2019 Newborn Screening & Genetic Testing Symposium
Chicago, Illinois
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Poster Abstracts

Poster abstracts with an "*" after the poster number were selected for brief oral presentations during the symposium.

P-001

Misclassification of VLCAD Carriers in Newborn Screening

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Background: The Iowa Newborn Screening (NBS) Program began screening for acylcarnitine C14:1 when it implemented expanded tandem mass spectrometry panel in 2004. If untreated, VLCAD can lead to liver failure, heart failure, or death. The current confirmatory testing recommendation for out-of-range C14:1 results lists functional studies (i.e., fibroblast fatty acid oxidation probe) as optional. Many experts also consider molecular testing optional. However, lack of use of both molecular and functional studies can lead to misclassification of VLCAD carriers as false positives.
Objective: 1) To calculate the incidence of VLCAD carriers in a population-based sample, 2) To examine the effect of a comprehensive VLCAD confirmatory testing algorithm on case classifications
Design/Methods: The data used in this analysis is from the Iowa Newborn Screening Program. From 2016-2017 (i.e., comprehensive testing period), nearly all infants with out-of-range C14:1 results underwent both molecular and functional studies. From 2005-2016 (i.e., variable testing period), different testing algorithms were used by each metabolic provider. For the comprehensive testing period, we calculated the incidence of VLCAD carriers, false positives, and true positives. For the variable testing period, we reviewed false positive cases to determine if they could represent misclassification of VLCAD carriers due to lack of comprehensive testing.
Results: During the comprehensive testing period, 48,651 specimens (approximately 45,500 births) were screened. Of these, there were 13 individuals with out-of-range C14:1 results that were classified as follows after confirmatory testing: Ten carriers (incidence of 1:4,550), zero true positive cases, zero FP, and three unclassifiable. During the variable testing period, a total of 486,566 specimens (approximately 448,500 births) were screened. There were 85individuals with out-of-range C14:1 results. Upon review of the classification of these cases, we found 32 individuals that may have been carriers but who did not receive genetic testing. Nearly all of these individuals were classified as false positives.
Conclusion(s): This study represents the first population based assessment of VLCAD NBS. Our findings suggest that many VLCAD carriers could be mistakenly classified as false positives. Since it is unknown if VLCAD carriers clinically overlap with affected homozygous individuals, we recommend comprehensive molecular and functional testing for all children with out-of-range C14:1 NBS results.

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P-002

CF Screening Efficacy and Seasonal Variation in California: 10-year Comparison of IRT Cutoffs Versus Daily Percentile for 1st-tier Testing

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Introduction: The California Genetic Disease Screening Program screens approximately 500,000 babies per year and identifies children diagnosed with Cystic Fibrosis (CF) and CF-Related Metabolic Syndrome (CRMS). When our 1st tier assay of immunoreactive trypsinogen (IRT) is higher than the cutoff, we then analyze a panel of 40 known disease-causing genetic mutations in the CFTR gene; if only one mutation is found, we sequence the CFTR gene. Our IRT cutoff has changed every couple of years to approximate a nominal 1.6% yearly IRT positive rate after a new reagent kit has been introduced. However, known seasonal variation in population IRT percentiles led us to develop a seasonal model to help set initial kit-based cutoffs. We wanted to compare model cutoffs with the alternative method of daily statewide and laboratory-based percentiles to see which method would identify or miss more CF cases near the IRT detection boundary. We attempted to answer which method is more efficacious since screen-positive bloodspots are costly to genotype.

Methods: We utilized an ARIMA model to fit monthly IRT screen-positive percentiles from 2007 to 2016. Model results were used to estimate regular seasonal expectations for percentiles. We then examined daily percentiles calculated statewide and by individual regional contract laboratory. We aligned known CF cases identified or missed by date, and noted percentiles where CF cases were missed by the two methods.

Results: The ARIMA model identified a regular, seasonal cycle ranging from 1.4% screen-positive in summer to 1.8% in winter. Using the adjustment factor derived from the model, we would have missed no CF cases near the IRT detection boundary; instead we had missed two cases in practice due to delays in new kit cutoff changes. In comparison, using a daily percentile of 1.6%, we would have missed 9 CF cases statewide and 15 CF cases if we calculated daily percentiles based on individual regional contract laboratories; small sample sizes contributed to some but not all missed results. We found that the equivalent daily percentile to emulate our current screening strategy was 3% statewide, or 4% based on individual laboratories.

Discussion: We found a regular seasonal pattern in IRT percentiles, which can be used to define stable cutoffs based on the first weeks of kit introduction. Daily variability in the screening population and in IRT results was great enough to lower screening efficacy for CF if we relied upon daily percentiles. If we went to a laboratory-based daily percentile, we would need to send more than double the number of specimens for molecular analysis to achieve the current detection rate. While the daily percentiles are more likely to have greater variability due to sample size, the population-based model focused on seasonal variation was more efficacious and led to improved screening performance.

