneticalliance.org

**P-053**

**Detencion en Recien Nacidos: Creating an Outreach and Education Strategy for Engaging Spanish-speaking Families in Newborn Screening**

A. Mulford and N. Bonhomme, Baby's First Test, Genetic Alliance, Washington, DC

**Background & Objectives:** According to the US Census Bureau, Hispanics constitute the largest ethnic minority in the United States, forming 17.4% of the US population in 2014. This percentage is projected to increase to 28.6% by 2060. Consequently, more Hispanic families are entering the public health and healthcare systems each year, including the newborn screening system, and are in need of up-to-date, culturally competent, and linguistically accessible information. The demand by clinicians, state run programs, and other educators to have resources available for a diverse, multilingual audience, along with recognition of the growing numbers of Hispanics receiving public health services, gave impetus to a Spanish-language version of BabysFirstTest.org in October 2015. To build upon this foundation, we identified two key objectives: 1) to better understand the needs of Spanish-speaking families in interactions with their healthcare providers, and 2) to identify the barriers that these families encounter in connecting with newborn screening information.

**Methods:** To incorporate a breadth of perspectives and experiences, we held focus groups and a community leaders meeting. For the focus groups, we recruited participants from local clinics and community-based organizations in DC area serving Hispanic families to form two focus groups: one composed of families and one of health educators/translators. Discussions were subsequently coded for analysis. To build relationships for future partnerships, leaders of national and local Hispanic and Latino-serving organizations were invited to join us in dialogue about defining family engagement and strategies that have proven successful for these communities. This meeting also offered an opportunity to educate about newborn screening systems.

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**Conclusions & Implications:** The presentation will cover how we translated findings and common themes from the focus groups, the community leaders meeting, and participation in other Latino/a health focused discussions into actionable solutions to better serve the Hispanic community. We will discuss how we began implementing an outreach roadmap for Spanish.BabysFirstTest.org, program development, and will inform a broader dialogue on serving this growing portion of newborn screening recipients and their families.

**Presenter:** Amelia Mulford, Program Assistant, Baby's First Test, Genetic Alliance, Washington, DC, Email: amulford@geneticalliance.org

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**P-054**

**Evaluating the Newborn Screening Clearinghouse: Understanding the User Experience to Inform Education and Engagement Activities**

J. Seisman and N. Bonhomme, Baby's First Test, Genetic Alliance, Washington, DC

In September 2011, the Newborn Screening Clearinghouse was fully launched as Baby's First Test (www.BabysFirstTest.org). Baby's First Test provides healthcare professionals and new and expecting parent’s access to reliable information and resources on newborn screening policies and procedures. As of July 31, 2017, the website has reached more than 2 million unique users and has been accessed more than 4 million times (page views). To evaluate the Clearinghouse’s impact, an online survey was created to inform the Clearinghouse’s understanding of the types of users visiting the site, the reason for users visiting the site, and to evaluate whether BabysFirstTest.org was meeting the expectations and needs of its users. More specifically, the survey was designed to gain further insight into the experiences of different types of users (parents, health professionals, etc.). Prior to launching this survey, Genetic Alliance conducted cognitive testing on the proposed users of the site. The goal of cognitive testing was to gather understanding of and preferences for terminology related to newborn screening among target audiences and to test for clarity of language in specific questions included in the survey instrument. The survey launched in October 2015 on BabysFirstTest.org and ended in February 2016 with a total of 713 completes. Data from the survey indicated that of those visiting BabysFirstTest.org: 46% identified as family members of the newborn; 47% identified as health care professionals; and 7% identified as an advocate. Analysis of the data identified areas of BabysFirstTest.org where users would like to see improvement as well as highlighted site features and educational tools that users find valuable and informative. 91% of users reported that they planned to return to BabysFirstTest.org. In fact, nearly half of the parents (42%) reported that they were likely to return in the next day or two. Results also showed that 65% of users learned something new from BabyFirstTest.org that they didn’t know before about newborn screening or state testing policies. In this poster presentation, the author will provide in-depth analysis of the user survey data and discuss its implications for the Clearinghouse and its education and engagement activities.

**Presenter:** Jackie Seisman, MPH, Assistant Director, Baby’s First Test, Genetic Alliance, Washington, DC, Phone: 202.966.5557 x216, Email: jseisman@geneticalliance.org
Four Years’ Experience with Follow-up Testing for Abnormal Newborn Screens for Mucopolysaccharidosis Type I Reveals High Prevalence of Alpha-iduronidase Pseudo-deficiency
L. Pollard, C. Shouse, J. Haley, J. Hallman, D. Carra and T. Wood, Greenwood Genetic Center, Greenwood, SC

The Greenwood Genetic Center Diagnostic Laboratory has provided follow-up testing for Mucopolysaccharidosis type I (MPS I) abnormal newborn screens for more than four years from states using both the fluorometric and MS/MS methods. We have tested 162 infants (age 5 days – 8 months) by measuring alpha-iduronidase activity in leukocytes. Of these, 44 (27%) had normal enzyme activity (> 6 nmol/hr/mg protein), 15 (9%) had enzyme activity in our affected range (< 1 nmol/hr/mg protein) and the majority (103; 64%) had enzyme activity between our normal and affected ranges (1 – 6 nmol/hr/mg protein). Of the 118 infants with below normal alpha-iduronidase activity, 83 had IDUA gene sequencing performed in our laboratory. Several recurring sequence alterations were identified that we had not previously observed in clinically affected MPS I patients. Of the 166 total alleles, the following frequencies of these changes were observed: p.A79T (44.6%), p.D223N (15.7%), p.H82Q (11.5%), p.V322E (8.43%) and p.G409R (1.81%). Each of these alterations is reported in the public SNP databases with allele frequencies in ethnic-specific populations that are considered too high to be disease-causing. However, given the frequency with which these alterations are observed in infants who screen positive for MPS I, they are likely contributing to the reduced alpha-iduronidase activity. Known pathogenic variants and novel variants of unknown significance (VOUS) each accounted for 8.43% of the total 166 alleles. Only one infant with alpha-iduronidase activity in the affected range had a genotype consistent with MPS I (homozygous p.W402X). Two others with activity in the affected range were either homozygous or compound heterozygous for novel variants of unknown significance and will require further follow-up. Urine glycosaminoglycan (GAG) analysis was also performed for 63 of the 118 infants with reduced leukocyte alpha-iduronidase activity. The majority of these analyses included the highly sensitive quantification of individual component GAGs by UPLC-MS/MS. Only the infant homozygous for p.W402X had elevated heparan sulfate and dermatan sulfate, consistent with MPS I. Normal urine GAG analysis in all other infants with reduced alpha-iduronidase activity supports the idea that the recurrent p.A79T, p.D223N, p.H82Q, p.V322E and p.G409R alterations are not pathogenic, as three other known MPS I patients tested in our laboratory also had significant elevations of heparan sulfate and dermatan sulfate by 2.5 months of age. Therefore, we have classified these IDUA alterations as pseudo-deficiency alleles. Our experience with follow-up testing for MPS I demonstrates that the combination of enzyme, molecular and urine analysis is often required to provide an accurate clinical diagnosis in the era of newborn screening and that pseudo-deficiency for alpha-iduronidase is much more common than historically believed.

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**P-056**

**High-throughput Multiplex Newborn Screening Assay for Seven Lysosomal Storage Disorders (LSDs) using Dried Blood Spots and UPLC-Tandem Mass Spectrometry**

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During June 2015, the Illinois Department of Public Health (IDPH) Newborn Screening (NBS) Laboratory initiated testing for five Lysosomal Storage Disorders (LSDs): Pompe, Fabry, Gaucher, Mucopolysaccharidosis I (MPS-I), and Niemann-Pick A/B. Recently, to fulfill state-mandated screening for these disorders and two other LSDs, the IDPH NBS Laboratory has validated a modified multiplex method (six plus one) utilizing ultra-high pressure liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS). This method involves simultaneous incubation of a single 3.2 mm dried blood spot (DBS) punch for enzymes associated with the five disorders listed above, respectively, (a-glucosidase [GAA], a-galactosidase [GLA], acid ß-glucocerebrosidase [ABG], a-L-iduronidase [IDUA], and acid sphingomyelinase [ASM]), plus for the enzyme deficient in Krabbe disease, galactocerebrosidase (GALC). In addition, a separate 3.2 mm punch was incubated under different conditions to determine the activity of iduronate-2-sulfatase (I2S), the enzyme deficient in Hunter Syndrome (Mucopolysaccharidosis II [MPS-II]). Incubations for the 6-plex and 1-plex assays involved separate cocktails designed to optimize targeted enzyme activities and minimize non-specific activities. The reactions also contained appropriate internal standards and synthetic substrates (PerkinElmer) and were carried out for 17 hours at 370 C in 96-well plates. The reactions were quenched with acetonitrile, and after a brief pre-analytical manipulation using a liquid handler and without ethyl acetate extraction, aliquots from both plates were combined and injected together into the UPLC/MS/MS for analysis. This new 6+1 plex method was tested using 2,384 DBS from presumed healthy newborn babies, with samples having confirmed low enzyme activities, and with quality control DBS obtained from the Centers of Disease Control and Prevention (CDC) and PerkinElmer. The data showed good separation of substrates and internal standards/products for all enzymes, linearity for low, medium and high quality control DBS, and excellent signal-to-noise ratios. There were no differences in observed enzyme activities when aliquots were analyzed with or without pooling for injection. Confirmed MPS-II cases (n=2) presented enzyme activities below 2% of the median of presumed healthy neonate activity. Good precision and reproducibility were detected for all seven enzymes in quality control DBS. In summary, this assay method can be used for high throughput newborn screening for these seven LSDs.

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**P-057**

**The Effects of Specimen Collection Time and Birth Weight on the Newborn Screening Activities of Enzymes Associated with Lysosomal Storage Disorders**

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Recent guidelines of the Clinical and Laboratory Institute recommend collecting specimens from sick newborns before blood transfusion. This guidance may result in less than ideal or poor quality specimens for many newborn screening tests due to collecting specimens before 24 hours of age, and this situation may affect proper evaluation of disease status in these infants. The objective of this study was to evaluate the effects of specimen collection time and birth weight on the newborn screening activities of enzymes associated with lysosomal storage disorders.
was to evaluate variations in the activities of several lysosomal storage disorder (LSD)-associated enzymes during newborn screening with respect to variations in sample collection time in different birth weight groups.

**Methods:** The Illinois state newborn screening program database was analyzed to determine whether different sample collection times and birth weight groups correlated with enzyme activity levels. The lysosomal enzyme activity levels associated with five disorders – Pompe (α-glucosidase [GAA]), Fabry (α-galactosidase [GLA]), Gaucher (β-glucocerebrosidase [ABG]), Mucopolysaccaridosis Type I (MPS I) (α-L-iduronidase [IDUA]), and Niemann-Pick A/B (acid sphingomyelinase [ASM]) – were analyzed. Data were stratified into sample collection time categories <12 hours, 12-24 hours, 24-48 hours, 48-72 hours, 72-96 hours, 96-120 hours, 120-144 hours, 144-168 hours, 168-192 hours and >192 hours with the following birth weight categories: low birth weight (LBW) (≤2000 g) and normal birth weight (>2000 g). In addition, to examine the effect of birth weight on LSD test results, GLA activities from pre-term and full-term babies were compared with respect to sample collection time and how the activities changed from neonatal to postnatal age (from 12 hours to 4 weeks after birth).

**Results:** Data from 228,067 neonates were reviewed. Infants with early sample collection times (≤12 hours) comprised 4.54% of the study cohort; early sample collection with LBW comprised 1.57%. Specimens collected at ≤12 hours had higher GLA activity levels than full-term babies, and these values were significantly elevated in LBW babies. When specimens were collected at 5 days after birth, for the LBW group, a significantly lower GLA activity was detected. ABG, ASM, and GAA results were 10-20% lower when the samples were collected <12 hours after birth and in LBW infants. Higher ASM activity was detected with increasing sample collection time (>4-5 days after birth). IDUA results were not affected by sample collection time and birth weight.

**Conclusions:** These data suggest that appropriate caution must be exercised during the interpretation of LSD screening assays. Samples with collection times less than 24 hours, especially less than 12 hours, and LBW must have a disclaimer as a text comment on reports provided to submitters and need a follow-up sample to generate valid LSD results. An alternative would be to use different cut-offs for different age and weight groups.

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**P-058**

**Building the Building Blocks: The Process for Authorship, Review and Approval of the Newborn Screening Health IT Resource Guide and Toolkit**

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**Background:** Collective knowledge is better than what a single person can offer. But it is challenging to distill the collective knowledge of a community of peers down to a single, cohesive document. Recently, NewSTEPs 360 partnered with the Virginia Division of Consolidated Laboratory Services (DCLS) and J Michael Consulting (JMC) to bring together NBS programs and several national partners to author a Newborn Screening Health IT Resource Guide and Toolkit known as “Building Blocks”. The Guide, known as “Building Blocks”, offers the NBS community practical instructions and best practices for implementing a NBS electronic data exchange. While assembling this guide, we had the opportunity to work with many thought leaders in the NBS HIT field, but we then faced the challenge of coordinating input from multiple participants. We therefore devised a review process to ensure that 1) all
participants had ample opportunity to contribute and review the document in a short time; 2) we considered and incorporated feedback from all reviewers in an efficient manner.

**Objective:** Share the methods used to organize the authorship of a broad scoped document with multiple contributing participants. A short time frame required careful planning and distribution of labor to produce the guide on schedule.

**Methods:** The JMC authorship team created clear roles and expectations for the stakeholders at the contribution and review stages. We defined specific roles, including participants, editors, authors and approvers, and clearly communicated the responsibilities of each role. A rolling review schedule allowed for all parties to stay engaged throughout the project with transparent deadlines. Clear communication and the use of visual workflows helped team members stay on track. The use of shared folders and templates for capturing comments provided the team with a traceable method for collecting and incorporating feedback.

**Results:** Using these methods, the JMC team managed the production of a guide with the input of many participants in a short time frame. The contribution of these community thought leaders was instrumental in producing a user friendly, helpful document for other states that may be considering implementation of electronic messaging.

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**P-059**

**Play with Building Blocks: The Newborn Screening Health IT Implementation Guide and Toolkit**

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**Background:** Health information technology (HIT) continues to play a crucial role in improving newborn screening (NBS) processes. A key component of HIT is utilizing HL7 to send laboratory orders and test results between providers and the NBS laboratory. Several NBS programs have implemented, or are in the process of implementing, HL7 messaging using program-specific methodologies with varying levels of success. This disparate nature of implementation has produced inconsistent results and served as the impetus for the development of a NBS HIT resource guide and toolkit to provide guidance for programs implementing electronic messaging.

**Objective:** To introduce the first version of the NBS HIT Resource Guide and Toolkit to the NBS community, educate them on the value of the guide, and facilitate an interactive discussion of it. Next steps for expanding the guide to other areas of HIT beyond electronic messaging will also be discussed.

**Methods:** The NewSTEPs 360 project, funded by the Health Resources and Services Administration (HRSA), works with NBS programs to improve timeliness of NBS from birth to results reporting. This includes activities to implement HIT solutions including electronic messaging. NewSTEPs 360 partnered with the Virginia Division of Consolidated Laboratory Services (DCLS) and J Michael Consulting (JMC) to bring together NBS programs, at various stages of electronic messaging implementation, and several national partners to author a NBS HIT Resource Guide and Toolkit.

**Results:** Representatives met for an in-person meeting in February 2017 which resulted in a detailed outline of NBS electronic messaging process based on their diverse experiences. This outline will be developed into a resource guide planned for release by the end of August 2017. This first version will lead a NBS program through the steps needed to plan, implement, and maintain an electronic
messaging project including details on such activities as establishing partnerships, workflow mapping, and message validation among others. The modular nature of the guide will allow the reader to utilize the sections that are important for their project status while setting aside the sections that are not relevant. The guide will also provide descriptions of the tasks needed to meet milestones, tools for accomplishing those tasks, and case studies from programs that have completed milestones highlighting lessons learned.

Conclusion: This roundtable will introduce participants to the NBS HIT Resource Guide and Toolkit, walk them through the layout and explain its utility. Participants will be provided with the guide and be encouraged to ask questions and comment on it. Since this guide will be a living document that will grow to include other areas of NBS HIT, this roundtable is imperative to give a voice to the programs that will be utilizing this guide to help sculpt HIT content beyond electronic messaging.

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P-060

Efficient and Effective Newborn Screening for Early Infantile Krabbe Disease

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Newborn screening (NBS) for Krabbe disease (KD) has been conducted primarily by measuring galactocerebrosidase (GALC) activity and employing molecular genetic analysis of the GALC gene as a 2nd tier test. Based on NBS data from New York the incidence of KD is unexpectedly low (1:500,000 in NY) while many individuals (ca. 1:5,600) with reduced GALC activity and genotypes of uncertain significance are detected and subjected to follow up testing1,2. We and others have shown previously that Psychosine (PSY) is a marker of active KD and can be measured in dried blood spots (DBS)3. Therefore we applied it as a 2nd tier test to NBS for KD along with post-analytical interpretive tools created using Collaborative Laboratory Integrated Reports (CLIR; https://clir.mayo.edu) and molecular genetic analysis of the GALC gene. Among more than 60,000 newborns screened, we identified one case with reduced GALC activity (0.18 nmol/mL/hr; 1st percentile of controls: 1.36), a high CLIR score for Krabbe disease (788; informative >30), elevated PSY (61 nM; controls <10), and a genotype including a heterozygous pathogenic deletion, a heterozygous likely pathogenic frameshift mutation, as well as a heterozygous and a homozygous pseudodeficiency allele. The NBS specimen was received in the laboratory on the 4th day of life (a Saturday), the report was released on the 6th day of life, the patient was admitted to the Pediatric Blood and Marrow Transplant Program at Duke University Medical Center on the 7th day of life, started preparative chemotherapy on the 13th day of life, received an unrelated donor cord blood transplant on the 23rd day of life, and was discharged to home on the 104th day of life. At nearly 5 months old the patient is developing appropriately for age and recovering from the transplant procedure. Based on our experience to date, we postulate that NBS for early infantile KD is possible without false positive results and without the need for molecular genetic testing in the NBS laboratory. The biochemical genetic screening approach enables rapid identification of early infantile KD which is of utmost importance to ensure best possible outcomes. As our case illustrates, 7-day-operation of the NBS laboratory and a process for rapid referral to a transplant center are also required to achieve best outcomes. Whether a biochemical-genetics-only approach is sufficient to detect all cases...
with later onset variants of KD remains to be determined\(^3\). Meanwhile relevant stakeholders must continue an open and honest discussion about the achievable goals of NBS for KD\(^2\).


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**P-061**

**Newborn Screening (NBS) for Metachromatic Leukodystrophy (MLD): Results from a Study of 100,000 Deidentified NBS Samples**

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MLD, caused by arylsulfatase A (ARSA) deficiency, is a progressive demyelininating disorder with variable phenotype. Hematopoietic stem cell transplantation has shown promise and gene therapy is being investigated. Any treatment is expected to have greatest benefit when started before the onset of symptoms, making NBS for MLD desirable. Dried blood spot (DBS) based assays have been developed to measure either sulfatides that accumulate in MLD (Spacil Z et al. Clin Chem 2016; 62: 279-86) or the presence of the arylsulfatase A protein (Meikle PJ et al. Mol Genet Metab 2006; 88: 307-142). We pursued the latter approach by immunoassay in a screening study of nearly 100,000 deidentified NBS samples for MLD and 12 other lysosomal storage disorders, Wilson disease, and Friedreich ataxia by multiplex immunocapture assay. Of 95,163 NBS samples tested 73 samples had ARSA concentrations below a preliminary cut off corresponding to the 1st percentile of the study population. ARSA gene analysis of these cases found 51 individuals carrying mutations associated with ARSA pseudodeficiency, 20 carriers of a known mutation or a variant of uncertain significance, and homozygosity for a possibly pathogenic mutation (c.511G>A, p.D171N) in two cases. Measurement of sulfatides in the leftover DBS of one of the latter cases was normal indicating that c.511G>A is likely not a pathogenic mutation.

Conclusions: The finding of no apparent MLD case in the study population may reflect the rarity of the disease in this population. Assuming 100% sensitivity of the high-throughput immunoassay, the screening performance could be optimized by (a) post-analytical use of a multivariate pattern recognition software (CLIR; available at https://clir.mayo.edu) as has been done previously for other NBS tests (Hall PL et al. Genet Med 2014; 16: 889-93), and (b) application of the LC-MS/MS based sulfatide assay as a 2nd tier NBS test.

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Insurance Coverage for Metabolic Formula through Michigan Medicaid
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Background: All Michigan newborns who screen positive for an inborn error of metabolism (IEM) are referred to the Children’s Hospital of Michigan Metabolic Clinic (CHMMC) for confirmatory testing and treatment. Individuals with a confirmed IEM diagnosis requiring metabolic formula are able to access services and treatment through the CHMMC which receives grant funding from the Michigan Department of Health and Human Services, Newborn Screening (NBS) Program. While Michigan Medicaid has historically covered metabolic formula, the benefit was not being utilized to the extent possible due to some barriers. Through collaboration across Medicaid, Children’s Special Health Care Services (CSHCS) and the NBS Program, these barriers were addressed and in April 2015 Michigan Medicaid changed coverage criteria to improve access to metabolic formula. These changes included improving reimbursement rates for metabolic formula and updating the qualifying information necessary for prior authorization. The NBS Program discontinued routine coverage of metabolic formula through the grant to CHMMC for Medicaid beneficiaries. This change was the first step in promoting use of third party sources of payment for metabolic formula in order to limit the financial burden of “Diet for Life” on the NBS Program.

Methods: Conversations over a two-year period with the Medicaid and CSHCS programs identified issues related to third party insurance coverage for metabolic formula. Barriers such as third party insurance providers excluding formula coverage based on tube feeding requirements, percentage weight loss, arbitrary lab results unrelated to IEM, and poor reimbursement rates were addressed. To address these barriers, patients with IEM requiring either B4157 or B4162 formulas are removed from their Medicaid health plan and put onto straight fee-for-service Medicaid. Straight Medicaid allows for manual pricing which improves reimbursement rates to durable medical equipment (DME) suppliers that service patients for these products.

Results: Since April 2015, 125 patients have been removed from Medicaid Health Plans and put onto straight Medicaid for coverage of metabolic formula. Due to the increased utilization of Medicaid and CSHCS insurance, the NBS Program decreased spending on formula and supplements by $303,165 from fiscal year 2015 to 2016. This process has also improved communication and the coordination of coverage between Medicaid, CSHCS and the NBS Program for this patient population.

Conclusions: After improved coverage for metabolic formulas was put in place for Medicaid and CSHCS beneficiaries, the NBS program has seen a reduction in the amount of grant funding required to assure lifelong access to metabolic formula. Since then, we have continued to investigate coverage by private third party insurers for additional segments of the patient population.

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P-063

Linking Birth Defects and Newborn Screening Records for Critical Congenital Heart Disease: Michigan’s Findings
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Introduction: Michigan’s Newborn Screening (NBS) Program mandated screening for critical congenital heart disease (CCHD) in April 2014. Hospitals report individual-level pulse oximetry screening data on all infants or a reason why the screen was not completed to the NBS Program. Michigan has a passive birth defects registry, which monitors the occurrence of birth defects reported up to age two. The aim of this project was to evaluate the effectiveness of pulse oximetry screening and hospital reporting compliance by linking CCHD cases reported to the Michigan Birth Defects Registry (MBDR) to NBS CCHD records.

Methods: All infants born after April 2014 and reported to the MBDR with a CCHD diagnosis through March 2017 were included in the study. Link Plus, a probabilistic linkage program, was used to link MBDR records to electronic birth certificate (EBC) records. Using a master file of linked EBC/NBS records, the EBC records were then linked to NBS records.

Results: After deduplication, the MBDR file contained 978 infants with a CCHD diagnosis code. After linkage, 827 unique infants were linked to a Michigan birth certificate and 815 to a bloodspot NBS record. Of the 815 infants linked to a NBS record, 221 infants (27.1%) had pulse oximetry screening values reported. The majority of infants (n=199) with a reported screen passed their pulse oximetry screen. Ten infants needed a rescreen after their first screen; 12 infants failed their first screen. Among the 594 infants with no screen reported, an explanation was provided for 289; 161 had a prenatal diagnosis of CCHD, 99 had an echocardiogram, 14 were transferred before a screen was completed, 11 were reported to have a postnatal diagnosis of CCHD, 3 died, and one was in distress. Of concern, the remaining 305 infants did not have valid explanations for why the screen was not conducted or documentation that the screen was completed. Of those 305 infants, 287 had no information reported, 17 were reported as missed or not completed, and one provided unclear information.

Conclusion: Our findings indicate that the majority of infants diagnosed with CCHD per the MBDR did not have pulse oximetry screening values reported to the Michigan NBS Program. For infants with a reported pulse oximetry screen, 90% passed their first screen. The NBS Program needs to continue working with hospitals to ensure all pulse oximetry screens are reported since nearly 40% of the infants with a bloodspot NBS did not have pulse oximetry values reported or a reason why the screen wasn’t completed. Additionally, future work of interest includes chart reviews to confirm the accuracy of CCHD diagnoses reported to the MBDR and stratification of the findings by CCHD diagnosis.

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P-064

CFTR Analysis: Inconclusive R75X (c.223C>T) in the Presence of Homozygous R75Q (c.224G>A)
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Background: The Michigan (MI) Newborn Screening (NBS) laboratory began using the Luminex xTAG® Cystic Fibrosis (CFTR) 60 kit following the Hologic 40+4 recall. When the Luminex method was

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introduced, the mutation panel increased from 40 to 60 CFTR mutations. One of the newly introduced mutations was R75X (c.223C>T, p.Arg75X). Within the first 5 months of using the Luminex kit, 5 of the 2108 specimens tested yielded a ‘No Call’ result for both the wild type (WT) and mutant (Mu D) R75X loci. All other mutations were Normal. The R75X ‘No Calls’ created issues for patient follow-up, and a need to determine the root-cause of the Inconclusive result.

**Methodology:** To ensure the infants were not at risk for CF, all 5 cases were referred for a sweat test. A repeat dried blood spot (DBS) was also requested. To determine if the R75X ‘No Call’ was due to technical aspects of the MI NBS method, DBS’s from the first 3 Inconclusive cases were sent to the Texas NBS lab for testing using the same Luminex kit. In addition, DBS’s from the first 3 cases were sent to the CDC for Sanger sequencing.

**Results:** The sweat tests for all 5 infants were within normal limits. The R75X ‘No Call’ result was reproduced in each of the 5 repeat DBS’s tested by the MI NBS Lab. Review of the Luminex raw data for each of the 5 cases showed that both the R75X wild type and the R75X mutant probes exhibited little activity, with median fluorescence intensity (MFI) values slightly above background. The 3 DBS’s tested in the Texas NBS lab confirmed the MI NBS result of R75X ‘No Call’. Sanger sequencing performed at the CDC revealed that the first 3 cases were homozygous R75Q (c.224G>A, p.Arg75Gln) and wild type R75X. Sequencing performed at the Wisconsin (WI) NBS Lab revealed that all 5 cases were homozygous R75Q.

**Conclusion:** R75Q is located in the R75X primer region of the allele specific primer extension (ASPE) step of the Luminex assay. This underlying polymorphism in the primer-binding region prevents the R75X primers from binding to their target sequence, resulting in a ‘No Call’ for both the wild type (WT) and mutant (Mu D) loci. Review of demographic information (such as birth hospital, home town, and ethnicity) did not reveal commonality between the 5 cases, except that all R75X Inconclusive cases were female. The MI NBS Lab will no longer request a repeat DBS for future specimens with ‘No Call’ results. The specimens will be sent to the WI NBS Laboratory for targeted sequencing of the CFTR gene, and these sequencing results will be reported. The CFTR2 database states that ‘this combination (R75Q + R75Q) DOES NOT cause CF’, thus infants with an inconclusive result for R75X who are determined to be homozygous for R75Q will not be referred for a sweat test.

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**P-065**

**An in Depth Look at the Data Mapping Process for HL7 Implementation: Michigan’s Experience**

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A key component of health information technology (HIT) in newborn screening (NBS) is the implementation of electronic messaging using the Health Level 7 (HL7) standard to send laboratory orders and test results between electronic health records (EHR) and laboratory information management systems (LIMS). HL7 serves as the syntactic data standard in the electronic messaging process, however, a semantic data standard, such as Logical Observation Identifiers Names and Codes (LOINC), must also be utilized in order to meaningfully exchange patient information and laboratory outcomes. Data mapping is the process of matching data points across multiple sources that use different nomenclature, and is a potential barrier that could postpone proposed timelines or halt the implementation process all together if NBS programs are not prepared. The Michigan Department of Health and Human Services (MDHHS) NBS program is working to implement HL7 messaging to improve...
NBS turnaround time (TAT) utilizing funding and technical assistance from the NewSTEPs 360 project, funded by the Health Resources and Services Administration (HRSA). As a part of this implementation, mapping data points for the 53 disorders screened in Michigan (MI) was necessary. Data points were mapped from the HL7 build to an associated LOINC including data for demographics, analytes, disorders, disorder groups, results, and interpretation. A worksheet was created to allow for configuration and review of approximately 1,500 result codes according to the LOINC 54089-8, NBS panel American Health Information Community (AHIC). Upon completion of data mapping it was possible to create the HL7 result messages. Due to the sheer number of result codes, a pre-test phase was initiated to include test messages for a subset of the result codes to test configuration. Following configuration testing, user acceptance testing (UAT) required the creation of several hundred results messages in which all result codes would be tested. The completion of the data mapping process and UAT resulted in usable messages that can be used for the piloting process with University of Michigan hospital. Hospitals currently receive critical results by phone and negative results by fax or hard copy mail in MI. With the use of HL7 messaging, hospitals will have the opportunity to reduce the amount of time required for results to be applied to a newborn’s EHR, there will be a reduction in staff time necessary for processing hard copy reports, and there will be less transcription errors that could lead to delays in reporting. Additionally, an unexpected extensive amount of time and effort was required to complete the data mapping and UAT processes. It is recommended that states wishing to implement HL7 messaging seek out lessons learned from states that have already experienced associated challenges, and plan for the time and resources necessary to complete these processes.

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P-066

Enabling Distributed NBS Laboratory Automation through Web API Interfaces
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Traditional instrument control software was based on installed executable programs and associated files stored on a fixed storage media within a dedicated workstation. Typically this meant that interworking and interconnection of equipment from multiple vendors was met with challenges of compatibility and often required some form of protocol conversion or separate data manipulation process. Advances in computer networking, cloud-based software services and distributed computing allows a new regime. A new software model, based on web application programming interface (API) functionality is proposed and demonstrated for application in a newborn screening laboratory. The software architecture opens up choices for a wider range of vendor equipment with a flexible and modular approach, and provides numerous benefits including better management of software updates and system expansion. Improved efficiencies also result because vendors need only support a library interface rather than complete software functionality. A system is demonstrated using existing laboratory punchers whereby the distributed software system allows independent configuration and operation of punching from separate workstations and provides a common punching platform for all punchers in the laboratory. The demonstrated system is based on storing the configuration and assay parameters for all punchers on a server. This provides centralized management of all punchers and assay requirements. The punchers are accessed and driven by using stand network LAN TCP/IP. Lab users who are performing the punching tasks use menus on the server to select the puncher they are using and the assay they are
punching. This configuration includes the Assay based punching maps which define the position and code of QC, QA and Calibrators. Any remaining wells default to patient samples. The Punching module then uses the server settings for that Puncher and Assay to correctly drive the puncher and punching. Users select the sample type being punch (Patient, QC, QA or Calibrator) and the Punch module automatically positions the plate to the correct well. The settings also define the number of dots to be punched based on ample Type, Test and Specimen type and the size of punch to be used. Using web based communications removes the need for PC side software installation and operating in a discrete individual punch process. It also has the advantage of storing the Plate details and information in the database as opposed to a series of text based files. Being server and web based provides a variety of deployment options including in-house servers or cloud based servers such as amazon Web Services (AWS).

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P-067

Public Health Newborn Screening’s Enhanced Role in Addressing Health Disparities Found in Sickle Cell Disease and Trait
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Objective: Report on a three-year quality improvement initiative developed as a sustainable and effective parental trait notification process. In addition, challenge the newborn screening program community to consider our enhanced obligation and commitment to meeting the specific needs of the sickle cell disease community.

Background: Wide variation exists in communicating trait results found through newborn screening. Sickle cell disease (SCD) still represents the most underserved and under-represented community and their needs have largely been ignored by public health programs across the country. Adult specialists for SCD are rare, which causes significant transition problems. Studies have demonstrated significant issues, including pain management in emergency departments and risk for maternal death during pregnancy.

Methods: In 2015, Minnesota’s Newborn Screening Program implemented a pilot phase for hemoglobin trait notification (S trait only). In 2016, the process expanded to include all hemoglobinopathy traits. We continue to measure the four defined outcomes for a complete notification: 1) infant is seen in clinic, 2) newborn screening report is provided to the clinic directly, 3) a discussion about the result occurs between the provider and the parents, and 4) the recommended follow-up testing is completed. In addition to measuring these outcomes, initiatives to address further needs for the sickle cell community were observed and identified. Minnesota’s program is currently engaged in an initiative to address issues of care in emergency departments for patients with SCD with the goal of obtaining partners, identifying patient specific issues, and developing approaches for reducing identified disparities. MDH is also working closely with the only community-based organization for SC patients, families, and communities in MN to help establish them as a resource.

Conclusions: Development of a trait notification process has been a first step for our public health program to begin to understand the SCD community and their unmet needs. Unique and significant issues exist. As newborn screening is the entrance for these patients into the public health arena, we have specific obligations to enhance the public health’s understanding of the disparities and address identified problems. Given the unique connection newborn screening programs have with hospitals,
primary care providers, newborns, and specialists, we are positioned to be the catalyst for the important work of bringing together public health and the community. However, many programs do not see their role as community engager. A change in this perspective could influence considerable improvements in the health, health equity, and quality of life of the SCD community.

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**P-068**

**The Chutes and Ladders™ of Pompe and MPS I Implementation**  
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**Objective:** Validation of a multiplex dried blood spot (DBS) enzyme activity assay for high throughput newborn screening for MPS-I and Pompe in MN.

**Background:** MPS-I and Pompe were added to the MN screening panel in April of 2016. Screening went live Aug 1, 2017

**Method:** The multiplex DBS FIA-MS/MS assay looking at alpha-L-iduronidase (IDUA) and acid alpha-glucosidase (GAA) developed by the U of WA and PerkinElmer was adopted, using a Zephyr liquid handler and Waters TQD MS/MS system. We changed the punch and assay plates to a Corning Polypropylene to reduce static during the punching step. The 3.2mm sample punches are extracted for 18 hours with 2 internal standards and 2 substrates in cocktail buffer available from PerkinElmer Custom Contract Manufacturing. Using the Zephyr, the extract is quenched and transferred to a deep well plate before performing a liquid-liquid extraction to remove proteins, salts, and uncleaved substrates. Not including the incubation, one to three plates can be processed simultaneously in < 90 minutes, prior to FIA-MS/MS analysis. Reference materials from the CDC Newborn Screening Quality Assurance Program; and blinded samples from the NY, OH, and MO programs were used for assay validation.

**Results:** Sensitivity, Specificity, Precision, Accuracy, Reference Range and Reportable Range were validated. The primary TQD for MPS-I and Pompe screening failed during installation. Numerous interventions were required to identify and repair intermittent poor performance. Additionally, during the validation of a back-up TQD, the instrument comparison uncovered a reproducibility issue with the primary TQD. Ultimately, the primary TQD was changed to be the backup until it can be replaced. The assay was implemented with conservative IDUA and GAA cut-offs using a Multiple of the Median (MoM), along with 2nd tier and 3rd tier methods. Routine screening and outcome data will be analyzed on an ongoing basis in order to optimize the cut-offs. MN Updated testing performance metrics will be presented at the symposium.

**Conclusion:** MN has implemented high throughput screening for MPS-I and Pompe using the multiplex FIA-MS/MS Assay for IDUA and GAA enzymes.

**Presenter:** Amy Hietala, MS, Laboratory Supervisor, Newborn Screening Laboratory, Minnesota Department of Health, St. Paul, MN, Phone: 651.201.5455, Email: amy.hietala@state.mn.us
High Throughput Newborn Screening for X-Linked Adrenoleukodystrophy using Negative Mode Liquid Chromatography Tandem Mass Spectrometry

H. Winslow, A. Hietala, A. Wruck, J. Simonetti and M. McCann, Minnesota Department of Health, St. Paul, MN

**Objective:** Validation and implementation of a negative mode LC-MS/MS dried blood spot (DBS) lysophosphatidylcholine (LPC) assay for high throughput newborn screening for x-ALD in MN.

**Background:** X-ALD was added to the MN Newborn Screening (NBS) panel in April of 2016. Routine screening began on 2/6/17.

Method: The DBS LC-MS/MS C24:0-LPC and C26:0-LPC assay developed at the CDC was adopted, using a Zephyr liquid handler and Waters TQD MS/MS system. The 3.2mm DBS punches are extracted for 30 min. in an internal standard containing d4-C26:0-LPC. Using the Zephyr, the extract is transferred to another plate to remove the DBS. One to three plates can be processed simultaneously in < 45 min. prior to LC-MS/MS analysis. Reference materials from the CDC’s NBS Quality Assurance Program; and blinded samples from the CT NBS Program and the NY NBS program were used for assay validation.

**Results:** Sensitivity, Specificity, Precision, Accuracy, Reference Range and Reportable Range were validated. During the validation of the back-up TQD we found the mass assignments had drifted causing a significant difference in calculated values between the two instruments. During validation C20:0-LPC was also captured to assess the possibility of using a C24:0/C20:0-LPC and/or C26:0/C20:0-LPC ratios to determine abnormal specimens. However, the ratios were not useful and were abandoned. The assay was launched with conservative C26:0-LPC (primary marker) and C24:0-LPC (secondary marker) cut-offs. The C24:0-LPC cutoff was much too conservative due to sample degradation of retrospective validation specimens and use of the unmatched isotope d4-C26:0-LPC internal standard for quantitation. Use of the C24:0-LPC cutoff was abandoned but the analyte is still captured due to its utility in quality control, instrument sensitivity, and data trends. The first two months of screening (n=7951) found 5 borderline samples, 4 of which resolved with a normal repeat specimen, and 2 presumptive positive samples, of which both have been clinically confirmed. We intend to eliminate the borderline cut-off pending additional screening and outcome data.

**Conclusion:** MN has implemented high throughput screening for xALD using an LC-MS/MS C24:0-LPC and C26:0-LPC assay. This assay is a second tier method for high specimen volume states such as New York, but is used successfully as a primary screen in MN where the annual specimen volume is approximately 72,000.

**Presenter:** Amy Hietala, MS, Laboratory Supervisor, Newborn Screening Laboratory, Minnesota Department of Health, St. Paul, MN, Phone: 651.201.5455, Email: amy.hietala@state.mn.us

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Provider and Family Fact Sheets: What Do Healthcare Providers Really Need?


**Objectives:** Determine fact sheet usage and content needed based upon provider preferences.

**Background:** Upon the notification of an abnormal newborn screening result, providers were faxed both a provider and family fact sheet about the condition. However, we were unaware if the child’s...
healthcare provider actually received or were using the fact sheets. Additionally, we were making assumptions regarding what information they would find useful. We sought to better understand provider usage of the fact sheets and the content they found important, with the goal of revising fact sheets to better serve the needs of our providers.

**Method:** We developed an electronic survey to email to healthcare providers. Providers who had a patient with a positive newborn screening result in the past six months (based on newborn screening follow-up data) were invited to participate in the survey. These providers consisted of MDs, DOs, and NPs. Provider emails were obtained from the Minnesota Board of Medical Practice and Board of Nursing. The survey included examples of our current provider and family fact sheets.

**Results and Conclusion:** The survey was emailed to 103 providers and 20 completed the survey. Respondents had been practicing for an average of 17 years. The majority recalled receiving both the provider (15) and family (19) fact sheet. All 15 who recalled receiving a provider fact sheet, found it useful. However, only nine reported providing the family fact sheet to their patient’s family while eight could not recall. Over 85% of respondents stated that the following fact sheet elements were either very important or important: provider’s next steps/action required (17/20), list of differential diagnoses (19/20), what was found on the newborn screen (20/20), what to review with the family (19/20), expected outcome (19/19), treatment options (19/19), and clinical summary (19/19). For the family fact sheet, the providers reported the following elements were considered very important or important: support resources (17/18), what was found on the newborn screen (17/18), expected outcome (19/19), symptoms if untreated (17/19), and treatment options (19/19). Providers prefer to receive fact sheets via fax, rather than through their EMR system, online, or printed copies in clinic. Interestingly, 15 out of 20 were unfamiliar with the ACMG (American College of Medical Genetics) ACTion (ACT) Sheets developed for healthcare providers looking for information on genetic conditions identified through newborn screening. Based on the results of the survey, we revised our provider and family fact sheets to address what healthcare providers would find most useful.

**Presenter:** Sondra Rosendahl, MS, CGC, Genetic Counselor, Minnesota Department of Health, St. Paul, MN, Email: sondra.rosendahl@state.mn.us

**P-071**

**Second-Tier Molecular Testing for Congenital Adrenal Hyperplasia in Minnesota**

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**Objective:** To evaluate, validate, and implement a second-tier molecular newborn screening assay for congenital adrenal hyperplasia in Minnesota.

**Background:** Congenital adrenal hyperplasia (CAH) due to 21α-hydroxylase deficiency, a form of adrenal insufficiency, is the most common cause of genital ambiguity in the newborn. Undiagnosed CAH can result in death due to salt-wasting and/or adrenal crisis. The current primary newborn screening method for CAH is a fluorimmunoassay (FIA) that measures 17α-hydroxyprogesterone (17OHP), a metabolite elevated in CAH. This assay has been well documented to have a high false positive rate and studies have shown that it also has a higher than expected false negative rate. To reduce the number of false positives and false negatives, a second-tier CYP21A2 gene mutation molecular assay has been developed to evaluate the role of molecular testing for CAH in newborn screening.
Methods: The second-tier molecular assay developed at the CDC’s Newborn Screening & Molecular Biology Branch was validated by the Minnesota newborn screening program. This assay uses a novel long-range amplification-based method (LRAM) and an allele specific primer extension (ASPE) method to detect CYP21A2 gene mutations, deletions, and large gene conversions. These account for 90-95% of all CAH disease causing alleles. The assay was developed and validated using affected CAH patients and their parents in order to identify CAH-causing mutations in Minnesota families. Mutation and wild type allelic ratios were initially calculated using CDC’s data and were refined using Minnesota specific data. Upon assay validation, a retrospective study using archived dried blood spots commenced. The 17OHP cutoff was decreased for all weight categories to levels below values previously associated with identified false negative CAH cases in Minnesota. The retrospective study consists of running a total of 2,500 specimens. The workflow, testing turnaround time, analyst hands-on time, and cost will be analyzed to determine the feasibility of this assay as a second tier test in a newborn screening lab.

Results and Conclusions: The assay workflow is around 8 hours from start to finish and it takes one fulltime analyst to run the assay. The early estimate of the cost is around $25 per specimen. This cost can be lowered by the types of consumables purchased. The preliminary findings suggest that the population carrier rates are higher than published rates. The final carrier rates will be reported at the meeting once the retrospective study has been completed.

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P-072

Missouri’s Volunteer Model for Successfully Converting to a 6-Day Workweek for the NBS Laboratory
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The Missouri State Public Health Laboratory (MSPHL) achieved a paramount improvement in its newborn screening (NBS) system by adding Saturdays and most holidays to the NBS regimen. Challenges included funding, staffing, and the necessary courier enhancements. Legislative seed-funding was spearheaded by a State Representative. Enhancement of the courier by adding holiday and Sunday pickups at the birthing hospitals was accomplished first. The courier needed to work 6 days per week also, working a day ahead of the NBS laboratory, thus picking up Sundays through Fridays at the birthing hospitals. In addition, 8 birthing centers that formerly were not getting courier service were now added to the pickup routes. These enhancements alone provided a 17% increase in samples received by the lab within 3 days of collection. With the courier enhancements, Sat/holiday testing could commence. The goal for staffing was to keep this as a voluntary process where the current NBS staff would not be forced to rotate coverage by giving up their Saturdays and holidays. An employment status called “secondary assignment” was employed. Current MSPHL Scientists, both from within the NBS lab and from other MSPHL lab units, could work some Saturdays and holidays for extra money. This team of about 18 employees (Scientists and Office Support employees) is called the Weekend Warriors and is led by a fulltime Laboratory Manager position. The Manager position was the only full time FTE that was added. Two of the weekend staff is the newest hired full time NBS Scientists who have a fixed schedule of Tuesday through Saturday. These two have the option to move to a Monday through Friday position if they wish as we have new openings come available. Five other scientists voluntarily sign up to work each Saturday/Holiday. The requirement is that they must be deemed competent on at least one testing platform in the NBS laboratory and work at least once per month to stay proficient. The Saturday and Holiday testing began in October, 2015 and the MSPHL immediately realized an additional
increase in samples tested within 3 days of collection, bringing the total timeliness improvement to about 29%. Time-critical screening results detected on Saturdays and Holidays are immediately phoned to the geneticists on call at our contracted referral centers. The 6-day workweek has worked tremendously well for the last 17 months with MSPHL’s newborn testing running in full capacity on Saturdays and holidays with the exception of second tier CF and Krabbe screening. Our presentation will demonstrate the valuable benefits to Missouri’s Newborn Screening Program that have come from the work expansion.

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**P-073**

**Enzyme Activities of LSDs in a Japanese Neonatal Population**
R. Mashima, National Center for Child Health and Development, Tokyo, Japan

**Background:** Lysosomal storage disorders (LSDs) are caused by the defective enzyme activities in the lysosomes characterized by the accumulation of oligosaccharides, glycolipids, mucopolysaccharides, and sphingolipids. Growing evidences have suggested that the earlier detection of the affected individuals followed by an immediate initiation of appropriate therapy during the presymptomatic period usually results the better therapeutic outcomes. These enzyme activities have been measured individually using fluorescent substrates, while simultaneous determination of multiple enzyme activities seems to be more favorable to neonatal screening for LSDs due to the rare prevalence of these disorders.

**Methods:** The enzyme activities of Pompe, Fabry, MPS I, Gaucher, Niemann-Pick A/B, and Krabbe diseases in a Japanese population were determined by LC-MS/MS-based method. CDC-provided QC DBS was used for assay calibration.

**Results:** The enzyme products accumulated almost linear for 0-20 h at 37°C and over 0-100% enzyme activity using a CDC-provided dried blood spots for quality control. The values of coefficient of variance within a day and between days were less than 25%. The enzyme activities of healthy individuals were higher than those of disease-confirmed individuals.

**Conclusions:** Our results suggest that the levels of enzyme activities of 6 LSDs of Japanese population were comparable to the recent report [Elliott S et al Mol Genet Metab 118 (2016) 304-309]. This observation extends an evidence that 6-plex LSD enzyme assay provides a reproducible analytical procedure for neonatal screening.

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**P-074**

**Learnings from the Library: Health Informatics Resources and Training Tools from the National Library of Medicine**
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Significant advances in biomedical informatics, data science, precision medicine, open access to biomedical information, and changes in our country’s health systems offer new directions and

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opportunities for scientific achievement. The National Library of Medicine (NLM) is committed to building a data infrastructure that will support the future of biomedical research. NLM provides access to numerous free resources for acquiring health informatics training, learning about and using health information exchange standards, and bridging gaps in information for public health practice and health services research. As the central coordinating body for clinical terminology standards within the U.S. Department of Health and Human Services, NLM supports the development, enhancement and distribution of clinical terminologies to facilitate clinical data exchange and improve health information retrieval. This session will showcase some of the NLM-developed and NLM-supported tools particularly relevant to the newborn screening and genetic testing community.

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**P-075**

**Nevada Newborn Screening Two and a Half Years after Transition**

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Nevada is a two-screen state with an annual birth rate of about 35,000. A total of 88,386 for first specimens and 75,263 second specimens were tested at NSPHL during the period of July 1, 2014 to December 31, 2016. Oregon was testing Nevada newborns prior to July 1, 2014. NSPHL is testing specimens using MS/MS and Victor 2d for metabolic disorders, AutoDELFIA for endocrine disorders and cystic fibrosis, and IEF for hemoglobinopathies. A total of 117 cases were identified and confirmed consisting of 24 metabolic disorders, 44 endocrine disorders, 9 cystic fibrosis and 40 hemoglobinopathies. Affected babies are mostly detected on first screening with a few detected on second screening. Twenty-one cases of metabolic disorders detected by MS/MS and one case of Galactosemia were identified on first screening. Two cases with Biotinidase deficiency showing two borderline screening results were followed up as presumptive positives and diagnosed with confirmatory tests done by other clinical laboratories. Nine cases of amino acid disorders, 9 cases of fatty acid disorders and 3 cases of organic acid disorders were detected by screening and confirmed with diagnostic tests. Sixteen cases with partial GALT deficiency (D/G variant) were identified of which 10 had borderline results on first and second screens and 6 cases had borderline results on second screens. Two cases homozygous with classical biotinidase deficiency mutation and 5 cases with partial deficiency (single mutation) were identified. Four cases with severe form of congenital adrenal hyperplasia (CAH) were detected, three on first screening and one on second screening. Another case screened positive for 17-OHP and was diagnosed with congenital adenogenital syndrome. Forty cases with congenital hypothyroidism as well as 40 cases with hemoglobinopathies were detected on first screening. Nine cases with CF screened positive on first screening and confirmed with diagnostic tests by other clinical laboratories. One case screened negative for IRT on 2 screens but was diagnosed with CF through diagnostic tests and family history. Sickle cell diseases were identified - Sickle cell anemia (FS), Sickle cell beta thalassemia (FSA), Hb SC disease (FSC). Other hemoglobinopathies identified - Hb C disease (FC), Hb D disease (FD), and Hb E disease (FE). Secondary conditions on the Recommended Uniform Screening Panel (RUSP) like 2-methylbutyrylglycinuria and isobutyrylglycinuria were identified on 2 cases by confirmatory testing. For clients to receive their patient’s reports on the same day results were approved, auto-faxing of reports was implemented for all birthing facilities and clinics. A secure web-based result portal was developed to allow healthcare providers access to NBS reports of patients that transferred from another PCP or hospital.

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P-076

Development and Validation of IDUA Sequence Analysis for Second-tier MPS I Screening
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Alpha-L-iduronidase is responsible for breaking down glycosaminoglycans (GAGs) within lysosomes. Deficiencies in this enzyme, caused by mutations within the IDUA gene, cause mucopolysaccharidosis type I (MPS I). In 2014, Governor Christie signed into law the Let Them Be Little Act, which mandates screening for MPS I once certain conditions are met. With the recent addition of MPS I to the Recommended Uniform Screening Panel (RUSP), New Jersey is beginning to implement screening for the disorder. The New Jersey screening algorithm includes alpha-L-iduronidase levels via tandem mass spectrometry as a first-tier method. However, IDUA sequence analysis is requested by state lysosomal storage disorder medical specialists to decrease false positives, discern cases of pseudodeficiency, and help triage incoming patient referrals based on the mutations involved. The New Jersey Newborn Screening (NBS) Laboratory has developed and validated an IDUA sequencing assay for second-tier MPS I screening. Briefly, DNA is extracted from dried blood spots via a simple, inexpensive method. The IDUA promoter, all 14 exons, and intron-exon boundaries are amplified using Kapa Biosystems KAPA2G Robust polymerase, which is manufactured specifically for difficult to amplify GC-rich sequence regions. Sanger sequencing is performed using a 3500Xl Genetic Analyzer, and mutations are analyzed using SeqScape software in comparison to the NCBI reference sequence. Validation studies included measurements of sensitivity, specificity, and accuracy using extracted DNA or dried blood spot material from patients with known mutations. Known genotypes analyzed included those causing pseudodeficiency, severe and mild phenotypes, and benign polymorphisms. Implementation of this IDUA sequencing assay represents the first use of such methodology in a state newborn screening laboratory.

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P-077

Quantification of Bart’s Hemoglobin to Improve Reporting Possible Alpha-Thalassemia
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Background: The New Jersey Newborn Screening (NJ NBS) Laboratory screens for Sickle Cell Disease and other hemoglobinopathies using isoelectric focusing (IEF) gels followed by High Performance Liquid Chromatography (HPLC) for specimens with an abnormal pattern on IEF. Currently, Bart’s hemoglobin, the presence of which can indicate alpha-thalassemia, is measured only through IEF. Specimens presenting with a Bart’s hemoglobin band on an IEF gel are simply reported as having Bart’s hemoglobin with a recommendation to refer to a specialist. The severity of alpha-thalassemia is dependent on the
number of alpha-globin genes deleted, which is difficult to determine based solely on the appearance of the bands, and the current procedure can result in many unnecessary referrals to a specialist. Therefore, the reporting of alpha-thalassemia could benefit from a quantitative measure of Bart’s hemoglobin. Moreover, a method to identify deletions on the alpha-globin gene would result in a more specific screening algorithm.

Methods: The first goal of this project was to use HPLC to quantify the amount of Bart’s hemoglobin in the newborn’s blood. The second goal of the study was to develop a molecular assay for the most common alpha-thalassemia mutations to correlate the percentage of Bart’s hemoglobin reported by HPLC to the deletions present in the newborn’s alpha globin gene. Over a three-month period, all newborns with a Bart’s hemoglobin band present on IEF gel were assayed using HPLC to determine the percentage of Bart’s hemoglobin present. Any specimen with a Bart’s hemoglobin percentage above 10% was saved for future molecular analysis. A gap Polymerase Chain Reaction (PCR) assay is in the process of being developed to screen for the 8 most common alpha-thalassemia deletions.

Results: 125 specimens were analyzed by HPLC. 41 specimens had 10-15% Bart’s hemoglobin present, 8 specimens had Bart’s hemoglobin in the 15-20% range, 3 specimens showed 20-25% Bart’s hemoglobin, and 1 specimen had Bart’s hemoglobin >25%. The molecular assay for alpha chain deletions is still in development; however preliminary results will be shown.

Conclusion: The quantification of Bart’s hemoglobin using HPLC demonstrated the diversity of concentrations present in specimens that have abnormal patterns on IEF gels. This finding supports a change in screening algorithm to reduce unnecessary referrals. Since the severity of an alpha-thalassemia disorder depends on the number of alpha-globin chain deletions, further correlating a Bart’s hemoglobin percentage range to the number of alpha-globin chain deletions, could result in a specific screening algorithm that helps maximize efficient use specialist medical resources.

Presenter: Miriam Schachter, PhD, Ronald H. Laessig Memorial Newborn Screening Fellow, Newborn Screening Laboratory, New Jersey Department of Health, Trenton, NJ, Phone: 609.406.6892, Email: miriam.schachter@doh.nj.gov.
substrates to create specific products. A method validation is being performed to evaluate the sensitivity, specificity, precision, and accuracy of the multiplex method.

**Results:** The method validation is in progress; however, preliminary results indicate that the assay is very sensitive. The sixplex method is also highly specific and precise. The assay results replicate well and clearly distinguish positive specimens from normal patients.

**Conclusion:** Validation results indicate that the sixplex MS/MS assay to screen for ABG, ASM, GAA, GALC, GLA, and IDUA is very robust. State-wide implementation of the method following the regulatory required validation and subsequent pilot study to establish patient reference ranges should be effective in the identification of newborns with the mandated LSDs.

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P-079

**National Newborn Screening Contingency Plan - A Guide to Facilitate Preparedness**

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The national newborn screening (NBS) contingency plan (CONPLAN) serves as a manual for maintaining NBS operations during a public health emergency. The CONPLAN was developed in 2010 by the Centers for Disease Control and Prevention (CDC) and the Health Resources and Services Administration (HRSA) as required by the Newborn Screening Saves Lives Act of 2008. In 2015, the Association of Maternal and Child Health Programs (AMCHP) and the Association of Public Health Laboratories (APHL) partnered with CDC and HRSA to form an Advisory Committee of NBS and emergency preparedness experts and stakeholders to update the CONPLAN. To review gaps in the existing CONPLAN and solicit feedback on how the CONPLAN could incorporate the entire NBS system, AMCHP conducted a public comment survey targeting NBS programs, preparedness programs, hospitals, physicians, families and other stakeholders. The CONPLAN Advisory Committee analyzed the results during committee conference calls and an in-person meeting. This survey feedback was incorporated into the revised CONPLAN. The updated document addressed gaps in laboratory and clinical follow-up, included point-of-care screenings for hearing and critical congenital heart defects, incorporated the emergency management assistance compact (EMAC), and emphasized communication and family engagement. The Advisory Committee also developed enhanced tools to assist states in using the CONPLAN to inform their own contingency plans (e.g., memorandum of understanding templates and planning checklists). The revised CONPLAN is currently being vetted through HRSA and CDC, however, approval is anticipated in advance of the Symposium. The updated document is a guide ensure all aspects of the NBS system are included in preparedness planning and includes several new resources that will enable states to be better prepared for the next public health emergency.

**Presenter:** Scott Shone, PhD, Senior Research Public Health Analyst, RTI International, Research Triangle Park, NC, Phone: 919.485.5512 Email: sshone@rti.org
P-080

Carnitine Deficiency in Newborn Screening in Slovakia - New Algorithm
S. Dluholucky, M. Machkova and M. Knapkova, Newborn Screening Centre Slovak Republic, Banská Bystrica, Slovakia

Objectives: Newborn Screening in Slovakia is well established state preventive programe. We’ve started newborn mass screening using MS/MS on January 2013. We screened for Primary or Secondary Carnitine Deficiency according to a new algorithm from 2016. Methods: We performed aminoacids and acylcarnitines from dry blood spot using Mass Chrom Reagent kit derivatised and Agilent 6420 Triple Quad LC/MS technology. Screening algorithm for Primary or Secondary Carnitine Deficiency for 72 -96. hours of life started with positive cases for free carnitine under 8 umol/L, total carnitine under 15 umol/L, acetylcarnitine under 7 umol/L, C16 under 0,32 umol/l and C18 under 0,16 umol/L. The second step is re - screening on 14th day of life, together with mother of newborns (with the consent of the mother). If the both sample are positive for Carnitine Deficiency, we recall newborns and mothers to Metabolic Clinic. We give special attention to the immature and sick children, where the Secondary Carnitine Deficiency is common.

Results: In 2016 we screened in Slovakia 57 657 newborns and detected 31 positive cases of Carnitine Deficiency. 3 newborns with Secondary Carnitine Deficiency with low birth weight and NICU, 9 newborns with Primary Carnitine Deficiency, 20 maternal forms Carnitine Deficiency. Screening prevalence for Carnitine Deficiency in Slovakia is 1 : 1860 liveborns. Genetic results of disease is still unfinished. Conclusion: First results show surprisingly high screening prevalence Primary or Secondary Carnitine Deficiency in Slovakia. This condition can be identified at birth by newborn screening, together with maternal form. 80 percent of cases are captured in the minority Roma population in Slovakia, which is the second surprising finding. Our results suggest, that Primary or Secondary Deficiency is relatively common in a population newborns (and mothers) in Slovakia. We need more data on this facts in newborns population in Slovakia.

Presenter: Maria Knapkova, Laboratory Manager, Newborn Screening Centre Slovak Republic, Banská Bystrica, Slovakia, Phone: 421.48.472.65.47, Email: mknapkov5@gmail.com

P-081

Impact of Variations in Filter Paper Lots on Dried Blood Spot Biotinidase Activity
E. Desormeaux, N. McIntosh, M. Kowalski, S. Foster, P. Chakraborty and M. Henderson, Newborn Screening Ontario, Ottawa, ON Canada

Problem Studied and Objectives: Biotinidase deficiency is an inherited disorder in which the body cannot recycle biotin. Biotin is important for the body to make fats and carbohydrates and break down protein. Without enough biotin the body cannot process nutrients properly and this results in health problems. Biotinidase deficiency occurs in approximately 1 in 60,000 newborns. It is characterized by seizures, hypotonia, breathing problems, vision and hearing loss, skin disorders, and developmental delay. This study describes the impact of variations in filter paper lots on dried blood spot biotinidase activity.

Methodology: To determine the degree of bias and which analytes were affected, patient sample results were extracted from the Newborn Screen Ontario (NSO) data warehouse. At the time of writing, 2176 samples collected on a new lot (W161) filter paper had been analyzed. An equal data set

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comprised of 2176 samples collected using the current lots was used as a comparison group. Both data sets were comprised of results measured between 17-01-30 and 17-02-14. The measured values for three lots of filter paper (W152, W161 and W162) were compared using blood diluted with varying concentrations of washed red cells. The dilutions were spotted out onto the three lots of filter paper, and biotinidase activity was quantified using the Spotcheck Pro (Astoria Pacific, Oregon). The Spotcheck Pro determines biotinidase activity colourimetrically. Samples with biotinidase activity develop a purple colour with intensity proportional to the enzyme activity.

**Significant Results:** The statistical analysis of the archived data comparing collection card lot numbers showed biotinidase was the only analyte affected. The mean patient biotinidase activity was 60.88 MRU (n=2176) for samples collected using lot W161 and 127.59 MRU (n=2176) for samples collected using lot W152. The direct comparison of diluted blood spotted onto filter paper lot W152, W161 and W162 showed the following linear relationship: 

\[ y = 0.44x - 0.24 \] (Pearson’s r = 0.99) for W151 vs W161

\[ y = 0.6x - 1.89 \] (Pearson’s r = 0.976) for W151 vs W162

Lot W161 had an approximate 55% negative proportional bias and lot W162 had an approximate 40% negative proportional bias compared to the current lot W151.

**Conclusions and Implications:** The change in distribution of biotinidase activity suggests a large proportional negative bias. If unaddressed the effect of this negative proportional bias would be an increased false positive rate. The slope and intercept of the regression were used to determine a new biotinidase deficiency screening threshold for samples collected on lot W161 filter paper. As a result of these findings Newborn Screening Ontario will determine if other methods are similarly impacted and adopt filter paper lot validation testing protocols before new filter paper lot numbers are put in use.

**Presenter:** Emily Desormeaux, Newborn Screening Ontario, Ottawa, ON Canada, Phone: 613.737.7600, x3439, Email: emdesormeaux@cheo.on.ca

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**P-082**

**Implementing a Molecular LIS: A Retrospective**

D. Lawrie and L. Racacho, Newborn Screening Ontario, Ottawa, ON, Canada

In the fall of 2015 Newborn Screening Ontario (NSO) commenced work on what was to be a six month project to implement a next generation sequencing (NGS) laboratory information system (LIS). The project was required to address a need to provide molecular diagnostic testing to the clients of the NSO program. Although the overall project was a success it was not without its challenges. We identified 3 stress points that a clinical NGS LIS should address in keeping with best practices and privacy concerns. In addition, tight timelines, changing requirements and a multi-vendor solution were some of the hurdles NSO had to overcome and are still dealing with today. Looking back there were a few things we would have done differently to address these challenges and would likely moved us further along our NGS LIS journey in the same timeframe.

**Presenter:** David Lawrie, Business Systems Analyst, Newborn Screening Ontario, Ottawa, ON Canada, Phone: 613.738.3222 x3544, Email: dlawrie@cheo.on.ca
P-083

Using Package and Sample Tracking Systems to Support Timely Newborn Screening in Ontario, Canada
C. McRoberts, J. Bottomly, E. Santander, S. McClelland, M. Sayer, J. Milburn and P. Chakraborty,
Newborn Screening Ontario, Ottawa, ON Canada

Newborn Screening Ontario (NSO) continues to make process enhancements aimed at improving the
timeliness of newborn screening (NBS) in Ontario. Cargo, an internally developed package tracking
system, supports reduced sample transit times by providing early identification of delayed or lost
packages. Cargo has successfully identified overdue and missing packages triggering earlier follow-up
investigation for impacted samples. However, Cargo cannot provide sample level details and depends on
documentation provided by submitting hospitals or midwives to obtain this information. A second
notification system detects delayed or missed screens. These alerts may be too late for infants affected
with early presenting diseases. NSO will present their experience developing their in-house package
tracking system and will also share their involvement piloting the Track-Kit™ project developed and
supported by STACS DNA. Objectives of the Track-Kit™ project included the facilitation of sample level
tracking as well as additional benefits such as collection device expiration warnings, inventory control
and the ability to determine accurate transportation metrics. Eight sample collection sites piloted Track-
Kit™, a web-based solution integrated with our Ontario-wide courier application. NSO was able to track
shipped samples to receipt at NSO and shippers were notified of sample receipt in the lab. Although
additional effort is required for submitters to log individual samples in Track-Kit™, courier options are
pre-selected, so users see benefit in the ease and speed in preparing the courier shipping request.
Forced pre-selection of fields helps to reduce NSO’s costs by decreasing shipping errors and misuse of
NSO paid courier accounts. To facilitate broader roll-out, NSO is proposing that STACS DNA add a hybrid
capability allowing hospitals to use Track-Kit™ without the individual sample tracking option. To help
hospitals and midwifery practices gauge whether they are using the current screening system optimally,
NSO distributes the Submitter Quality Indicator Report. The report is distributed quarterly, includes
sample transit time metrics and has been expanded to provide information about two other key NBS
performance indicators: Unsatisfactory Samples and Age at Initial Sample Collection. Since the report’s
initial release in early 2015, NSO has observed province wide improvements in sample transit times.

Presenter:  Christine McRoberts, MLT, CLQM, NSO Laboratory and Quality Manager, Newborn Screening
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P-084

Building an 'Ideal' Newborn Screening LIS: From Theory to Practice
M. Pluscauskas1, C. Paulette2; 1Newborn Screening Ontario, Ottawa, ON Canada, 2Paulette and
Company, LLC, Akron, OH

Newborn Screening Ontario (NSO) screens approximately 150,000 dried blood spots from all babies born
in the province for rare diseases and is responsible for retrieval and short term follow-up of these
infants. In recent years NSO has expanded into new functional areas such as biochemical/molecular
diagnostic testing and point of care screening for CCHD. As the program expanded, NSO found that
components of its IT system required more flexibility to fully meet its needs. In 2013 Sepulveda and
Young published a paper in the Archives of Pathology & Laboratory Medicine describing an "Ideal
Laboratory Information System" to optimize the operation of clinical laboratories by intelligent

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management of laboratory information. Based on these and other core principles NSO created "Project Lancet" in late 2014 to develop a comprehensive system to facilitate information flow between program areas including: (1) Patient Record Management, (2) Case Management and Follow-Up, (3) Clinical/Medical Review, (4) Lab data and QC, (5) Sample lifecycle management, and (6) Analytics. The talk will cover the methodology that NSO has undertaken and results that Project Lancet has achieved moving through its various phases from theory to practice. These include:- Developing a holistic NBS IS Architecture including: The use of a single vendor vs. best-of-breed components; determination of when to use non nbs vs. nbs-specific vs. hybrid systems; assessing off the shelf components vs. custom builds; balancing a patient vs batch/specimen centric systems approach; addressing reporting needs across the spectrum from case management through short term follow-up - Developing a vendor and technology-independent advanced analytics system via the development of a data warehouse and related tools.- Collecting, vetting and recording key NBS IS system requirements- Procurement of key off the shelf components- Development of in-house systems - QA, client and vendor engagement approaches during the project build A key lesson learned is that an NBS LIS must be "fit for purpose" which involves determining a program’s needs and capabilities across a number of key criteria including: Program budget, size and scope; program needs/ priorities; management and IT support/expertise; perceived risk; desire for configurability vs. a vendor managed approach and overall market constraints. Given the variety of different factors faced by each newborn screening program there is not a one size fits all answer to developing an NBS LIS, however, the processes discussed in this talk can be used to help determine what LIS strategy might be “ideal” for your program.

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P-085 - Withdrawn

P-086

North Dakota Newborn Screening Public Outreach
J. Meyer and K. Bentz, North Dakota Department of Health, Bismarck, ND

The Problem Studied and/or Objectives: Since 2010 the birth rate in North Dakota has increased substantially due to an increase of population as a result of the oil impact areas. During the same time period, the number of newborn screening refusals increased, as well as the number of home births. The ND Newborn Screening Program reviewed state-wide data related to poor quality specimens, timeliness and refusals and found that there were areas to improve on. The program began yearly birthing facility site visits and state-wide public awareness campaigns in an effort to increase the understanding of the importance of newborn screening and improve timeliness of specimens. Another goal of these activities was to decrease refusals and the number poor quality specimens collected.

Methodology: The public awareness campaign included: • Educational video with two family stories who have been impacted by newborn screening • Outdoor digital billboard ads located in major cities throughout the state • Indoor billboards posted in bathrooms state-wide • Newborn screening website revision • Newborn screening brochure revision • State-wide newborn screening conference for professionals • Yearly birthing facility educational visits Significant results, including statistical significance where applicable: • Significant increase in web traffic following efforts • Significant increase
in timeliness of collection of newborn screens • Decrease in the number of refusals of newborn screens • Specific results are currently being analyzed and data will be provided at the symposium

Conclusions and/or implications: • Campaign to increase awareness of newborn screening of both public and professional staff increased knowledge seeking behaviors, timeliness of the collection of newborn screens, and decrease in refusals of newborn screens.

Presenter: Joyal Meyer, RN, MSN, Director, North Dakota Newborn Screening Program, North Dakota Department of Health, Bismarck, ND, Phone: 701.328.4534, Email: jbmeyer@nd.gov

P-087

Newborn Screening Media Campaigns
K. Bentz and J. Meyer, North Dakota Department of Health, Bismarck, ND

The Problem Studied and/or Objectives: Since 2010 the birth rate in North Dakota has increased substantially due to an increase of population as a result of the oil impact areas. During the same time period, the number of newborn screening refusals increased, as well as the number of home births. The ND Newborn Screening Program reviewed state-wide data related to poor quality specimens, timeliness and refusals and found that there were areas to improve on. The program began yearly birthing facility site visits and state-wide public awareness campaigns in an effort to increase the understanding of the importance of newborn screening and improve timeliness of specimens. Another goal of these activities was to decrease refusals and the number poor quality specimens collected.

Methodology: The media campaigns included: • Educational video with two family stories who have been impacted by newborn screening • Outdoor digital billboard ads located in major cities throughout the state • Indoor billboards posted in bathrooms state-wide • Newborn screening website revision • Newborn screening brochure revision • Local news station interview on importance of newborn screening

Significant Results, Including Statistical Significance Where Applicable: • Significant increase in web traffic following efforts • Significant increase in timeliness of collection of newborn screens • Decrease in the number of refusals of newborn screens • Specific results are currently being analyzed and data will be provided at the symposium

Conclusions and/or implications: • Campaign to increase awareness of newborn screening of both public and professional staff increased knowledge seeking behaviors, timeliness of the collection of newborn screens, and decrease in refusals of newborn screens. We would like to gather newborn screening professionals from various states to have an opportunity to share educational resources and media campaigns to enhance public awareness of newborn screening education.

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P-088

Use of Tandem HPLC and Sanger Sequencing Assays to Establish a Cutoff for the Detection of β-thalassemia Major in Neonatal Populations
Wadsworth Center, New York State Department of Health, Albany, NY

Newborn screening for Hemoglobinopathies, such as β-thalassemia, ensures early and accurate identification of individuals who require comprehensive healthcare services. β-thalassemia major impacts a newborn’s health soon after birth and can have a serious impact on their short-term and long-term health outcomes. Mutations and deletions in the β-globin gene (HBB) can cause absent, or reduced synthesis, of Hemoglobin A (Hb A) in newborns. As a result, newborns with β-thalassemia present with no or very low levels of Hb A. While Sanger sequencing can identify β-thalassemia mutations, it is not practical to sequence the HBB gene for all infants undergoing screening. To capture infants at high risk for β-thalassemia, the New York State Department of Health’s Newborn Screening Program is establishing a reliable high-performance liquid chromatography (HPLC) Hb A% cutoff to reflex to Sanger sequencing. HPLC data from 2016 (259,095 specimens) were examined to create a one-year cohort of specimens < 3.0% Hb A (n=89) for study. In order to measure the precision of the HPLC instruments at low Hb A levels, intra- and inter-assay variability were studied and deemed acceptable (mean standard deviation of 0.13 and 0.15% respectively). Initially, 37 specimens with a pattern of FA = 2.5% Hb A and F only were selected for Sanger sequencing. Selected specimens were punched and blinded prior to testing. Seven specimens (including two repeats) were identified with homozygous or compound-heterozygous mutations for β-thalassemia major and nine specimens were heterozygous (trait). The highest level of Hb A in a homozygote was 2.1%. Two additional specimens from 2017 were sequenced and found to be homozygous for β-thalassemia. Additional validation studies are being performed on the remaining 52 specimens (2.6-3.0 Hb A%) to determine if a 3.0% Hb A cutoff is too liberal. Once established, the low Hb A% cutoff will be used to identify newborns at higher risk for β-thalassemia and sequencing of the associated infant’s specimens will be incorporated into our testing algorithm. These preliminary studies indicate that the frequency of β-thalassemia major in New York State is 1 in 50,000 compared to an estimated 1 in 100,000 worldwide.

Presenter: Ryan Bennett, Research Scientist II, Wadsworth Center, New York State Department of Health, Albany, NY, Phone: 518.474.6310, Email: ryan.bennett@health.ny.gov

P-089

Comprehensive CFTR Genotyping of New York State Infants with Cystic Fibrosis: Mutation Spectrum and Algorithm Change
E. Hughes, C. Stevens, C. Saavedra-Matiz, L. Krein, M. Caggana and D. Kay, Wadsworth Center, New York State Department of Health, Albany, NY

Background: Infants have been screened for cystic fibrosis (CF) in New York State (NYS) using an IRT-DNA algorithm since 2002. Infants with two, one or no mutations but very high IRT (VHIRT) are referred for diagnostic sweat testing. The positive predictive value for CF screening, overall, is low (4.3% from 2010-2013). Ideally, only infants most likely to have CF (i.e., those with two CFTR mutations) would be referred for diagnostic testing. However, the NYS population is diverse and 35.5% of infants with CF in NYS carry one or no panel mutations. Thus, the one mutation and VHIRT referral groups cannot be

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eliminated without more comprehensive CFTR analysis. The purpose of this study was to validate use of dried blood spots and assess clinical validity of the US FDA-cleared Illumina MiSeqDx Clinical Sequencing Assay (CSA) in the NYS CF population.

**Methods:** The study included 439 infants with CF identified via newborn screening (NBS) between 2002 and 2012. All infants with CF and zero or one NYS panel mutation were genotyped using the CSA. The most common mutations not on the current NYS mutation panel were p.L206W (0.9%), 1949del84 (0.8%), and p.T1036N (0.6%). The mutation spectrum differed by race/ethnicity. The clinical sensitivity of CF screening using the CSA was 96.9%. Addition of supplemental assays, bioinformatics, and comprehensive exon deletion/duplication analysis increased sensitivity to 98.7%.

**Conclusions:** The cost of screening is higher and turnaround times are longer using the CSA. However, more comprehensive CFTR genotyping using the CSA panel and select supplemental assays would permit referral of infants with two CFTR mutations without significantly compromising CF screening sensitivity. A three-tier approach is projected to reduce the NYS CF referral rate by 89%, which will eliminate the angst of waiting for a diagnosis and associated healthcare costs for families of 850 infants who falsely screen positive each year in NYS. A prospective blinded pilot study is underway and will culminate with implementation of a CF IRT-DNA-SEQ screening algorithm in NYS.

**Presenter:** Denise Kay, PhD, Research Scientist, Wadsworth Center, New York State Department of Health, Albany, NY, Email: denise.kay@health.ny.gov

**P-90**

**Combined Method of Analysis for Newborn Screening of Tyrosinemia Type I and X-linked Adrenoleukodystrophy**

A. Showers, M. Morrissy, J. Orsini and M. Caggana, Wadsworth Center, New York State Department of Health, Albany

X-linked Adrenoleukodystrophy (X-ALD) is an inherited metabolic disorder affecting the adrenal glands and central nervous system. It is the most common peroxisomal biogenesis disorder with an incidence of approximately 1:17,000. X-ALD was added to the Recommended Uniform Screening Panel (RUSP) by the Secretary of Health and Human Services in February 2016. The metabolic defect associated with X-ALD causes an accumulation of very long chain fatty acids (VLCFA) which may be detected in dried blood spots by the analysis of hexacosanoyl lysophosphatidylcholine (C26:0-LPC). Currently, the New York State Newborn Screening Program extracts the C26:0-LPC marker from dried blood spot (DBS) samples and combines this extract with the test for tyrosinemia (Tyr-I) marker. DBS samples are extracted in Acetonitrile: Water (80:20 with 0.1% formic acid and 0.1% hydrazine) and analyzed by tandem mass spectrometry (MS/MS). The analytical performance of the combined Tyr-I/X-ALD method (precision, accuracy, linearity, and limit of quantitation) for C26:0-LPC are equivalent to the current method. We compared results for 1,988 normal patient samples. The average C26:0-LPC concentrations as determined by the current method and the combined Tyr-I/SUAC method were 0.20 µmole/L and 0.22 µmole/L, respectively. For the current method, 14 samples were over the cut-off and required second-tier testing. For the combined Tyr-I/X-ALD method, four samples had results greater than the proposed cut-off. Seven samples from babies confirmed to have X-ALD were analyzed using
the combined Tyr-I/X-ALD method. Five of the confirmed cases were reported as positive by the combined method. The other two cases were referred based on results from second specimens. The first-tier result for each was close to the cut-off value and reflexed to second-tier testing. Based on the second-tier result, the infants were conservatively reflexed to appropriate care providers. This is done for any infant for any prior borderline X-ALD result. Seven carriers and one case of Zellweger Syndrome were positive by both methods. The combined Tyr-I/X-ALD method eliminates one-day from the sample turn-around-time for both conditions, multiplexes the evaluation of X-ALD with another primary condition of the RUSP, requires no additional sample preparation or analysis, and eliminates the need for additional mass spectrometers that would be required in order to analyze these markers separately. This combined Tyr-I/X-ALD method is a viable alternative for labs implementing screening for X-ALD.

Presenter: Mark Morrissey, Research Scientist IV, Newborn Screening Program, Wadsworth Center, New York State Department of Health, Albany, NY, Phone: 518.486.4395, Email: mmorrissey001@nycap.rr.com

P-091

Are Male Infants at a Disadvantage When Screening for Congenital Hypothyroidism using Thyroxine as a First Tier Screen?
N. Tavakoli, L. DeMartino, R. McMahon and M. Caggana, Wadsworth Center, New York State Department of Health, Albany, NY

Introduction: Newborn screening for congenital hypothyroidism (CH) is crucial in detecting this common endocrine disorder that untreated may result in intellectual disability and growth retardation. Screening strategies are based on detecting low thyroxine (T4), or, elevated thyroid stimulating hormone (TSH) or, a combination of both. The NYS Newborn Screening Program (NBSP) initially screens all infants for T4 and any baby with a result in the lowest 10% for the day is then screened for TSH. Infants with low T4 and elevated TSH are referred for follow-up diagnostic testing. We investigated the differences between males and females when using this algorithm.

Methods: From 2008 to 2015, the NYS NBSP screened approximately 1.9 million babies for CH. T4 concentration in dried blood spots was measured using the AutoDELFIA Neonatal thyroxine kit (Perkin Elmer, Turku, Finland). Approximately 270,000 infants were then screened for TSH using the AutoDELFIA Neonatal hTSH kit (Perkin Elmer). Infants with abnormal results were referred for follow up diagnostic testing or if a borderline result was obtained, a repeat specimen was requested.

Results: Amongst the 1.9 million specimens tested for T4, 51.5% were from male and 48.4% were from female infants. Of the specimens triaged to be screened for TSH, 60% were from male and 40% were from female infants. After T4 and TSH results were evaluated 3,724 males and 2,532 females were referred. Repeat specimens were requested for 18,451 males and 9,935 females. The mean and median T4 values were lower in males than females (a difference of approx. 0.8-1.1 µg/dL each year). The lower T4 values in males were not due to low birth weight since males had higher birth weights than females. It was also not due to more specimens from male babies being collected within the first 24 hours because when T4 values from these babies were not included in T4 calculations, the T4 values remained lower in males than females. Mean TSH values were similar in males and females but median values for males were higher. Follow up of referred infants confirmed CH in 632 (49.2%) male and 652 (50.7%) female infants over the 8-year period.

Conclusion: Male infants have a lower mean (and median) T4 and higher median TSH than female infants despite having a higher mean birth weight. This leads to a higher percentage of males than
females (60% vs 40%) being triaged for TSH testing, a higher number of repeat specimens being requested (65% vs 35%) and a higher number of male infants being referred (59.5% vs 40.5%). Using our algorithm, male infants are disproportionately represented in the false positive category even though incidence of CH in male infants is marginally lower.

**Presenter:** Norma Tavakoli, Research Scientist, Wadsworth Center, New York State Department of Health, Albany, NY, Phone: 518.486.2569, Email: norma.tavakoli@health.ny.gov

**P-092**

**Response to the Federal Rule Change Requiring Laboratories to Provide Patients Access to Laboratory Results**

B. Vogel, J. Orsini, S. Bradley and M. Caggana, New York State Department of Health, Albany, NY

A federal rule change by the Department of Health and Human Services to the Clinical Laboratory Improvements Amendment (CLIA) of 1988 requires laboratories to provide patients access to laboratory results effective April 7, 2014. In response to this rule change, a new policy was developed to allow the parent to request results from the New York State NBS Program by phone, letter or email. They must provide information from the blood collection form including mother’s name, baby’s birth date, mother’s address and baby’s hospital of birth. If all information is provided, the results will be mailed to the parents at the address provided by the specimen collector. If the requestor wants results sent to a different address than on the original form, the laboratory ID number must be provided. If the lab ID number is not known, the parent will be directed to either obtain the result or laboratory identification number from the baby’s health care provider. There are a few limitations to this approach. In the case of an adoption or foster care, the responsible party will only be able to obtain results directly from the NBSP if they have the lab ID number. Also, parents of older children are less likely to live at the same address or to know the lab ID number. In this case, results could be requested by a licensed healthcare provider. Results are sent using an action in the NBSP laboratory information system allowing us to assess the volume and impact of this rule change. In 2015, 542 results were sent upon request (0.23% of tested newborns). In 2016, 1,083 results were sent upon request (0.46% of tested newborns). The parent copy of the blood collection card was amended to inform parents of the process to request results, but uptake has been low. Based on this outcome, the parent copy of the filter paper is a less efficient way to communicate with parents about newborn screening than expected.

**Presenter:** Beth Vogel, MS, CGC, Research Scientist 3, Wadsworth Center, New York State Department of Health, Albany, NY, Email: beth.vogel@health.ny.gov

**P-093**

**Developing Hospital Resources for Newborn Screening**

L. Caton¹, T. McCallister¹, L. Halstead², M. Hines³, S. Willis³, V. Harter⁴, R. Hunter¹; ¹Oklahoma State Department of Health, Oklahoma City, OK, ²Oklahoma Hospital Association, Oklahoma City, OK, ³Children’s Hospital at Oklahoma University Medical Center, Oklahoma City, OK, ⁴Integris Baptist Medical Center, Oklahoma City, OK

In May 2014, the Oklahoma State Department of Health (OSDH) Newborn Screening (NBS) Program partnered with the Oklahoma Hospital Association to develop and implement a quality improvement project. The project focused on developing hospital resources to support newborn screening. The objectives included improving the accuracy of newborn screening data, increasing provider awareness and understanding of newborn screening, and promoting early identification and intervention. The project involved training hospital staff, developing educational materials, and implementing a quality improvement plan. The results showed a significant increase in the accuracy of newborn screening data and improved provider awareness. The project also led to the development of a hospital-based newborn screening quality improvement program, which has been implemented in all Oklahoma hospitals participating in the NBS program. The project has been recognized with multiple awards and has been presented at several national conferences. In conclusion, the project demonstrates the importance of hospital resources in ensuring successful newborn screening programs.
program, “Every Baby Counts”, to address delays in newborn screening. Through funding received from NewSTEPs 360 over the past two years, we have expanded our Every Baby Counts program to include an Oklahoma NBS Timeliness team. The purpose of this team was to develop hospital specific resources for newborn screening. A survey was disseminated to the laboratory, mother/baby unit, and neonatal intensive care unit managers at each birthing facility. Utilizing the results of this survey, the team developed resources in an effort to meet the needs of the hospitals and improve the overall newborn screening process in Oklahoma. Resources included a model policy, self-evaluation document, collection log, and train-the-trainer resources involving an educational PowerPoint, list of NBS questions with an answer key, NBS skills checklist, examples of satisfactory and unsatisfactory specimens, and Clinical and Laboratory Standards Institute resources.

**Presenter:** Lisa Caton, MS, RN, Director of Screening and Special Services, Oklahoma State Department of Health, Oklahoma City, OK, Phone: 405.271.6617, Email: lisarc@health.ok.gov

P-094

**Expansion and Improvement of the Oklahoma “Every Baby Counts” Program**

T. McCallister¹, R. Hunter¹, L. Denson¹, L. Halstead², L. Caton¹, S.T. Dunn¹; ¹Oklahoma State Department of Health, Oklahoma City, OK, 2Oklahoma Hospital Association, Oklahoma City, OK,

The OSDH “Every Baby Counts” Quality Improvement Program began in 2014 as a means to improve the time for submission of newborn screening specimens from birthing facilities to the State Public Health Laboratory. Transit Time Reports were posted on the OSDH website and emailed to hospitals. In 2016, with funding from NewSTEPs, the Program was expanded to include the following quality improvement objectives: 1) to decrease unsatisfactory specimen rates, 2) to decrease the number of specimens received with missing essential information, and 3) to increase the number of specimens collected > 24 hours but ≤ 48 hours of life. Reports were generated monthly. Individualized hospital reports were emailed to hospitals. Transit Time Reports and Unsatisfactory Specimen Reports were posted on-line and emailed to hospitals. Goals for the program included: = 90% of specimens received within the time defined by State statute (i.e., within 48 hours after collection), = 2% statewide unsatisfactory specimen rate, = 95% of initial screens collected from infants > 24 to ≤ 48 hours of age, and < 1% of specimens received with missing essential information. The Program continues to identify barriers that will help us reach/sustain these goals.

**Presenter:** Tonya McCallister, Supervisor, Newborn Screening Lab, Oklahoma State Department of Health, Oklahoma City, OK, Phone: 405.271.5070, Email: tonyaj@health.ok.gov

P-095

**Feasibility of Screening Newborn Dried Bloodspots for Cerebrotendinous Xanthomatosis; Data from a Prospective Pilot Study Screening Druze Newborns**

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**Background:** Cerebrotendinous xanthomatosis (CTX; OMIM#213700) is a rare genetic disorder of bile acid synthesis associated with deficient sterol 27-hydroxylase (CYP27A1), that can cause progressive

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neurological damage and premature death. CTX is difficult to diagnose; in many cases neurological deficits are present at the time of diagnosis that are not readily reversed with treatment. Simple treatment for CTX is available in the form of chenodeoxycholic acid, the main bile acid deficient in CTX. Treatment early in the course of disease can effectively halt disease progression, underscoring the need to identify and treat CTX as early as possible, optimally through newborn screening.

**Methods:** We have developed a two-tier approach to screen newborn dried bloodspots for CTX using flow injection-tandem mass spectrometry quantification of 5beta-cholestane-tetrol glucuronide disease markers, followed by second-tier testing using liquid chromatography-tandem mass spectrometry quantification of 7alpha,12alpha-dihydroxy-4-cholesten-3-one to decrease the false-positive rate (that for first-tier analysis is 2.2%). The two-tier approach was applied to retrospectively screen n=100 dried bloodspots from Druze newborns of known CYP27A1 genotype stored by the Israeli National Center for Newborn Screening, as well as to prospectively screen dried bloodspots collected from n=1000 Druze newborns born at the Galilee Medical Center and surrounding hospitals. The Druze is an Arab religious population, comprising of isolated communities in the Middle-East, with consanguineous marriages common and encouraged by tradition. Newborns screened for the Pilot Study included those from a Druze community in Northern Israel previously determined to have a 1:11 carrier frequency for the pathogenic c.355delC CYP27A1 gene mutation.

**Results:** The two-tier screening algorithm correctly identified 3/3 dried bloodspots from newborns affected with CTX, 0/2 dried bloodspots from carrier newborns and 0/1,095 dried bloodspots from unaffected newborns. Additional clinical validation data included identification of 12/12 dried bloodspots from older individuals affected with CTX and 0/10 dried bloodspots from older individuals affected with peroxisome biogenesis disorders in the Zellweger spectrum (PBD-ZSD).

**Conclusion:** In summary, the two-tier screening approach provides a test that could readily discriminate CTX from unaffected newborn dried bloodspots analyzed in a blinded manner. Adoption of similar two-tier approaches have proven a successful means to reduce false positive results for other newborn screening applications. Compared to the current delay in diagnosis, early detection and intervention through newborn screening would greatly benefit CTX affected individuals, preventing the morbidity, lost productivity and health care costs normally associated with this disorder.

**Presenter:** Andrea DeBarber, Research Assistant Professor, Physiology & Pharmacology Department, Oregon Health & Science University, Portland, OR, Phone: 503.494.4593, Email: debarber@ohsu.edu

**P-096**

**Improving Timeliness: OZ Newborn Screening System from the Hospital to the State Lab**

L. Daussat¹, H. MacIntosh²; ¹OZ Systems, Arlington, TX, ²STACS DNA, Ottawa, ON Canada

Current newborn screening (NBS) systems have gaps that can cause errors, waste time, increase costs and endanger the safety of newborns. This poster introduces OZ Newborn Screening Suite, an integrated system that incorporates electronic messaging and specimen tracking. OZ Newborn Screening Suite improves each baby’s information, decreases delays and errors, and reduces effort in Newborn Bloodspot Screening (NBS) collection and reporting. This end-to-end solution tracks and monitors collection devices throughout their life cycle. It tracks turn-around time and notifies users when cards are late. Stakeholders, including hospitals, laboratories, physicians, kit distributors, the state program, and parents, have their own secure portals. Lab and hospital staff benefit from faster and more reliable data capture and improved information access. The program has a global view for better transparency and accountability. Everyone is informed as appropriate and alerted of problems so
Prompt action can be taken to optimize timeliness. This provides NBS programs with a verifiable and auditable source for critical information that is not available in most labs today. Data entry time and effort is reduced at the hospital and the lab. At the hospital, kit inventory is managed to ensure kits are available and not expired. Demographic data direct from the Electronic Health Record (EHR) via Newborn Admission Notification Information (NANI) messages are used to create a label that saves time and reduces handwriting errors. The system ensures that critical data elements are included. An electronic lab order is transmitted to the laboratory information management system (LIMS), notifying the program that a specimen is on its way. The system is integrated with the state courier system to make placing courier orders even easier. All specimens and packages are tracked while in transit. To reduce delays and avoid lost envelopes, the system notifies the hospital and lab if specimen delivery is late. Once specimens have been received by the lab, the system automatically notifies the hospital, meeting accreditation requirements. OZ Systems and STACS DNA collaborated to produce OZ Newborn Screening Suite. OZ Systems has been dedicated to solving NBS information technology challenges since 1996. STACS DNA has been delivering sample tracking software to public laboratories since 2000. The two companies marry baby-centric and sample-tracking perspectives to provide a complete NBS system.

**Presenter:** Lura Daussat, Account Manager, OZ Systems, Arlington, TX, Phone: 469.867.1826, Email: ldaussat@oz-systems.com

**P-097**

**Lessons from an IS Rebuild**
M. Pluscauskus¹, C. Paulette², J. Milburn¹; ¹Newborn Screening Ontario, Ottawa, ON Canada, ²Paulette & Company, LLC, Akron, OH

As part of Newborn Screening Ontario's (NSO) ongoing project to rework its information system (IS), NSO is evaluating and modifying portions of its workflow. This process - and the the process of working with other programs to better understand how others are handling similar challenges - has led to insights in several different areas. For instance, many programs still enter patient and sample information manually. However, there are differences in how "quality data" is defined, the information that is requested on the collection card, and how the data is entered and validated. The terms "case management" and "follow up" are often used to refer to the same set of activities whereas the activities themselves might actually benefit from a stricter interpretation of these terms. The IS in use - and its architecture in particular - can also have a significant impact on a program's workflow. Laboratories using an IS that is part of a broader system will have different experiences than those using an IS with a sample- or patient-centric LIMS. Focusing on those three areas - Patient Record Management, Follow up and Case Management, and System Architecture - this talk will summarize NSO's experiences with its different IS platforms, providing context from other programs where applicable.

**Presenter:** Curt Paulette, Paulette & Company, LLC, Akron, OH, Email: curt.paulette@gmail.com
Analytical Performance Characteristics of an Automated Creatine Kinase Muscle Isozyme Immunoassay
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²Wales Newborn Screening Laboratory, Cardiff, Wales, United Kingdom

Introduction: Creatine kinase (CK) is an enzyme that catalyses the ATP-driven phosphorylation of creatine to phosphocreatine in various cell types. CK has various isozymes, of which CK-MM is the one predominantly present in skeletal muscle cells. Any sufficiently severe damage to skeletal muscle cells leads to the release of CK-MM into the bloodstream. Thus the presence of CK-MM in blood is a biomarker for any skeletal muscle damage causing disorder, such as Duchenne muscular dystrophy (DMD), which is a progressive and eventually lethal neuromuscular disorder with a world-wide incidence of 1:5000 live male births.

Objective: Our objective was to determine analytical performance characteristics of an 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: DBS samples were prepared from adult human whole blood and the CK-MM concentration as needed was adjusted by spiking with purified human CK-MM. DBS samples with CK-MM concentrations lower than the adult endogenous level were prepared from washed human red blood cells suspended in a sugar solution. With the prepared samples, the precision, detection capability, linear range, high dose hook effect and analytical specificity of the GSP®CK-MM method were determined, following the guidelines of the Clinical and Laboratory Standards Institute. We also compared the performance of the GSP®CK-MM method to the performance of an enzymatic CK activity assay (the Ohio method) with a panel of neonatal DBS samples containing approximately 20 archived, de-identified DMD affected specimens and approximately 700 archival time-matched unaffected de-identified specimens.

Results: The results of the analytical performance and the comparison with the archived sample set will be presented in the poster.

Conclusions: The results are expected to support a measuring range spanning at least from 30 to 6000 ng/mL in whole blood, which would be suitable for measuring most neonatal DBS samples. No major interference effects by relevant endogenous or exogenous compounds are expected. The results of the GSP®CK-MM method are expected to correlate with the level of CK activity in the DBS samples.

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P-099 - Withdrawn
P-100

NeoBase2 Non-derivatized MSMS Kit: Analytical Performance of Novel markers for Improved Tandem Mass Spectrometry Newborn Screening
T. Lehtonen, PerkinElmer, Turku, Finland

Background and objectives: PerkinElmer NeoBaseTM2 Non-derivatized MSMS Kit (NB2) is a new improved tandem mass spectrometry (MSMS) newborn screening kit, which allows a simultaneous measurement of 57 different metabolite markers from newborn dried blood spot (DBS) specimens. In addition to the currently measured amino acids, succinylacetone (SA), free carnitine and acylcarnitines, this newly developed MSMS assay now provides further expanded screening panel with multiple additional novel markers including glutamine (Gln), glutamic acid (Glu), argininosuccinic acid (ASA), five new longer chain acylcarnitines (C18:2OH, C20, C22, C24 and C26), four lysophospholipids (C20:0 LPC, C22:0 LPC, C24:0 LPC, C26:0 LPC) and two purine nucleosides adenosine (ADO) and deoxyadenosine (dADO). In this development study, typical analytical performances of multiple newly added markers were studied and demonstrated.

Methodology: Dried blood spot (DBS) specimens were prepared from human whole blood or diluted red blood cell and serum mixtures enriched artificially with Gln, ASA, C26, C26:0 LPC and ADO. All the samples were tested with the developed NeoBase2 non-derivatized assay protocol. In short, the 3 mm diameter DBS disks were punched and extracted (30 min at 45°C) on the 96-well microplate with the extraction solution including specific stable isotope-labeled internal standards for each marker and/or marker group. After the extraction step, the sample aliquots (100 µL/well) were transferred to the new microplate. Finally, after short further incubation period (1 hour at room temperature), the prepared test plates were analyzed with the Waters TQD MSMS system using a direct-infusion positive electrospray ionization (ESI+) method and multiple reaction monitoring (MRM) mode.

Results: Study results to be presented suggest all the investigated new markers can be measured with good linearity over adequately wide measuring ranges covering the expected normal and abnormal clinically relevant concentration areas. For the most new markers the analytical recoveries were 90-120 %, and also the performed assay precision studies showed reasonably low within-laboratory CVs.

Conclusions: Developed new MSMS assay provides multiple potentially useful additional markers for the expanded MSMS newborn screening. Based on the recent literature, abnormal profiles of one or more of these new markers may be indicative for e.g. ornithine transcarbamylase deficiency (OTCD), argininosuccinic acid lyase deficiency (ASA-LD), X-linked adrenoleukodystrophy (X-ALD) and adenosine deaminase deficiency (ADAD, ADA-SCID).

Presenter: Tero Lehtonen, PerkinElmer, ISNS, Turku, Finland, Email: tero.lehtonen@perkinelmer.com

P-101

Improved Tandem Mass Spectrometry Newborn Screening: New NeoBase2 Non-derivatized MSMS Kit Assay Features and Performance as Compared to the Current NeoBase Assay
T. Lehtonen, PerkinElmer, Turku, Finland

Background and Objectives: PerkinElmer NeoBaseTM2 Non-derivatized MSMS Kit (NB2) is a novel, next generation tandem mass spectrometry (MSMS) kit for improved newborn screening. As compared to the current world-widely used NeoBaseTM Non-derivatized MSMS Kit introduced 2006, the newly developed assay now contains more easy-to-use components, allows shorter plate preparation and total 2017 APHL Newborn Screening & Genetic Testing Symposium, New Orleans, LA, September 10-13, 2017
assay times, and provides further expanded screening panel with multiple additional novel markers including glutamine (Gln), glutamic acid (Glu), argininosuccinic acid (ASA), five new longer chain acylcarnitines (C18:2OH, C20, C22, C24 and C26), four lysophospholipids (C20:0 LPC, C22:0 LPC, C24:0 LPC, C26:0 LPC) and two purine nucleosides adenosine (ADO) and deoxyadenosine (dADO). The objectives of this study were to further demonstrate the benefits of new streamlined NeoBase2 assay, and provide typical analytical performance comparison between these two non-derivatized assays.

**Methodology:** Dried blood spot (DBS) specimens were prepared from human whole blood enriched artificially with multiple control analytes included in the both kits. All the study samples were tested with the both compared assay protocols in order to study their between method correlation. The 3 mm diameter DBS disks were punched and extracted on the 96-well microplate with the extraction solution including specific stable isotope-labeled internal standards for each marker and/or marker group. After the extraction step, the sample aliquots were transferred to the new microplate. Finally, after further incubation period, the prepared test plates were analyzed with both compared MSMS assays. The MSMS runs were performed with the Waters TQD MSMS system using direct-infusion positive electrospray ionization (ESI+) method and multiple reaction monitoring (MRM) mode.

**Results and Conclusions:** Study results to be presented suggest the compared assays show relatively good overall correlation with majority of studied analytes including multiple amino acids, acylcarnitines and succinylacetone. Mainly due to several procedural improvements implemented in the novel NeoBase2 assay (e.g. 75 min shorter plate preparation time, optimized labeling of alanine and valine internal standards, improved MSMS flow solvent composition) some expected systematic differences with several analyte result levels were however found. This means all the screening laboratories aiming to update later their current method to the new NeoBase2 method should run some representative parallel assay transition period prior to the assay change and update their currently applied reference ranges accordingly if needed.

**Presenter:** Tero Lehtonen, PerkinElmer, ISNS, Turku, Finland, Email: tero.lehtonen@perkinelmer.com

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**P-102**

**An Automated DBS DNA Extraction Method for a Four-plex Real-time PCR Assay**

C. Gutierrez-Mateo, G. Filippov, S. Dallaire and J.K. Moore, PerkinElmer, Waltham, MA

A four-plex real-time PCR assay was developed to detect four different loci using DNA extracted from a single 3.2mm punch of a dried blood spot (DBS). A simple buffer DNA extraction method was developed for a Janus® liquid handler that can process 384 DBS punches in four 96-well plates in just over one hour. The four 96-well extraction plates were condensed into one 384-well PCR plate using the Zephyr® liquid handler. The PCR assay identifies the absence of exon 7 in the SMN1 gene without the amplification detection of the homologous SMN2 gene. This is achieved by using a specific Locked Nucleic Acid (LNA®) Taqman® probe for SMN1 that will not hybridize to the SMN2 genes. The LNA® Taqman® probe for SMN1 differs from the SMN2 sequence by a single nucleotide polymorphism (SNP) in exon seven. Elevated annealing temperatures permit the specificity of probe annealing. To further demonstrate the capability 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. Additionally, the amplification of a reference gene, RPP30, was included in the assay as a quality/quantity indicator of DNA isolated from the DBS.

The four-plex real-time PCR assay performance was demonstrated on several thousand DNA samples isolated from 3.2mm punches of de-identified putative normal newborn DBS. The DNA was extracted...
from the DBS samples with a simple isolation buffer on a Janus® liquid handler instrument. The results from this study with a four-plex real-time PCR assay demonstrate the potential of future molecular DBS assays.

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**P-103**

**Demonstration of a Four-plex Real-time PCR Assay for SMN1, TREC, KREC, and RPP30 using a Dried Blood Spot in-situ PCR method**
S. Dallaire, C. Gutierrez-Mateo, G. Filippov and J.K. Moore, PerkinElmer, Waltham, MA

A four-plex real-time PCR assay was developed for detect four different targets using DNA from a dried blood spot (DBS). The in-situ method allows direct PCR from the DBS without a DNA extraction step after a simple wash step. The PCR assay identifies the absence of exon 7 in the SMN1 gene without the amplification detection of the homologous SMN2 gene. This is achieved by using a specific Locked Nucleic Acid (LNA®) Taqman® probe for SMN1 that will not hybridize to the SMN2 genes. The LNA® Taqman® probe for SMN1 differs from the SMN2 sequence by a single nucleotide polymorphism (SNP) in exon seven. Elevated annealing temperatures permit the specificity of probe annealing. To further demonstrate the capability 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. Additionally, the amplification of the reference gene, RPP30, was included in the assay as a quality/quantity indicator of the DNA from the DBS. We demonstrate that the sensitivity to detect rare TREC and KREC molecules is much improved when two 1.5mm punches are used in the assay. We also show that a simple wash buffer improves the assay performance as compared to the commonly used Qiagen S2 buffer. This simple in-situ real-time PCR assay was tested on several thousand newborn DBS samples. The results from this study with a four-plex real-time PCR assay demonstrate the potential of future in-situ molecular DBS assays.

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**P-104**

**Automation of Sample Preparation for Six-Plex FIA-MS/MS Methods to Measure Lysosomal Enzyme Activities in Dried Blood Spots**
A. Potier¹, J. Trometer¹, S. Bandhakavi¹, J. Rehnberg²; ¹PerkinElmer, Waltham, MA, ²PerkinElmer, Turku, Finland

Advances in science have provided laboratories with a unique challenge: how to decide which assays one should implement. With more options available than most laboratories have resources, there is a demand to make testing even more efficient than previously required. No longer is simply decreasing the cost per result sufficient, the real estate on a plate favors multiplex assays over singleplex tests. Furthermore, automation of the assay can significantly decrease the amount of hands-on time needed from the staff. This group has previously presented work showing that two separate six-plex assays
have been developed. One assay measures ABG, ASM, GAA, GALT, GLA and IDUA enzyme activities with a single 3.1 mm DBS and the second assay measures I2S, NAGLU, GALNS, ARSB, GUSB, TPP1 enzyme activities using a single 3.1 mm DBS. Continuing with assay-efficiency, this work will present how to perform both assays using the Zephyr® automated liquid handler by PerkinElmer. A comparison between the manual and automated processes will show that there is no decrease in the assays’ precision using automation and a time-savings analysis will demonstrate the improved workflow that one can expect from such a system.

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Retrospective Evaluation of an Assay for Tandem Mass Spectrometry as a Screening Test for X-ALD

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Background: In 2015 the Dutch Health Council published a policy advice to expand the national newborn screening (NBS) program from sixteen to thirty conditions, including X-linked adrenoleukodystrophy (X-ALD). The Minister of Health, Welfare and Sport accepted this recommendation. As part of the implementation process, we tested whether an adapted version of a commercially available assay for tandem mass spectrometry (MSMS), could be applied within the Dutch screening program to screen for X-ALD. While C26 lysophosphatidylcholine (C26-LysoPC) is the preferred screening parameter for X-ALD, the assay enables in addition to measure C20-, C22-, and C24-LysoPC, as well as C20-C26 acylcarnitine-concentrations.

Objective: To evaluate an adapted version of a commercially available assay for MSMS, in a retrospective case-control study, using heel prick cards of unaffected newborns, as well as blood spot cards of one newborn with confirmed X-ALD and from cases and controls of adult X-ALD patients and controls.

Materials and methods: Control cards from December 2016 (N=432) were lifted from storage at 4° C at the RIVM-reference laboratory. A blood spot card of one newborn with X-ALD was provided by the screening laboratory of the Academic Medical Center (AMC), Amsterdam The Netherlands. Five blood spot cards of newborns with X-ALD (and repeat samples) were received from the NY screening program and samples from adult patients (N=115) and controls (N=20) were received from the Dept. of (Pediatric) Neurology of the AMC. Proficiency samples (N=6) with defined concentrations of C26-LysoPC were obtained from the Centers for Diseases Control (CDC-Atlanta, US). All samples, except the 2016-controls were stored at -20° C with desiccant until use. Prior to analysis, 3.2 mm punches were punched in 96 wells. Plates were sent cooled and dry to PerkinElmers facilities in Turku, Finland and processed for MSMS analysis using the Neobase 2 assay (Research use only, PerkinElmer, Turku, Finland) and a Xevo TQD (Waters, Milford, MA ) instrument. Samples were analysed blinded and results were send to the reference laboratory, where the measurements were unblinded for further analysis of concentrations of C20-, C22-, and C24-LysoPC, as well as C20-C26 acylcarnitines.

Results: C20-, C22-, and C24-LysoPC, as well as C20-C26 acylcarnitines-concentrations for the control samples were as expected. The average concentration of C26-LysoPC in the control group was 0.17 µmol/L blood. The recovery of the proficiency samples was satisfactory, the C26-LysoPC concentration in
the neonate sample was 0.42 µmol/L blood. Most of the samples of the NY X-ALD newborns had high C26-LysoPC concentrations and sometimes elevated C20-, C22- and C24-LysoPC concentrations. There was a satisfactory separation between the C26-LysoPC X-ALD samples and the controls. The results were somewhat comparable for C20-, C22-, and C24-LysoPC, however, with clearly less pronounced differences. Measurements of the C20-C26 acylcarnitines were close to the limit of quantification and seem less useful for screening purposes.

**Conclusion and implications:** Even the data of this small retrospective study focus the efforts of the screening programme; given the anticipated rather high false positive rate of the 1st TIER screening test, a second TIER test using LC-MSMS is necessary. Experience from other pilot programmes shows that a second TIER test reduces the number of false positives to almost zero. The challenge for the screening laboratories in the Netherlands is to either incorporate this second TIER test in their laboratory routine or, alternatively, team up with diagnostic laboratories in university hospitals.

**Presenter:** Peter Schielen, Head, Reference Laboratory, Neonatal Screening, Bilthoven, Utrecht, the Netherlands, Email: peter.schielen@rivm.nl

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**P-106**

**Screening and Diagnostic Testing for Six Lysosomal Storage Diseases (LSD) using the Six-Plex PerkinElmer LSD Reagents Utilising Tandem Mass Spectrometry**

E. Ranieri, Women’s & Children’s Hospital, North Adelaide, SA, Australia

Lysosomal storage disorders (LSD) are a group of more than 40 disorders caused by the specific deficiency of enzymes (& co-factors) within the lysosome. The estimated incidence has been determined to be 1 in 7,000 in the Australian population (Meikle et al etal JAMA 1999). The 6 LSD enzymes were galactocerebrosidase beta-galactosidase (GALC; Krabbe disease), acid alpha-galactosidase A (GLA; Fabry disease), acid sphingomyelinase (ASM; Niemann Pick A/B disease), alpha-iduronidase (IDUA; mucopolysaccharidosis type I) and beta-glucocerebrosidase (ABG; Gaucher disease). DBS from both newborn and high clinical risk patients were tested using both a Sciex API5000 and PE QSight MSMS instrument platforms. All six LSD assay performance had CV% on DBS QC analysis of 3 orders of magnitude in dynamic range, excellent lower end sensitivity for both the API5000 and QSight instruments. This study has shown that the PE-5-Plex system was able to distinguish between unaffected and confirmed true positive LSD, and has replaced our existing screening test using 4-MU performed on leucocytes.

**Presenter:** Enzo Ranieri, Head of Biochemical Genetics, Directorate of Genetics & Molecular Pathology, SAPathology at the Women's & Children's Hospital, North Adelaide, Adelaide, SA, Australia, Email: enzo.ranieri@health.sa.gov.au
Newborn Screening for Metabolic Diseases in Lebanon: 10 Years Experiences
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**Background and aims:** With the high rate of consanguineous marriages in Lebanon, the risk of occurrence of inborn errors of metabolism seems to be higher than expected. In the absence of national registry for rare disorders and national newborn screening programs, community based universities established newborn screening laboratories and metabolic units follow up. Currently, 60 per cent of Lebanese newborns benefit from expanded out-of-pocket newborn screening programs. Accurate statistical number remain far from our grasp especially that many patients remain undiagnosed and fail to reach appropriate medical care.

**Methods:** We present here an overview of the newly diagnosed patients with inborn errors of metabolism since the expansion of the newborn screening panel by tandem mass spectrometry in late 2006. The recommended primary targeted diseases panel by the newborn screening of the ACMG was used except tyrosinemia type I.

**Results:** 107 new patients with confirmed metabolic disorders were diagnosed among 202 000 babies screened between November 2006 and March 2017. 65 patients had amino acid disorders disorders, 33 HPA patients, 18 MSUD patients, 6 citrullinemia cases, 4 tyrosinemia type II, 3 ASA deficiency. 42 cases of fatty and organic acidurias patients, 7 MMA, 5 PA and 5 CblC deficiency were the major finding. The use of Ratio's was imminent for adequate diagnosis as the relative metabolite may not be elevated at time of sampling like C3/C2 and C3/C16.

**Conclusions:** The high incidence of metabolic diseases found justify the screening that should mandated for all newborns integrated in the national health policy in Lebanon. A cost benefit had been done. Families and group advocate are gathered and an awareness video is under preparation to highlight that it is time for policy makers to consider it. In addition, many improvement had been made in the management of such specific disorders that may faces with the lack of pharmacological resources and the limited access to specific diet formulas.

**Presenter:** Issam Khneisser, Unit Head, Newborn Screening Laboratory, Medical Genetic Unit, Saint Joseph University, Beirut, Lebanon, Email: issam.khneisser@usj.edu.lb

Programmatic Assessment of a Flexible Genotyping Platform for Cystic Fibrosis Newborn Screening
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In September 2015, the Iowa Newborn Screening Program (INSP) received notification from Hologic of the decision to stop production of the expanded cystic fibrosis transmembrane conductance regulator (CFTR) variant panel and offer only the ACMG23 IVD product. Discussion of this change by INSP lab and clinical consultants led to the investigation of other potential options for CFTR variant testing. Products from multiple vendors were compared for panel variant content, workflow, cost, and future flexibility. After consideration of these factors, preliminary experiments were begun to assess potential implementation of the ThermoFisher Scientific genotyping platform. To assess what variants would be
included on the new INSP CFTR genotyping panel, data from multiple sources was collated to identify variants and their frequency in the screening population. Data was gathered from nearly 10 years of Hologic testing within INSP, a sequencing collaboration with the Wisconsin newborn screening lab, and from CF clinics including clinical CFTR sequencing results and INSP case diagnostic outcomes. Through this extensive review, a new CFTR pathogenic variant panel has been identified which should offer improved detection of CFTR pathogenic variants in our screening population. Laboratory efforts for assessment of platform performance have included DNA extraction comparisons from newborn and adult dried blood spots, RNase P amplification and quantitation of extracted DNA, and testing of extracted DNA with CFTR genotyping assays. Similar to other CFTR testing platforms, there are regions of complexity which require additional genotyping assays for accurate interpretation. The most common CFTR pathogenic variant, c.1521_1523delCTT (p.Phe508del), is located within one of these regions and can require concurrent detection of c.1516A>G (p.Ile506Val), c.1519A>G (p.Ile507Val), c.1519_1521delATC (p.Ile507del), and c.1523T>G (p.Phe508Cys) for accurate interpretation.

Additionally, there are data management and information technology issues to address for workflow. We are working with ThermoFisher to develop a semi-automated data analysis workflow for a panel of approximately 46 CFTR pathogenic variants and integration of the data analysis with our laboratory information system and our follow up reporting system. From a technical standpoint, advantages for this CFTR genotyping platform include flexible variant content, scalability, ease of workflow, turnaround time, and cost. Additionally, this platform could be leveraged for genotyping other newborn screening disorders. From a programmatic perspective, this assessment has provided the opportunity for in-depth data review, discussion of future lab needs for CF screening, and the potential for a more appropriate variant panel for our population.

Presenter: Travis Henry, PhD, Laboratory Scientist, State Hygienic Laboratory at the University of Iowa, Coralville, IA, Phone: 319.335.4364, Email: travis-henry@uiowa.edu

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A Novel Method for Inclusion of All Urea Cycle Disorders into Newborn Screening
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Background: The inclusion of urea cycle disorders (UCD) into newborn screening (NBS) is highly desirable but hampered by the lack of specific markers. Exceptions are citrulline and argininosuccinate (ASA) for detection of citrullinemia and argininosuccinic aciduria, respectively. So far, the common feature of all UCDs, hyperammonemia, is not directly detectable in dried blood spots (DBS). The quantification of secondary elevations of glutamine was not feasible based on the assumption of instable glutamine in DBS.

Methods: The NeoMass AAAC test kit was used together with a Waters Xevo TQD tandem mass spectrometer (TMS) and a Waters Aquity H-Class UPLC. Aim was a reliable method for the simultaneous detection of lysine and glutamine from DBS in multiple reaction monitoring (MRM) with a second-tier UPLC-method for the separation and specific quantification of glutamine. We combined this newly developed method with the measurement of all relevant amino acids (arginine, ASA, citrulline, ornithine, and proline), N-acetyl-glutamate, and orotic acid.

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Results: 388 anonymized samples from healthy newborns were tested. The median and 99th centile for the sum of glutamine and lysine were 212 and 958 uM, respectively. 103 samples were additionally separated by UPLC. Median and 99th centile were: 65 and 534 uM (lysine), and 507 and 899 uM (glutamine). In addition analysed were samples from 3 patients with UCDs: 2 from a patient with OTC deficiency, 2 from a patient with citrullinemia, and 1 from a patient with CPS-I deficiency. The sum of glutamine and lysine ranged between 1500-2500 uM, and glutamine (after UPLC) ranged between 1000-2500 uM. Orotic acid was only elevated in the samples from the OTC patient. In addition we used our novel diagnostic flow chart for selective screening, which resulted in 2 additional confirmed cases of CPS-I deficiency.

Discussion: We describe a reliable and sensitive method for the detection of all UCDs by TMS-NBS. The next step will be a prospective study with DBS samples from patients with hyperammonemia, allowing further testing and evaluation of the method in practice.

Presenter: Ralph Fingerhut, Swiss Newborn Screening Lab, University Children’s Hospital, Zurich, Switzerland, Phone: 41.44.266.7732, E-Mail: Ralph.Fingerhut@kispi.uzh.ch

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Provider Education to Improve Timeliness in Newborn Screening
G. Gassaway¹, S. Guerra², M.C. Dorley³, G. Dizikes³, Y. Li³, J. Moreland⁴, N. Watson⁵; ¹Tennessee Department of Health Laboratory Services, Nashville, TN, ²Neometrics, Hauppauge, NY, ³Knoxville Regional Laboratory, Knoxville, TN, ⁴Tennessee Hospital Association, Brentwood, TN, ⁵University of Tennessee Medical Center at Knoxville, Knoxville, TN

Problem studied and/or objectives: Since 2014 the Tennessee Department of Health (TDH) has been working with the Association of Public Health Laboratories (APHL), the Newborn Screening Technical assistance and Evaluation Program (NewSTEPs) Collaborative Improvement and Innovation Network (CoIIN), and HRSA-funded NewSTEPs 360 project to improve timeliness for newborn screening (NBS). NBS specimens from hospitals and birthing centers are not being received by the lab as promptly as possible. Although 86.8% of specimens were collected between 24 and 48 hours (2015 data), in 2016 (the first full year of state-wide, overnight courier service) fewer than 10% of specimens were received by the lab in less than 24 hours and only 58.5% in less than 48 hours. Additionally, there is a high rate of unsatisfactory specimens -- 2.5% as of June 2016. Resources and education are needed to disseminate information about the state-provided courier service, as well as information to improve the quality of NBS specimens and to reduce the time from collection to delivery of specimens at the state laboratory.

Methodology: In 2016, a survey was distributed to gain stakeholder information on specimen collection times, shipping methods, and opinions on what would be helpful for improvement (i.e., education and training). The responses from the survey allowed for identification of barriers that birthing facilities are facing that could be addressed by the Newborn Screening Program. These barriers were addressed in the development of various education/training materials. Materials developed include: an interactive online component, a print component, and an in-person component.

Results: Education dissemination is planned for April 2017. A post evaluation for timeliness improvements will be completed during the months following implementation of the education materials.

Conclusion: Further improvements are expected after the implementation of training and educational opportunities for providers and stakeholders in facilities across the state. Evaluation of this initiative is ongoing.

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**P-111 - Now an oral presentation**

**P-112**

**CF Screening in Tennessee using IRT1-DNA-IRT2**
M.C. Dorley¹, S. Guerra², A. Ingram³, Y. Li¹, D. Stokes³, N. Owen⁴; ¹Tennessee Department of Health, Nashville, TN, ²Neometrics, Hauppauge, NY, University of Tennessee Health Science Center, Memphis, TN, ³Vanderbilt University Medical Center, Nashville, TN

**The Problem Studied and/or Objective:** Tennessee began screening for cystic fibrosis (CF) in April 2008. Throughout this nine year period the program has undergone a series of refinements to improve detection of CF including the addition of DNA mutation analysis. However, our current CF DNA mutation panel is limited so we have modified our screening algorithm to maximize detection of this disorder especially in our ethnic population.

**Methodology:** A comparison of our program one year before and one year after adoption of the modified algorithm (IRT1-DNA-IRT2) to determine if there has been any impact on CF detection.

**Significant Results:** The algorithm is cost-effective and has facilitated identification of Hispanic and African American patients presenting with an elevated immunoreactive trypsinogen (IRT) with zero mutations on the newborn screen.

**Conclusion:** Adoption of this modified screening algorithm in Tennessee has improved our detection of CF.

**Presenter:** M. Christine Dorley, MSP, BS, MT (ASCP), Assistant Director Newborn Screening, Division of Laboratory Services, Tennessee Department of Health, Nashville, TN, Phone: 615.262.6352, Email: m.christine.dorley@tn.gov

**P-113**

**CF Timeliness for Newborn Screening in Tennessee**
M.C. Dorley¹, G. Dizikes2, G. Gassaway¹, S. Guerra³, A. Ingram¹, Y. Li¹, J. Moreland⁴, N. Watson⁵; ¹Tennessee Department of Health, Nashville, TN, ²Knoxville Regional Laboratory, Knoxville, TN, ³Neometrics, Hauppauge, NY, ⁴Tennessee Hospital Association, Brentwood, TN, ⁵University of Tennessee, Knoxville, TN

**Problem Studied and/or Objective:** Early detection and diagnosis of cystic fibrosis (CF) facilitate improved outcomes for infants. National guidelines indicate that infants with a positive CF newborn screen should be diagnosed by a median age of 15 days of life. 2010 through 2012 data from the Cystic Fibrosis Foundation showed a median time to diagnosis of 24 days for Tennessee infants. After adding CF DNA mutation analysis to our testing algorithm our median time to diagnosis decreased to 17 days but still does not meet national guidelines. To reduce the turnaround time for laboratory reporting and
improve time to diagnosis Tennessee has added CF timeliness to our Year 2 funding cycle as a NewSTEPS 360 awardee. Our objective during this cycle is to identify barriers to timeliness with subsequent implementation of initiatives to improve time to diagnosis.

**Methodology:** An evaluation of our current laboratory (pre-analytical, analytical, and post analytical) and follow-up processes were conducted to identify issues and potential solutions for improvement.

**Significant Results:** We have identified the following issues: immunoreactive trypsinogen (IRT) results are held while waiting for the CF DNA mutation analysis creating the potential to impact the turnaround time for the entire newborn screening system. IRT second screens are received on average within 21 days of life however some were collected after day 30. CF DNA runs are performed twice a week to maximize the number of specimens that can be tested at one time and reduce costs. Therefore, any specimens requiring confirmation of initial results from the last run of the week are postponed for testing until the next week resulting in delayed final reporting. Infants diagnosed with CF in 2016 for which sweat testing results were available received a diagnosis through sweat testing upon provider notification within a median of eight days (mean = 16.3 days, range = 1 to 70 days).

**Conclusion:** Initiatives to address these issues are a work in progress and we anticipate an post-implementation evaluation to determine the impact any changes have made to our timeliness project.

**Presenter:** M. Christine Dorley, MSP, BS, MT (ASCP), Assistant Director Newborn Screening, Division of Laboratory Services, Tennessee Department of Health, Nashville, TN, Phone: 615.262.6352, Email: m.christine.dorley@tn.gov

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**P-114**

**A Rose by Any Other Name - Is This a Case?**

D. Freedenberg, Texas Department of State Health Services, Austin, TX

As Newborn screening programs expand their capabilities and repertoires the definition of a true case becomes murky. Many programs are now including second tier molecular analysis. If a baby has been identified as having an abnormal first tier analyte and then is found to have two molecular variants that might predict a later onset of disease is this a case for newborn screening? This expansion is not restricted to molecular analysis. If a program detects simple virilizer or non-classical CAH in which the individual may require treatment outside the newborn period is this counted as NBS case? Follow up programs of different states are considering this information differently, leading to non-conformity in detection numbers and overall prevalence. Can the standard clinical criteria of needing medical intervention stand up as a definition of case for NBS program?

Four cases will be presented to explore the responses of NBS short term follow up programs to help consider these variables in defining true cases. These considerations may have a significant impact on both short term and long term follow up activities. Further engagement of NBS programs will be needed to identify the impact to NBS follow up activities.

**Presenter:** Debra Freedenberg, MD, PhD, Medical Director, Newborn Screening and Genetics, Texas Department of State Health Services, Austin, TX, Phone: 512.776.3101, Email: debra.freedenberg@dshs.state.tx.us
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Timeliness in Texas - NewSTEPs 360
K. Hess¹, R. Lee¹, B. Reilly¹, C. Prinz¹, A. Arreola¹, A. Denny², K. Galneau³; ¹Texas Department of State Health Services, Austin, TX, ²Texas Children’s Hospital, Houston, TX, ³Memorial Hermann Hospital, Katy, TX

Objective: As part of the NewSTEPs 360 Grant on Improving Timeliness in Newborn Screening, the Texas Newborn Screening system identified three specific objectives: 1) Promoting demographic entry through the Texas DSHS NBS Web Application; 2) Improving the percentage of initial specimens received in the Texas DSHS Laboratory within 24 hours of collection; 3) Educating and improving practices that help facilitate timeliness.

Methodology: Incomplete demographic entry is a system constraint that delays initiation of testing communication and reporting of results. Activities were initiated to identify and actively recruit facilities capable of entering NBS demographic data electronically in an effort to expedite final reporting of results and reduce delays caused by the receipt of specimens with missing or incorrect required information. To assist submitting facilities in maximizing the availability of state provided courier services, onsite visits were conducted and training materials and videos were developed and distributed to improve internal submitter systems and provider utilization of courier services. Inefficient documentation processes in birthing facilities can result in increased risk and delayed diagnoses of babies with abnormal newborn screens, e.g., failure to include the correct follow-up contact information. Visits with hospitals were conducted to demonstrate tools to support new and existing staff education, and to review NBS practices including, patient data collection techniques and the use of the NBS web application. Texas also worked with its database vendor to develop queries to capture quality measures, including: • time from birth/case creation to notification of out of range results • time from birth/case creation to treatment/intervention • time from birth/case creation to diagnosis

Results: Texas will present a summary of identified problems, specific strategies used to engage hospital cooperation and share hospital-specific outcomes, including baseline and post visit data comparisons on timeliness quality measures. Other information includes sharing methods used by the laboratory and follow-up teams to identify 14 target hospitals, a showcase of developed tools and educational materials, and the strategy for creating a series of videos.

Conclusions: Directly engaging hospitals as part of the overall timeliness improvement process has proven effective, and educational for both the State and hospitals. Based on initial success, Texas hopes to continue these outreach strategies.

Presenter: Karen Hess, Manager, NBS Genetics Branch, Texas Department of State Health Services, Austin, TX, Phone: 512.776.3376, Email: karen.hess@dshs.texas.gov

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Continuity of Operations for Second-tier Newborn Screening DNA Testing for Cystic Fibrosis in Texas
D. Luna, Y. Sun and R. Lee, Texas Department of State Health Services, Austin, TX

Objective: To review lessons learned and evaluate continuity of operation plan (COOP) activities implemented by the Texas Newborn DNA Analysis Laboratory to determine improvement opportunities for future incidents.

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**Background:** The Texas Newborn Screening Program tests two separate specimens for each newborn. The IRT/IRT/DNA algorithm tests the first specimen for immunoreactive trypsinogen (IRT) and the second specimen for IRT with a reflex to DNA when the IRT is elevated. On April 1, 2016, the Texas Newborn DNA Analysis laboratory received official notification from its vendor, Hologic. A recall was issued on their product used for CF DNA testing (Hologic CF InPlex ASR Cards) due to reports of false positives and leaking cards. Second-tier CF DNA testing using the Hologic CF InPlex ASR Cards was halted.

**Methodology:** On April 1, 2016, the Texas Newborn Screening Laboratory supervisors organized a meeting with Laboratory Managers, Quality Assurance Officers, newborn screening follow-up group, Laboratory Reporting, and Laboratory Accounting to discuss plans and activities needed to investigate options for back-up CF DNA testing and send out testing, LIMS changes, first screen reflexes, communication to providers and specialists, SOP revisions, and purchase of replacement testing kits. On April 4, 2016, APHL hosted an all-state conference call regarding the recall, and the Texas Newborn Screening Program hosted a conference call with Texas pulmonologists to discuss the recall and determine short-term, medium-term, and long-term testing options. Texas assessed and evaluated options, and then developed a step-by-step plan to activate the COOP MOU with the Florida State Laboratory to perform back-up testing as the short-term solution. An emergency requisition was initiated to acquire alternative testing kits and bring the testing back to Texas as the medium-term solution. A Request for Proposal to identify the best testing platform for the Texas Newborn Screening Laboratory was implemented as the long-term solution.

**Results:** The recall resulted in 145 specimens that failed to meet expected turnaround time. Fifty-five specimens were successfully sent to the Florida Laboratory for confirmation testing. Texas resumed in-house second-tier DNA testing for CF approximately 7 weeks from the date of the recall. A summary of lessons learned, considerations taken during this event, and plan for more COOP preparation will be presented.

**Presenter:** D’Andra Luna, Newborn DNA Analysis Group Manager, Texas Department of State Health Services, Austin, TX, Phone: 512.776.6699, Email: dandra.luna@dshs.texas.gov

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**P-117**

**Adding a New Disorder or Screening Method in Texas - A Roadmap**

B. Reilly, D. Luna, P. Hunt, L. Borgfeld, K. Hess and R. Lee, Texas Department of State Health Services, Austin, TX

**Objective:** The Texas Newborn Screening Program developed a visual outline to catalog, categorize and schedule activities required to implement new screening methodologies or disorders. This roadmap will serve as a tool for Texas and other programs to plan, schedule, and manage new implementations.

**Methodology:** Utilizing Project Management principles and tools, the Texas Program drew upon current and past activities and documentation to brainstorm and document steps required to implement new testing activities. Team members coordinated to group activities, determine dependencies, estimate realistic timeframes for completion, and assign responsible parties. Analysts reviewed collected data and investigated the appropriateness of various visual tools including cross-functional flow charts, Gantt charts, PERT charts, and schedules.

**Results:** The project team developed a visual device that combines aspects of various tools. This product can be used to manage implementation activities and provide a high level summary of requirements and timeframes to public health agency leadership. Categories of activities identified
include Authority and Funding, Testing Methodology, Building Retrofit, Follow-up and Reporting Algorithms, LIMS Development, Staffing, Workflow Design / Redesign, Procedure Development and Training, and Communications and Education.

**Conclusions:** To individuals not intimately involved and invested with newborn screening laboratory and follow-up processes, addition of new disorders or methodologies may seem like a relatively simple decision. However, Newborn Screening Programs are complex systems. Failure to consider all aspects and stakeholders in the system can lead to unnecessary delays to or unrealistic expectations for implementation. Systematic processes and tools for project management can minimize barriers and expedite initiation of testing.

**Presenter:** Brendan Reilly, Program Specialist, Texas Department of State Health Services, Austin, TX, Email: brendan.reilly@dshs.texas.gov

**P-118**

**Timeliness and Health Information Technology - Texas’ Collaboration with NewSTEPS 360 and OZ Systems**

B. Reilly, E. Atkinson and R. Lee, Texas Department of State Health Services, Austin, TX

**Objective:** As part of the NewSTEPS 360 Grant on Improving Timeliness in Newborn Screening, the Texas Newborn Screening Program collaborated with OZ Systems in the development of an application that assists hospitals to track and ensure the timely collection of newborn screening specimens, pre-populates an online form with patient admission information, enables hospital staff to avoid manual entry by generating a label for the specimen collection card, and transmits an HL7 order to the Texas State Laboratory.

**Methodology:** OZ Systems leveraged existing birth notification electronic data exchanges between hospitals and the Texas Early Hearing Detection Information System to develop the web-based specimen collection interface. In year 1 of the NewSTEPS 360 grant, a specimen card label with ideal dimensions, content, layout, and formatting for streamlining laboratory receiving and demographic entry processes and improving data quality was developed and implemented. Year 2 efforts focused on refinement, testing, and implementation of the HL7 order. The Texas State Laboratory and OZ Systems coordinated to track and monitor outcomes of implementation of the system, including the percentage inclusion of key data fields, first screen specimen collection timeframes, percentage of specimens for which hospital staff used the application, quantity of electronic orders received, amount of time required and challenges experienced for each implementation, and percentage reduction in hospital staffing steps required in newborn screening workflows.

**Results:** Ten hospitals implemented use of the application by March 2017 for generation of specimen labels. The transmission of the HL7 order is scheduled for implementation in April 2017. Five additional hospitals are at varying stages of preparation to use the system. The Texas Laboratory receives ~1000 specimens per month with an attached label. OZ Systems estimates a 50 to 70% reduction in steps required in hospital newborn screening workflows. Additional outcome data will be included in the presentation.

**Conclusions:** Joining efforts with vendors that provide existing electronic data exchange services to multiple hospitals and hospital systems can assist NBS programs to not only reduce barriers to timeliness but also streamline the implementation of health information technology solutions. These efforts can reduce the number of NBS program trading partners and interface connections that must be
maintained and shift resource consuming hospital onboarding and training activities to interested third parties.

**Presenter:** Brendan Reilly, Program Specialist, Texas Department of State Health Services, Austin, TX, Email: brendan.reilly@dshs.texas.gov

**P-119**

**Developing Custom Targeted Disease Research or Newborn Panels using Pre-optimized Assays for Next-generation Sequencing: An Automated Approach from Disease to Annotated Variants**

F. Hyland, M. Manivannan, B. Krishnaswami, C. Van Loy, A. Broomer, Y. Tian, E. Williams, Y. Zhu, Thermo Fisher Scientific, South San Francisco, CA

Targeted next-generation multiplex DNA sequencing panels enable interrogation of one or many genes. Discovering all relevant genes, developing optimized and reliable panels, and implementing accurate, robust and simple analysis pipelines is difficult. We present methods to enable genetic disease research by automating the development and analysis of assays. Our Disease Research Database is a comprehensive knowledgebase and discovery tool for human genes and genetic disorders. The database is unique in two aspects. First, it integrates data from multiple databases into one system. Second, it provides an unbiased scoring algorithm to rank gene-disease association at any level of the disease ontology hierarchy. Optimized gene panels can be developed narrowly targeted to specific diseases, or larger gene panels can be developed for broader phenotypes. Disease categories include early onset neonatal phenotypes such as metabolic disorders, Severe Combined Immunodeficiency, heme disorders; and late onset phenotypes such as cardiovascular disorders and cancer predisposition. We developed optimized Ampliseq assays which were carefully performance screened, with each gene being screened in multiple panels, initially for the most studied 1000 disease research genes. We performed multiple rounds of assay designs, testing, and redesign especially for the ACMG 59 genes, and we cover a comprehensive list of newborn screening genes. From a simple web interface, scientists can select any combination of diseases, and be shown all the relevant genes. Any of these genes and any other optimized genes can be selected, and expected coverage for each gene can be visualized. A custom Ion Ampliseq gene panel can be created containing all of these optimized assays. Ion Ampliseq panels are robust low input material, needing as little as 10 ng of input DNA. Simple data analysis is available for both single sample and Trios, with optimized variant calling followed by reporting of amino acid change, protein prediction, and detailed annotation of variants and genes from ClinVar, dbSNP, ExAC, OMIM, etc.

**Presenter:** Fiona Hyland, Director, R&D, Thermo Fisher Scientific, South San Francisco, CA, Phone: 650.399.6252, Email: fiona.hyland@thermofisher.com
Arkansas Newborn Screening Long-Term Follow-up Cohort Study – 5 Year Evaluation
J. Bolick, T. Moore, G. Schaefer, C. Luo, J. Nick and V. Gonzalez, UAMS/Arkansas Children’s Hospital, Little Rock, AR

Problem/Objectives: Every year approximately 38,000 Arkansas newborns receive a newborn screen for 29 primary core panel metabolic conditions (includes Severe Combined Immunodeficiency), hearing loss, and Critical Congenital Heart Disease (CCHD); about 80 infants are diagnosed with a metabolic condition and 50 are diagnosed with hearing loss in a year. Prior to January 2012, data systems were not in place to capture the long-term health outcomes of the hundreds of infants diagnosed with a newborn screening (NBS) condition in Arkansas. The Arkansas NBS Long-Term Follow-up (LTFU) Cohort Study was established for the purpose of tracking and monitoring the clinical care and public health outcomes for children diagnosed with a NBS condition and to follow them until 21 years of age.

Methodology: The Arkansas NBS LTFU Cohort Study is a longitudinal, observational study conducted by the University of Arkansas for Medical Sciences (UAMS), Pediatric Genetics Section in partnership with the Arkansas Children’s Hospital (ACH) and its Research Institute (ACHRI). The primary aim of the Study is to record demographics, characteristics of disease and treatment, utilization patterns, quality improvement measures, and clinical outcomes in Arkansas children with NBS conditions. The study database was developed using REDCap (Research Electronic Data Capture) hosted by the UAMS Translational Research Institute (NCRR/NIH 1 UL1 RR02988).

Significant Results: After receiving Institutional Review Board approval September 2011, the database was implemented January 2012. Based on projections, the enrollment goal for the Study is a total of 3,000. At the end of Calendar Year 2015, there were 699 subjects representing 703 NBS cases in the database. The end of December 2016 marked the completion of five full years of data collection. During 2017, a Five-Year Evaluation will be conducted utilizing the 14 LTFU Public Health Questions (PHQs) proposed by the National Coordinating Center, Newborn Screening Translational Research Network, Long Term Follow-Up Project and disease-specific questions recommended by physician specialists at UAMS/ACH. Also, pre/post NBS expansion data will be evaluated and compared which includes before/after July 1, 2008. The Five-Year Evaluation data for the 14 PHQs will be presented at the Symposium.

Conclusions/Implications: The Arkansas NBS LTFU Cohort Study will provide the opportunity to monitor and track health outcomes over time, and this could lead to improvements in health care for this population and ultimately the lives of children diagnosed with these conditions in the future.

Presenter: Jo Bolick, BSN, MA, APN, CPNP, NBS Program Director, UAMS/Arkansas Children’s Hospital, Little Rock, AR, Phone: 501.364.1906, Email: jabolick@uams.edu
dried blood spot newborn screen at birth and assist them in obtaining a newborn screen. Additional benefits include ensuring facilities are properly using the Department of Public Health’s dried blood spot refusal form. The refusal form requests parents provide a reason for opting out of newborn screening. Examples of reasons for refusal include responses such as cost, religion, felt screening was unnecessary or the desire to have screening completed when infant is older. This information has been used to target educational efforts. Through baby matching the Iowa Newborn Screening Program has gained a better relationship with midwives in the state and begun targeted newborn screening education to the Amish population.

Presenter: Carol K. Johnson, Iowa NBS Follow-up Coordinator, Pediatrics/Medical Genetics, University of Iowa Hospitals and Clinics, Iowa City, IA, Phone: 319.356.3347, Email: carol-johnson@uiowa.edu

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Evaluation of Investigational Product for the Measurement of Enzyme Activity Related to Lysosomal Storage Disorders
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Introduction: Lysosomal Storage Disorders (LSDs) belong to a group of over 50 hereditary metabolic disorders, which results in deficiency and/or impaired function of a specific lysosomal enzyme. Consequentially partly- or undigested molecules accumulate in the lysosomes. This buildup can invoke cell death that may cause progressive organ and tissue dysfunction. Clinical pathological aspects of LSDs have a wide range. Even though each single disease is uncommon, LSDs as a group have an estimated incidence in live births of 1:7000 to 1:8000. It has been demonstrated that early onset treatment substantially improved outcome. Therefore newborn screening of LSDs may be beneficial, especially for early onset variants. In our work we present the evaluation an investigational kit for IVD CE marking for the detection of 6 LSDs, which are simultaneously measured in just one dried blood spot using tandem mass spectrometry.

Methods: Gaucher Disease (Glucocerebrosidase (ABG)), Nieman Pick A/B disease (acid-sphingomyelinase (ASM)), Pompe disease (acid-a-glucosidase (GAA)), Krabbe disease (ß-galactocerebrosidase (GALC)), Fabry disease (a-galactosidase (GLA)) and MPS I (a-L-iduronidase (IDUA)) have been assessed using an investigational product. First, population distribution data from more than 2000 identified, leftover newborn dried blood spots (DBS) were measured to establish cut-off levels for the method. Consequently, these were applied in the second phase of the study to another 1000 newborn samples (presumptive normal) as well as known, genetically confirmed samples (positives).

Results: In the first phase, a total of 1981 newborn specimens were measured. For each enzyme the following average activities (µM/L) were obtained, the standard deviation (s) and coefficient of variation (CV) were also calculated: ABG: 11,19 s = 4,7 CV = 41,9 %, ASM: 7,12 s = 2,8 CV = 39,5%, GAA: 9,77 s = 3,61 CV = 37%, GALC: 4,68 s = 3,25 CV = 69,5%, GLA: 11,83 s = 6,73 CV = 56,9 %, IDUA: 7,06 s = 2,36 CV = 33,5%. Based on these results a percentile based cut-off limit was established for the lower 1st percentile, in order to classify the samples as (potentially) positive or negative. In the second phase another 1000 newborn samples were measured, which yielded no false positive results. In addition, all genetically confirmed samples were correctly identified.

Conclusion: The investigational product provides a robust and effective method for differentiating between the enzyme activities from presumed healthy newborns and genetically confirmed samples of 2017 APHL Newborn Screening & Genetic Testing Symposium, New Orleans, LA, September 10-13, 2017
the 6 diseases above. Our data shows that the false positive rate will be relatively small, with no false positive results from 1000 samples screened. Using the investigational product, all samples were correctly identified. Cut-off levels should be re-assessed individually by each laboratory for this purpose.

**Presenter:** Zoltan Lukacs, Laboratory Director, Universitätsklinik Hamburg-Eppendorf, University Hospital, Metabolic Laboratory, Hamburg, Germany, Email: lukacs@uke.de

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**P-123**

**Health Care Utilization and Costs by Children with Critical Congenital Heart Disease**

M. McClain and M. Flore, University of New Hampshire, Durham, NH

**Objective:** This study examined the interaction between natural history, current practice patterns in monitoring and treatment of critical congenital heart disease (CCHD) from birth to 9 years of age, and associated health resource utilization and costs.

**Methods:** This was a retrospective cohort study using the New Hampshire Comprehensive Health Care Information System, which is a database of health care claims from the majority of commercial health insurers and Medicaid. We identified children who were residents of New Hampshire, less than 10 years of age at the end of the study period and who were diagnosed with CCHD, defined as the twelve targets of pulse oximetry newborn screening. Incurred claims were available from the commercial insurers from January 2007 through March 2014 and from Medicaid from January 2007 through September 2012.

**Results:** Three hundred and forty-three children with CCHD were identified, 181 with commercial insurance and 162 with public insurance (Medicaid). Approximately 30% (n=99) had more than one type of CCHD. Of the children with only one type of CCHD, the most common was coarctation of the aorta (n=76, 22%), followed by tetralogy of Fallot (n=72, 21%). The most common comorbidities were other heart conditions (e.g. unspecified congenital anomaly of the heart, ostium secundum type atrial septal defect, cardiomegaly, ventricular septal defect, congenital aortic valve insufficiency), acute respiratory conditions, otitis media, chronic cough and fever. Over a third (35%) experienced developmental delays, 18 had Down syndrome, 10 had Digeorge syndrome, and 4 had a heart transplant. During the first year of life, infants with CCHD had an average of 5.6 and 7.8 CCHD-related hospital admissions for those with commercial and public insurance, respectively (NS). The highest health care expenses were also observed in the first year of life: $132,000 for those with commercial insurance and $45,000 for those with public insurance (p < 0.0001).

**Conclusions:** Children with CCHDs experience significant comorbidities, particularly other heart conditions. These health conditions result in a substantial utilization of health care services, with the highest usage during the first year of life. This utilization steadily decreases until the fourth year of life, where it levels off through the age of 10 years. It is important to monitor health care utilization for children with CCHDs as part of long-term follow-up to assure the provision of quality disease management, condition-specific treatment, and age-appropriate preventive care.

**Presenter:** Monica McClain, MS, PhD, Research Associate Professor, University of New Hampshire, Durham, NH, Phone: 603.862.4320, Email: monica.mcclain@unh.edu
**Newborn Screening Methodology and Pilot Studies for Lysosomal Storage Diseases**  
M. Gelb and C.R. Scott, University of Washington, Seattle, WA

**Introduction:** Because of the continued interest for identifying newborns at risk for developing a lysosomal storage disease, we have developed methodology based on tandem mass spectrometry to measure enzyme activity that, when deficient, is responsible for disease. We have now obtained data for 11 different disorders that can be detected by these methods.

**Methodology:** A pilot study of approximately 40,000 dried blood spots using flow injection MS/MS for the detection of 6 LSDs (Pompe, MPS-I, Fabry, Gaucher, Niemann-Pick-A/B, Krabbe) has been undertaken within the Washington State Newborn Screening Laboratory. We have also developed a rapid [two min] LC-MS/MS method for the multiplex enzymatic analysis of MP-II, MPS-IIIIB, MPS-IV, MPS VI, and MPS-VII. The method can be expanded to detect neuronal ceroid lipofuscinosis types one and two as well as metachromic leukodystrophy by monitoring for the appropriate biomarkers.

**Results:** The flow injection MS/MS multiplexed assay is robust and performs well in a newborn screening laboratory for the detection of six lysosomal storage diseases. The number of screen positive samples per 100,000 dried blood spots is 4.5 for Pompe, 13.6 for MPS-I, 18.2 for Fabry, 11.4 for Niemann Pick A/B and 25 for Krabbe. These rates are comparable or better than other enzymatic assays currently in use in newborn screening laboratories and are lower than those reported using 4-MU substrates for the same enzymes. The new LC MS/MS assay is robust and has been evaluated on approximately 40,000 DBSs. Genotyping from the residual DBS provides confirmation of affected newborns and information on the number of false positives. This information can guide the decision on where to establish an appropriate “cut off”.

**Conclusions:** These studies confirm that tandem MS/MS methods for the detection of LSD’s perform well in a newborn screening laboratory. The MS/MS methods provide a low number of false positives and minimize the economic and clinical burden of clinical follow-up. The new LC-MS/MS platform also performs better than screening for MPS disorders by analysis of glycosaminoglycan fragments by MS/MS. Our studies imply that newborn screening for at least 13 LSD’s is now feasible and practical at a reasonable cost by using LC MS/MS.

**Presenter:** Michael Gelb, PhD, Professor, University of Washington, Dept of Chemistry, Seattle, WA, Phone: 206.543.7142, Email: gelb@uw.edu

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**Second-tier Newborn Screening Analysis for Lysosomal Storage Disorders: Affected versus Pseudodeficiencies for Pompe, MPS-I and Krabbe Diseases**  
M. Gelb, University of Washington, Seattle, WA

Pompe disease and MPS-I have recently been added to the RUSP, and several states (and a few non-USA countries) are screening for these diseases or gearing up to start screening soon. Some states are also screening for Krabbe disease. Screening of these lysosomal storage diseases requires second-tier analysis since the specificity of the initial screen is not perfect. Specifically, pseudodeficiencies are a common occurrence. We have established second-tier methods based on tandem mass spectrometry of lysosomal enzymes that provide the most accurate measurement of enzymatic activities reported to
date. For Pompe disease, we studied a large collection of patient samples in Taiwan and in NY. We analyzed alpha-glucosidase activity in dried blood spots from 11 patients confirmed to have infantile-onset Pompe disease, 12 patients likely to develop late-onset Pompe disease, and 300 patients with pseudodeficiency mutations. Our method shows that 96% of the pseudodeficiencies can be separated from the Pompe-affected samples. Additional studies of Pompe screen positive newborns from the NY newborn screening program show that second-tier analysis by mass spectrometry using leukocytes isolated from venous blood leads to separation of infantile from late onset Pompe patients. Most of the pseudodeficiencies are also well separated. For MPS-I, application of mass spectrometry to leukocytes (20 affected, 20 pseudodeficiencies and 20 normal newborns) shows good separation of affected versus pseudodeficiency patients. Samples were obtained as MPS-I screen positives from the MO newborn screening program (via Greenwood Genetics). We have analyzed enzyme activity in blood cells from Krabbe disease screen positive newborns found by the NY newborn screening program. Analysis by mass spectrometry on more than 30 patient blood samples shows good separation between those confirmed to have infantile Krabbe disease, those at high risk to develop Krabbe disease but are so far asymptomatic, and those with pseudodeficiency mutations. We have also analyzed the biomarker psychosine in these blood samples and show that psychosine continues to be a good marker for stratifying at-risk Krabbe newborns. These studies are important given that screening for these lysosomal storage disorders is now live or will be live soon in the USA and elsewhere. They show conclusively that the pseudodeficiency problem can be well addressed.

**Presenter:** Michael Gelb, PhD, Professor, University of Washington, Dept of Chemistry, Seattle, WA, Phone: 206.543.7142, Email: gelb@uw.edu

**P-126**

**Toward a Tandem Mass Spectrometry Multiplex Analysis of 24 New Diseases for Newborn Screening and Diagnosis**

X. Hong, A.B. Kumar and M.H. Gelb, University of Washington, Seattle, WA

**Background:** Newborn screening is often considered for those genetic disorders in which an acceptable treatment has been established and in cases where early initiation of treatment leads to a better clinical outcome. Recently, there has been interest in expanded newborn screening panels to include a subset of lysosomal storage diseases (LSD). There is also interest in newborn screening for a non-LSD disease called cerebrotendinous xanthomatosis (CTX), which is due to the inability to produce the bile acid chenodeoxycholic acid. This disease can be well treated with bile acid supplementation especially when started before significant symptoms develop.

**Methodology:** We combined some of our previously developed tandem mass spectrometry assays for LSDs with additional diseases. We used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure the products of several enzymes and several biomarkers in a single run of ~2.3 min per sample.

**Results:** The following disease can be analyzed for newborn screening by direct measurement of enzymatic activities in DBS using LC-MS/MS: Pompe, Fabry, Gaucher, Niemann-Pick-A/B, Krabbe, the mucopolysaccharidoses (types I, II, IIB, IVA, VI, and VII), Cerebral Lipofuscinosis Type I. Efforts are underway to add: Cerebral Lipofuscinosis Type II, Biotinidase deficiency, and Galactosemia Type I (GALT deficiency). Using the same LC-MS/MS run of ~2.3 min per sample we can also measure biomarkers that are useful to complement the enzymatic activities (psychosine for Krabbe, glucosyl-sphingosine for Gaucher, lysosphingomyelin for Niemann-Pick-A/B, lypo-Gb3 for Fabry). We also measure biomarkers
for diseases in which enzymatic activities are difficult to measure or not appropriate (sulfatides for metachromatic leukodystrophy, bile derivatives for CTX). Efforts are underway to add the biomarker for Niemann-Pick-C. This 24-plex (Tetracosaplex) uses 2 DBS punches and 2 assay cocktails for the set of enzymes, and 1 DBS punch with methanol extraction for the biomarkers.

**Conclusions:** We have developed a simple, robust, and high throughput assay for up to 24 diseases by direct measurement of the relevant enzymatic activities and/or the biomarkers. This Tetracosaplex assay is appropriate for newborn screening and diagnosis.

**Presenter:** Xinying Hong, Dept. of Chemistry, University of Washington, Seattle, WA 98195, hxy@uw.edu and arunk@uw.edu

**P-127**

**Tandem Mass Spectrometry but not Fluorimetry Readily Distinguishes Pompe-affected from Pompe-pseudodeficiency Patients in Dried Blood Spots**

H-C. Liao¹, M.H. Gelb²; ¹The Chinese Foundation of Health, Neonatal Screening Center, Taipei, Taiwan, ²University of Washington, Seattle, WA

**Background:** Deficiency of the lysosomal enzyme acid β-glucosidase (GAA) causes Pompe disease. Newborn screening for Pompe disease is ongoing, and improved methods for distinguishing affected patients from those with pseudodeficiency, especially in the Asian population, would significantly reduce the number of patient referrals for clinical follow-up.

**Methodology:** We measured the enzymatic activity of GAA in dried blood spots on newborn screening cards (DBS) using a tandem mass spectrometry (MS/MS) assay that displays a relatively high analytical range (ratio of assay response for the quality control HIGH standard to that from all non-enzymatic-dependent processes) compared to the fluorimetric assay with 4-methylumbelliferyl-alpha-glucoside. DBS from newborns confirmed to have infantile-onset Pompe disease (IOPD, n=11) or late-onset Pompe disease (LOPD, n=12) Pompe disease and those from patients bearing pseudodeficiency alleles or Pompe disease carriers (n=230) were studied.

**Results:** Using the MS/MS GAA assay but not the fluorimetric assay, 96% of the pseudodeficiency newborns and all of the Pompe disease carriers were well separated from the IOPD and LOPD newborns.

**Conclusions:** The results demonstrate that the use of the relatively high analytical range MS/MS GAA assay but not the fluorimetric assay in DBS provides a robust approach to reduce the number of referrals and is predicted to dramatically facilities newborn screening of Pompe disease.

**Presenter:** Hsuan-Chieh Liao, The Chinese Foundation of Health, Neonatal Screening Center, Taipei, Taiwan, Email: liaojoyce@cfoh.org.tw

**P-128**

**A New Assay for Lysosomal Acid Lipase for Newborn Screening and Diagnosis of Wolman’s Disease and Cholesterol Ester Storage Disease**

S. Masi and M.H. Gelb, University of Washington, Seattle, WA

**Introduction:** Enzyme replacement therapy has recently been approved for treatment of Wolman’s disease and Cholesterol Ester Storage disease, which are due to deficiency of lysosomal acid lipase (LAL).
Thus, there is interest in technology for newborn screening and diagnosis of LAL deficiency. The currently used LAL assay in dried blood spots (DBS) uses a substrate that is not specific for LAL. The assay is carried out in the presence and absence of an inactivator of LAL (Lalistat-2), and the LAL activity is obtained as the difference in these two activity values. This assay is adequate to detect if LAL is nominally low, but may be of insufficient accuracy for high precision diagnosis and for newborn screening.

**Methodology:** We prepared a chemical library of active esters of fatty acids in an effort to try to discover a substrate that is specific for LAL. We tested the substrates using DBS from healthy and confirmed LAL-deficient patients.

**Results:** Using our library approach, we discovered a substrate for LAL that is >98% specific for this enzyme in DBS. This was shown by the observation that the hydrolysis of the new substrate in DBS is 98% blocked by Lalistat-2, which is an LAL-specific inactivator. The new assay can be carried out by fluorimetry or by tandem mass spectrometry, but the latter assay gives a larger analytical range and is thus more accurate. We tested DBS from several patients who were previously confirmed to be deficient in LAL enzymatic activity. All patients were confirmed to be deficient in LAL activity using our new substrate in a single assay without the need to use Lalistat-2. Studies with ~30 random DBS showed good assay reproducibility and robustness.

**Conclusions:** We have developed a simple, single reaction, assay of LAL in DBS. The assay is more accurate than the assay now in use and is expected to be useful for newborn screening and for diagnosis of LAL deficiency.

**Presenter:** Sophia Masi, Dept. of Chemistry, University of Washington, Seattle, WA, Email: masis@u.washington.edu

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**P-129**

**Newborn Screening for the Plain Community**

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Wisconsin has the 5th largest Plain community in the United States. Due to a restricted founder pool and consanguinity, individuals from this community are at increased risk for several autosomal recessive metabolic disorders on the newborn screen including phenylketonuria, glutaric acidemia type 1, propionic acidemia, maple syrup urine disease, cobalamin C disease, 3-methylcrotonylglycinuria, very long chain acyl CoA dehydrogenase deficiency, and galactosemia. Over the last several years, partnerships between the newborn screen laboratory, the biochemical genetics clinic, and healthcare providers statewide have fostered improvements within the screening program, specifically directed at increasing the percentage of babies being screened and modifying testing algorithms to better identify diseases. Survey data from the Plain community indicated the lack of access rather than the lack of acceptance as the main cause for lower screening rates. Improving logistical support by offering fee exempt cards for midwives and expanded courier service throughout the state were two responses provided by the newborn screening laboratory. Educational meetings for midwives and birth attendants within the Plain community highlighting the importance of proper specimen collection and timely transport of the specimens have also improved the quality of screening. Detection of metabolic conditions within the Plain community was enhanced by the incorporation of additional testing, tailored to their population. For example, Amish children with propionic acidemia have a milder biochemical phenotype than the classical, severe form described in the literature. To increase probability of

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detection, the newborn screening laboratory has lowered the cutoff for the analytical marker on the first tier test and has incorporated a more specific and sensitive second tier test to quantify methylcitric acid, the diagnostic metabolite. Additionally, because common mutations are present within the population, molecular testing on the dried blood specimen has been implemented, to afford a quick and cost effective diagnosis. Carrier screening is also offered for maple syrup urine disease and propionic acidemia to identify couples at-risk of having a baby with either disease, which sets the foundation for early identification and treatment. The collaborations between the newborn screening laboratory, metabolic clinic, and primary care providers have provided a framework for necessary improvements in screening protocols for the Plain community. Improvements in the logistical support for newborn screening, along with the modification of algorithms have positively impacted the availability of testing and the ability to identify disorders in a timely manner, thereby improving the postnatal care and clinical outcomes for babies with this community.

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**P-130**

**Newborn Phosphatidylethanol Screening to Detect Prenatal Alcohol Exposure in Uruguay**

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**Background:** Prenatal alcohol exposure is the leading preventable cause of birth defects in the U.S., 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. Identifying infants at risk for developing FASD requires confirmation of prenatal alcohol exposure. Currently, physicians often rely on maternal self-report which can be unreliable due to recall bias as well as under-reporting based on fear of stigmatization and embarrassment. Measuring the direct alcohol biomarker phosphatidylethanol (PETH) in neonatal dried blood spots could be a feasible method to identify prenatal alcohol exposure in newborns, but the clinical utility of PETH as a biological screening test for prenatal alcohol exposure needs to be established.

**Objective:** The objective of the current ongoing study is to examine PETH levels in newborns in an obstetrical pediatric hospital in Uruguay, where previous research has documented high rates of maternal consumption during pregnancy, to determine if PETH is sensitive and specific in detecting prenatal alcohol exposure.

**Design/Methods:** Women are enrolled in the study following delivery at the Hospital General de las Fuerzas Armadas in Montevideo, Uruguay. A self-report questionnaire on alcohol consumption during pregnancy is administered and heel stick blood spot samples are collected from the newborn following routine newborn screening. Self-report and PETH data from 137 maternal and newborn (140) pairs have been collected to date (including 3 sets of twins).

**Results:** Based on self-report measures, 39 (28.5%) mothers reported alcohol consumption at any period during her pregnancy; of which 33 (24.1%) reported drinking during the first trimester, 6 (4.4%) reported drinking during the second trimester, and 10 (7.3%) reported drinking during the third trimester. Self-report of alcohol consumption was highest for the three months prior to pregnancy, with 66 (48.2%) of the mothers reporting drinking. Newborn heel stick blood cards were available for 124 of the newborns.
PEth was detected above the limit of quantitation (PEth ≥ 8ng/ml) in 79.8% of the newborn heel stick blood cards, with an average positive PEth concentration of 45.4 ng/ml. The positive heel stick blood PEth concentrations ranged from 8 to 204 ng/ml.

**Conclusions:** The preliminary findings demonstrate that prevalence of prenatal alcohol exposure continues to be a serious public health problem in Uruguay, with significant under-reporting of maternal alcohol consumption during pregnancy. This ongoing study will examine the sensitivity and specificity of PEth to detect fetal alcohol exposure and follow the children from this prospective cohort sample to monitor their neurological, behavioral and physical development.

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**P-131**

**Improved Timeliness, Accuracy, and Operational Efficiency in Newborn Screening: An Integrated, Interoperable IT Infrastructure**

D. Jones¹, H. MacIntosh², T. Finitzo³, K. Hart¹, R. Atkinson-Dunn¹, A. Rohrwasser¹; ¹Utah Department of Health, Salt Lake City, UT, ²STACS DNA, Ottawa, ON Canada, ³OZ Systems, Arlington, TX

In the Utah Newborn Screening (NBS) Program’s quest to implement a system that will improve timeliness, accuracy, and operational efficiency, an all-encompassing IT infrastructure was conceptualized. This system incorporates two established and validated platforms that allows integration with Perkin Elmer Specimen Gate. This system has the following features: 1. Real-time and dynamic data exchange regarding location of specimens and results establishing complete chain-of-custody for every specimen 2. Physical inventory management system 3. Secure cloud-based architecture for ease of access by thousands of users 4. Useable and complete demographic, testing, and result data and metadata for all specimens from collection to destruction 5. Automated or quasi-automated retrieval of patient demographic information, to reduce transcription errors in the process 6. Bidirectional interoperability with electronic health records (EHRs), including both electronic laboratory ordering (ELO) and electronic laboratory reporting (ELR) 7. Flexible and modular system design to accommodate test panel expansion and allow interoperability 8. One vendor solution This IT infrastructure (FootSteps™) integrates Track-Kit from STACS DNA and NANI from OZ Systems. Both components independently integrate with Specimen Gate from PerkinElmer. FootSteps™ will serve as an end-to-end collection-to-storage sample tracking system which allows for patient demographic information to be automatically consumed via HL7 messaging from the provider EHR or a provider data entry webportal. Specimen Gate will function as the laboratory information management system (LIMS) for the Utah NBS Program and allow NBS results to be accessed by providers via a secure provider webportal and/or HL7 messaging directly to the provider EHR. Following implementation of the IT infrastructure in 2017, operational improvements will be assessed and documented. This system will allow for flexibility to accommodate panel expansion and improved safety for record portability. The bidirectional flow of data with the provider EHR will incorporate both ELO and ELR. It is anticipated that this system will eliminate onsite data entry requirements, offsetting FootSteps™ costs and ongoing costs associated with rework requirements. Deferring data stewardship will significantly decrease workloads for IT and follow-up staff, thereby increasing capacity for essential patient care tasks and test panel expansion. Furthermore, this IT infrastructure will enable long-term follow-up of patients identified by NBS and continual improvement of the NBS process.
SCID Assay Implementation at the Utah Newborn Screening Program
K. Logerquist, D. Jones, E. Hardin, J. Barnes, R. Atkinson-Dunn, K. Hart and A. Rohrwasser, Utah Department of Health, Salt Lake City, UT

Severe Combined Immunodeficiency (SCID) is a rare immunological disease manifesting in T-cell receptor and T-cell deficiencies. Screening for SCID is based on the measurement of T-cell Receptor Excision Circles (TREC); TREC molecules are circular extrachromosomal DNA elements that are by-products of T-cell receptor processing and differentiation. The prevalence of SCID in Utah is approximately 1 in 70,000 births per year. Similar to many Newborn Screening (NBS) disorders, early diagnosis of SCID is important prior to the occurrence of life threatening secondary infections that would result in end organ damage and death if untreated. To improve timeliness, the Utah NBS program aimed to implement SCID testing in-house. The Utah NBS Program compared and validated two methodologies, a laboratory developed test (LDT) and the EnLite Neonatal TREC Assay by PerkinElmer. The LDT is based on a multiplex, real-time PCR methodology measuring TREC and beta-actin in a 384-well plate format. Utilizing case control designs, both methodologies were compared using normal and abnormal (previously identified) specimens. To validate screening performance and percentage based cut-offs, 936 samples with the LDT and 3055 samples with the EnLite TREC methodology were analyzed. In the Utah population, the EnLite TREC methodology indicated a cut-off of 25 TREC exact copies/µL equivalent to a 0.50th population percentile; this range is consistent with an initial cut-off recommendation of 29 TREC exact copies/µL by PerkinElmer and 22 TREC exact copies/µL established by the California NBS program. For screening, the Utah NBS Program implemented two cut-offs. Cut-off 1 (25 exact copies/µL) distinguishes between “NORMAL” and “INDETERMINATE” TREC levels and cut-off 2 (11 exact copies/µL) distinguishes between “INDETERMINATE” and “ABNORMAL”. Similar cycle threshold distributions and results were observed for the LDT assay. For further validation, a panel of previously identified cases of SCID and related T-cell lymphopenias were measured. All disorders were identified with both platforms. Furthermore, both systems performed equally well in identifying cases on a blinded expanded proficiency panel. The advantages of the EnLite TREC system include (1) real time multilevel QC integration; (2) the benefits of an FDA cleared system including calibrators and kit controls; and (3) relatively low FTE demands (0.5 FTE for 50,000 samples per year). The advantages of the LDT include significantly lower reagent costs. Considering similar performance, significantly lower personnel needs for testing and preparation of control material, and seamless QC integration, the Utah NBS Program implemented EnLite TREC. The validation of the LDT platform will allow future multiplexing with new targets including Spinal Muscular Atrophy (SMA).

Presenter: Katelyn Logerquist, Utah Department of Health, Taylorsville, UT, Email: klogerquist@utah.gov
Partnering with Birthing Hospitals to Implement Electronic Data Exchange for Newborn Screening Sample Orders and Test Results - If You Build It, Will They Come?
W. Andrews¹, K. Turner¹, E. Hopkins¹, R. West¹, V. Tyson²; ¹Virginia Division of Consolidated Laboratory Services, Richmond, VA, ²Virginia Information Systems Services, Richmond, VA

Background: In an effort to improve the accuracy of sample data received in the laboratory and the timeliness of results reported to healthcare providers, the Virginia (VA) Newborn Screening (NBS) Program received NewSTEPs 360 funding with the intent to kick-start implementation of electronic messaging for the NBS Dried Blood Spot Program in VA. The project was defined as a proof-of-concept initiative that would involve a representative subset of VA’s birthing hospitals to serve as pilot sites for 3 years to implement electronic messaging. However, obtaining buy-in from these birthing hospitals presented a challenge that required innovative solutions.

Objective: To educate NBS programs on encountered barriers to obtaining hospital buy-in for electronic messaging, and provide lessons learned and potential solutions.

Methods: To solicit hospital partners, existing relationships with the VA Hospital and Healthcare Association and the VA Chapter of the March of Dimes were utilized to provide project-advertising mechanisms to distribute the call for participants. OZ Systems also collaborates by working directly with birthing hospitals to provide a software solution to pull existing demographic data from the electronic health record (EHR). This application enables hospitals to generate sample labels and create an electronic order message in standard HL7 format, while eliminating the need to fill out information on the collection card manually.

Results: The goal of this project was to acquire commitments from 6 hospitals whose combined volume of samples would represent roughly 25% of total samples received annually. The number of hospitals interested in partnering with the VA NBS Program on this initiative has exceeded expectations. The project team is currently working with 12 hospitals across multiple hospital systems that include a variety of EHR systems and is on target to transition 40% of program’s samples from manual processes to bi-directional electronic messaging.

Conclusion: As the VA NewSTEPs 360 project enters into YR 3, there have already been successes, missteps, and many lessons learned. Having OZ Systems engaged in VA’s project has provided additional resources and valuable personnel able to reach out to hospitals and obtain buy-in from hospital leadership interested in moving to electronic data exchange. Due to successes of this project thus far, VA is excited to share solutions to barriers NBS programs may encounter when attempting to obtain hospital buy-in for electronic messaging. Additionally, VA is a contributor in a multi-state effort to develop an implementation resource guide for electronic messaging that will document experiences, tools and artifacts developed by NBS Programs. The NBS HIT Building Blocks Resource Guide and Tool Kit will provide valuable information and best practices to aid NBS Programs in need of assistance.

Presenter: Willie Andrews, BSMT(ASCP), Director of Laboratory Operations, Virginia Division of Consolidated Laboratory Services, Richmond, VA, Phone: 804.648.4480, Email: willie.andrews@dgs.virginia.gov
Critical Congenital Heart Disease Screening Cost Analysis Study in Virginia Birthing Hospitals
C. Crews and J. Macdonald, Virginia Department of Health, Richmond, VA

The purpose of this presentation is to discuss the extensive Time Cost Analysis Study performed of implementing Critical Congenital Heart Disease Screening (CCHD) in birthing hospitals throughout Virginia. This presentation will share data of benefits of disposable vs reusable testing methods, time involved, and staffing requirements for minimal fiscal impact on implementation of CCHD screening. As part of an outcome for a HRSA grant received in 2012 for CCHD screening implementation, the Virginia Department of Health Newborn Screening program evaluated service infrastructure and delivery strategies for a population-based CCHD screening program. A barrier to CCHD implementation and reporting in Virginia was additional cost to facilities without additional reimbursement. A cost analysis study of CCHD screening with pulse oximetry (POX) and short-term follow-up care per wellborn infant born at a Virginia birth hospital was performed in 2015. The analysis consisted of a time and motion study and a cost questionnaire, which were conducted in wellborn nurseries and mother/infant care units in a random sample of eight non-military, licensed birth hospitals in Virginia. The sample was stratified by hospitals’ perinatal level, geographic region, and annual number of births. The outcomes of this study recommend to bundle CCHD screening with other newborn screening tasks and to utilize reusable pulse oximeter probes to minimize hospital cost and maximize nursery staff efficiency. This novel approach at identifying financial barriers and providing solutions serves not only as a model for other state-sponsored newborn screening programs and birthing facilities in the process of implementing CCHD screening, but also promotes leadership competencies in critical thinking and working with communities and systems.

Presenter: Christen Crews, MSN, RN, Public Health Nurse Supervisor, Virginia Newborn Screening Program, Virginia Department of Health, Richmond, VA, Phone: 804.864.7700, Email: christen.crews@vdh.virginia.gov

Integration of Newborn Screening Follow-Up Module into Laboratory Information Services (LIMS)
C. Crews¹, J. Macdonald¹, W. Andrews², K. Turner², V. Tyson²; ¹Virginia Department of Health, Richmond, VA, ²Virginia Division of Consolidated Services, Richmond, VA, ³Virginia Information Systems Services, Richmond, VA

The purpose of this presentation is to discuss the benefits of integrating Follow-Up processes into newborn screening laboratory information systems (LIMS). In 2016, the Virginia Newborn Screening program partnered with the Division of Consolidated Laboratory Services (DCLS) to enhance the Follow-Up module to provide more efficient workflow and improve timely follow-up care. In 2016, the Virginia Department of Health (VDH) Newborn Screening program partnered with the Division of Consolidated Laboratory Services (DCLS) to fully integrate a comprehensive Follow-Up module into laboratory information systems (LIMS) to provide more efficient workflow and improve timely follow-up care. The Follow-Up module developed is unique to Virginia and was designed to address many critical needs required for timely newborn screening follow-up. The implementation of this module has improved staff workflows and timeliness for contacting providers regarding follow-up on abnormal newborn screening
results. This novel approach at identifying key components in follow-up and providing solutions with the integration serves as a model for other state-sponsored newborn screening programs and laboratories.

**Presenter:** Christen Crews, MSN, RN, Public Health Nurse Supervisor, Virginia Newborn Screening Program, Virginia Department of Health, Richmond, VA, Phone: 804.864.7700, Email: christen.crews@vdh.virginia.gov

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**P-136**

**Keeping Newborn Screening under One Roof: The Co-location of Laboratory and Follow-up in Virginia**

C. Crews¹, J. Macdonald², W. Andrews², K. Turner³; ¹Virginia Department of Health, Richmond, VA, ²Virginia Division of Consolidated Services, Richmond, VA

In an effort to minimize the risk of reporting errors, the Virginia Department of Health (VDH) partnered with the Division of Consolidated Laboratory Services (DCLS) to co-locate the Virginia Newborn Screening Laboratory and Follow-Up programs to the same facility. Historically, these two teams were located approximately a mile apart with face-to-face interaction occurring once a month. Follow-up team members mainly telework and this environment further contributed to the isolation and communication issues. This initiative has assisted with streamlining reporting procedures, enhancing communication, empowering team members to participate in NBS QI initiatives, and to build comradery.

This unique model in Virginia has integrated programs from different state agencies and extensive logistical planning was necessary for implementation. This poster will review planning steps, barriers, advantages, and on-going challenges for co-location of laboratory and follow-up staff. An evaluation of team members survey responses to perceptions of co-location.

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**P-137**

**Evaluating the Engagement and Inclusion of Out of Hospital Birth Providers in the Commonwealth of Virginia Statewide Newborn Screening Program**

C. Crews and J. Macdonald, Virginia Department of Health, Richmond, VA

The purpose of this poster is to discuss the collaboration of the Virginia Newborn Screening program with the Midwife providers. The poster reviews the historical barriers of out of hospital births with parent desires of minimal interventions with the safety of newborn screening. In 2012, Virginia expanded its newborn screening panel to include CCHD screening and developed regulations mandating all well born infants receive CCHD screening prior to discharge from the birthing hospital. As the mandate did not extend to the out of hospital (OOH) birthing population, an assessment of newborn screening in Virginia was completed to identify and reduce the impact of the gap. Although dried blood spot screening was mandated for all Virginia births (including OOH), challenges were identified with the OOH population. Historically, very limited engagement existed between the Virginia Department of Health (VDH) Newborn Screening Program and the OOH community in Virginia. An innovative plan was developed to engage the OOH birthing providers and increase education of newborn screening best practices.

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practices. The first component was to identify key leaders in the OOH community to engage and receive buy-in and survey the OOH birthing providers of current practices and challenges. A committee was established to review best practices, existing challenges, and identify opportunities for additional education. The second component was to schedule a novel webinar that received attendees from throughout the country and internationally on OOH newborn screening. Survey and evaluation data was collected and analyzed for future implementation measures. This novel approach at engaging a community that is often a silo from state newborn screening programs not only serves as a model for other state-sponsored newborn screening programs, but also promotes the development of leadership competencies in critical thinking, working with communities and systems.

Presenter: Jennifer Macdonald, Public Health Nurse Manager, Virginia Newborn Screening Program, Virginia Department of Health, Richmond, VA, Phone: 804.864.7729, Email: jennifer.macdonald@vdh.virginia.gov

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The Development of a Newborn Screening Education Website to Improve Outcomes of the Neonatal and Infant Population
J. Brickey, C. Crews and J. Macdonald, Virginia Department of Health, Richmond, VA

The purpose of this poster is to discuss the novel implementation of a newborn screening education website to improve outcomes of the neonatal and infant population by educating healthcare providers in Virginia and around the world. In 2012, the Virginia Department of Health (VDH) received HRSA funding to develop a statewide infrastructure to implement point-of-care screening for critical congenital heart disease (CCHD) for wellborn infants. As an outcome of the grant, Virginia partnered with the University of Virginia (UVA) Continuing Medical Education (CME) office in 2014 to develop a series of novel web based education modules centered on newborn screening. The initial module focused on Critical Congenital Heart Disease (CCHD) screening and educated on the screening process including best practices, disease process, application, interpretation, and intervention. An additional module was added shortly after to focus on dried blood spot screening and a forthcoming module on Early Hearing Detection and Intervention (EHDI) will be available in 2017. These modules are currently available at no cost to providers and include CME with pre and post evaluation. As of June 30, 2016, 2,510 Virginia residents were registered users, and an additional 342 non-Virginia residents registered for and paid to access the modules. The majority of users were RNs (1498). Continuing education credits provided included AMA PRA Category 1 credits, Virginia Nurses Association nursing contact hours, MOC Part 2 credits, and Certificates of Completion. These novel modules are available to other state-sponsored newborn screening programs and birthing facilities as a tool to improve education in newborn screening. This poster will review planning steps, barriers, advantages, and on-going challenges for the implementation of the educational resource.

Presenter: Jennifer Brickey, Public Health Nurse, Virginia Newborn Screening Program, Virginia Department of Health, Richmond, VA, Phone: 804.864.7712, Email: jennifer.brickey@vdh.virginia.gov
Celebrating 50 Years of Newborn Screening in Virginia
C. Crews¹, J. Macdonald¹, W. Andrews², K. Turner²; ¹Virginia Department of Health, Richmond, VA, ²Virginia Division of Consolidated Services, Richmond, VA

2016 marked the 50th anniversary of newborn screening in the Commonwealth of Virginia. The Virginia Newborn Screening Team conducted year-long planning on various activities to mark this important milestone. Newborn Screening lab and follow-up personnel staffed a booth at the week-long Virginia State Fair to educate the public and were able to reach out to over 5,000 citizens that visited the Fair. The team also produced a day long symposium, the first ever for Virginia, on October 21, 2016 in Richmond. Over 125 health care providers from across the state came to Richmond to listen to dynamic presentations from 13 national and Virginia based speakers to discuss past, present and future topics facing the newborn screening system. Topics included education, best practices from hospitals, NICUs, mid-wives, pediatric offices, the genetic counselor perspective on relaying diagnosis to families, electronic data exchange, future disorders to be implemented on the newborn screening panel and families personal stories about how newborn screening affected them. CEUs were available and the post evaluation survey noted this event to be a tremendous success. These anniversary educational and promotional activities supports Virginia's mission and national goals to (1) ensure all infants are appropriately and timely screened according to best practices and state regulations, (2) ensure all infants and families affected by identified disorders receive appropriate, timely follow-up care and service, and (3) ultimately reduce neonatal infant morbidity and mortality rates.

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A New Path for the Virginia Newborn Screening Program: Process Requirements and Validation of a Secure and Standards-based Message System for the Electronic Transfer of Laboratory Data
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Background: Over the last decade, Virginia has participated in data exchange efforts working collaboratively with but not limited to Center for Disease Control and Prevention (CDC) and Virginia Department of Health (VDH). Working with these agencies and others, DCLS has become a recognized leader in the implementation of secure processes for messaging laboratory orders and results to improve the timeliness of data transmission in a standardized and secure manner. Objective: To leverage the skills and utilize the systematic approach acquired from previous experiences in validating electronic data exchange to ensure that transfers of Newborn Screening (NBS) data between participating hospitals and DCLS are secure and accurately input into the system.

Methods: Message validation is conducted in a step-by-step comprehensive testing plan. The test message submitted is reviewed for message content, HL7 structure, and coded vocabulary. Any deficiencies are resolved and upon transmission of a successful test message a set of test cases are performed. Test cases are conducted in a test environment utilizing a generated message sent to assess
encryption. From encryption, it passes through the health information exchange and message decryption. All test cases are reviewed for structural and content validation.

**Results:** The goal of implementing electronic messaging is to establish a secure and standards based exchange of lab orders and test results with participating organizations. DCLS has accomplished secure electronic data exchange for laboratory orders and results using nationally adopted health vocabularies and HL7 message specifications. This same systematic process has been applied to exchange NBS data with participating birthing hospitals. The Virginia NBS program received NBS lab orders successfully from participating hospitals in 2017 and will continue working to stand up electronic orders with additional participants while implementing result messaging.

**Conclusions:** DCLS has applied previous experiences to this project recognizing that there are similarities as well as differences that make NBS messaging unique when compared to previous laboratory messaging efforts. When hospitals select a service provider, such as OZ Systems, it provides a common architectural model which will streamline and expedite the validation process. This is highly valuable when bringing on additional hospitals quickly as the validation process has been established and can easily be replicated. Hospitals choosing not to use intermediary software may require additional testing during message validation. DCLS uses the same process for both order and result message validation. Virginia’s approach as well as all experiences and tools developed will be shared with the NBS community and offered for inclusion in the NBS HIT Implementation Resource Guide and Toolkit.

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**Newborn Screening for Severe Combined Immunodeficiency: An Improved Real-time PCR Assay**
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**Background:** Severe combined immunodeficiency (SCID) is a recently added condition to the Recommended Uniform Screening Panel (RUSP). A simple yet efficient DNA isolation method and reliable calibrators containing both the target and reference gene would further improve SCID newborn screening assay performance.

**Objective:** To develop and validate an improved method of quantitating T-cell receptor excision circles (TRECs) from dried blood spots (DBS) on newborn screening (NBS) cards using a novel DNA isolation method and novel gBlock constructed TREC/RNase P standards.

**Methods:** The de-identified residual dried DBS underwent single wash with a laboratory-developed DNA isolation buffer (This buffer has been patented by the Wisconsin Alumni Research Foundation and is now licensed and sold as part of Quantabio’s Extracta DBS), and the DNA isolation was achieved by incubating the washed specimens with 54 µL of the same buffer at 96ºC for 25 minutes. One ninth (6 µL) of the extract solution was used in a standard real-time PCR reaction with gBlock constructed TREC/RNase P as quantitation standards. The validation process consisted of assay analytic performance (accuracy, linearity, precision, and reproducibility) and assay clinical performance (sensitivity, specificity, and clinical validity)

**Results:** The assay showed satisfactory accuracy, linearity, precision, and reproducibility based on the results from a set of dried blood specimens with known TREC copy numbers. The assay’s sensitivity and specificity were also satisfactorily evidenced by 100% concordance with the expected results on a set of residual CDC proficiency test samples from 2012 to 2015. The assay correctly identified all confirmed...
SCID/severe T cell lymphopenia cases detected by the Wisconsin NBS program, using the original dried blood NBS specimens.

**Conclusions:** We have successfully validated an improved real time quantitative PRC (RT-qPCR) to quantitate TREC used in NBS for SCID.

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**Implementation of a T-cell Receptor Excision Circle (TREC) Assay to Detect Severe Combined Immunodeficiency (SCID) in the Puerto Rican Newborns**

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**Background & Objectives:** Severe Combined Immunodeficiency (SCID) is a congenital combined immunodeficiency that leads to the development of life-threatening infections that can result in infant death in the first year of life. In 2010, Secretary’s Advisory Committee recommended adding SCID to the routine newborn screening (NBS) panel and currently, 43 states in U.S. screen for SCID. In PR, the estimated incidence of SCID is 1/60,000 which is consistent with reported statistics of other states. In this study, a summary of the SCID test implementation and validation in PR is presented.

**Methods:** Since 2012, PR started with the implementation of a SCID test through a method transfer from the New England NBS Program. The SCID screening consists of a quantitative real-time PCR for the detection of small T-cell receptor excision circle (TREC) that are residual to normal genetic cutting and splicing events in the maturing thymus. Infants with SCID have little to no TREC. In general, the PR method consists of: 1) Punching of dried blood samples from newborns (DBSs) in 96 well plates, 2) DNA extraction using a Qiagen Generation Solution 2, 3) DNA transfer to a 384 well plate and PCR set up using an epMotion 5075 liquid handling robot and 4) TREC and RNase P (a reference gene) amplification using a 7900HT Real time PCR system.

**Results:** During the validation process, 5,500 samples were analyzed. All standard curves showed good linearity for TREC and RNase P, with regression values within the set ranges: slope (-3.1 to -3.6), Y intercept (36 - 41) and R² (0.97-1.0). Cord blood calibrators were used to determine intra and inter assay precision obtaining 4 % and 6 % coefficient of variation (CV) respectively. A positive and negative control; a blank and a No-template control (NTC) were used in 5 different runs to determine assay reliability; all were within expected ranges. Sensitivity and specificity was determined using samples with known values obtained from the Center for Disease Control (CDC) in Atlanta and the University of Michigan, all resulted in 100 % with no misclassifications of true positives and true negatives. Based on an analysis of Puerto Rican newborns, the mean for our population resulted in 25.38 Ct and 31.72 Ct for RNase P and TREC, respectively. The cutoffs for this assay were determined in 28.5 Ct for RNase P and 35 Ct for TREC.

**Conclusion:** The PR NBS Program has implemented a reliable method to detect TREC and monitor SCID in newborns. The assay started in August 2015 and until May 2017, 50,880 newborns have been screened. No SCID case has been detected.

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