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P-003

New Online Course on Analytical Validation of a Biochemical Genetic Test Using Liquid Chromatography - Tandem Mass Spectrometry

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Introduction: Biochemical genetic tests are highly specialized laboratory procedures for the evaluation, diagnosis, and clinical management of inherited metabolic diseases. Over the last two decades, the advent of expanded newborn screening for inherited metabolic disorders using tandem mass spectrometry (MS/MS) and other technologies has presented an increasing demand for biochemical genetic testing in diagnosing presumptive cases detected through newborn screening. However, biochemical genetic tests are highly complex procedures performed on a wide variety of patient specimen types. Biochemical genetics laboratories need easily accessible training materials in developing and implementing new tests using the MS/MS technologies.
Method: Since 2013, CDC has been collaborating with the Association of Public Health Laboratories (APHL) to help genetic and newborn screening laboratories in their quality improvement activities. Findings from two discussion groups held in 2013 identified topic areas in which training resources are desired, including user-friendly online courses on developing and validating new test procedures. (https://www.aphl.org/aboutAPHL/publications/Documents/CDCRecommendationsGoodLabPractices_OCT2014.pdf). To meet this need, experts in biochemical genetic testing and MS/MS technologies were enlisted to develop a new online course titled "Validating a New Test Using Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS) in a Biochemical Genetics Laboratory." Pilot testing was conducted by laboratory professionals to ensure its usefulness for the target audience.
Results: This intermediate-level multimedia online course discusses a stepwise plan for setting up and validating a new LC-MS/MS based assay for application in a biochemical genetics laboratory. A specific example of quantifying methylmalonic acid in human plasma is used to describe each of the steps required to develop and validate the method. An outline of the validation report is provided that includes how to review, interpret and document validation tests, quality control results, and proficiency tests. Continuing education credits are available from this course free of charge, including 1.5 hours of the ASCLS P.A.C.E. credit. The course is expected to be publicly available on CDC TRAIN website at https://www.train.org/cdctrain/home in Spring 2019.
Conclusions: This online training module is intended to help laboratory professionals understand the regulatory environment of a biochemical genetics laboratory, define the steps required to develop and validate an assay based on LC-MS/MS, and describe the contents of the validation report. Course evaluation results and feedback from the participants will be closely monitored to assess the utility and learning outcomes.

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P-004

Update on the Online Course to Help Laboratory Professionals and Healthcare Providers Improve Preanalytic Processes of Biochemical Genetic Testing

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Introduction: Biochemical genetic tests are associated with a wide range of preanalytic 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 preanalytic phase might be the most error-prone during the total testing process in many laboratory disciplines including genetic testing.

Method: CDC published a guideline "Good Laboratory Practices for Biochemical Genetic Testing and Newborn Screening for Inherited Metabolic Disorders" in 2012 (http://www.cdc.gov/mmwr/pdf/rr/rr6102.pdf). Since 2013, CDC has been collaborating with the Association of Public Health Laboratories (APHL) to help genetic and newborn screening laboratories in their quality improvement activities. Findings from two discussion groups held in 2013 identified a need for training to supplement the guideline (https://www.aphl.org/aboutAPHL/publications/Documents/CDCRecommendationsGoodLabPractices_OCT2014.pdf). To meet this need, an online training course titled "Good Laboratory Practice Recommendations for Biochemical Genetic Testing: Preanalytic Phase" was developed under the APHL-CDC cooperative agreement to help laboratory professionals and healthcare providers improve preanalytic practices for biochemical genetic testing.

Results: This multimedia online course has been publicly available on CDC TRAIN website at https://www.train.org/cdctrain/course/1072447 since December 2017. The course consists of 3 lessons on quality assurance for test requisitions, specimen collection and submission; laboratory-clinician communications; and preanalytic quality assessment. Several case scenarios are included to illustrate how the recommended practices can be used to improve preanalytic quality and patient outcomes. Continuing education credits are available from this course free of charge, including 1.5 hours of the ASCLS P.A.C.E. credit and 1.5 contact hours for Florida Laboratory Licensees. As of September 25, 2018, 119 learners have completed the course with post test scores averaging 97%, reflecting a significant knowledge improvement from their average pre-test scores (63%). Approximately 89% of the participants stated that this training course addressed a gap in their knowledge or skills.

Conclusions: The quality improvement practices discussed in this course are helpful not only for biochemical genetic testing but also for many other laboratory areas. Knowledge improvement and evaluation results will be continuously monitored to assess the effectiveness of this online course and to inform future training development needs.

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P-005

APHL Newborn Screening Health Information Technology (HIT) User Groups

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Background: In January 2018, four health information technology (HIT) user groups, PerkinElmer, Natus/ Neometrics, OZ and interoperability, were established as smaller workgroups of the larger APHL Newborn Screening (NBS) HIT workgroup. User groups provide a structured and moderated forum where participants can candidly discuss NBS activities as they relate to Information Management Systems (inclusive of the laboratory and follow-up program). User groups identify topics of interest for discussion, share experiences, identify challenges and solutions and share information gathered with the wider NBS community, including vendors.
Objective: To describe the four APHL NBS HIT user groups and describe the topics of discussion, experiences, challenges and potential solutions in 2018.
Methods: The APHL NBS HIT user groups consist of participants from state NBS programs who work with information management systems, and include a moderator who solicits topic areas and moderates discussions during each call. The user groups meet via video teleconference bimonthly or as needed, where members discuss issues and provide consensus-based solutions.
Results: As of September 2018, there are 14 total members on the Natus/Neometrics group, 17 on PerkinElmer, 21 on Interoperability and nine on OZ, although participant engagement varies by month. The Natus/Neometrics group has had four calls as of September 2018, and meets on a bimonthly basis. The top priority of the group has been the development of a change request form to send formal requests to Natus, such as description of and reason for the change, module(s) involved, severity of the change, as well as the impact on time, cost, resources and quality. The PerkinElmer group has had five calls as of September 2018, and meets on a bimonthly basis. This group has frequently discussed challenges relative to IT burden placed on NBS programs and the need for proper staffing of NBS programs, which was elevated to the broader HIT workgroup. Most recently, the group has been reviewing pain points and wish list items in preparation for PerkinElmer's next-gen software. The Interoperability group has had eight calls as of September 2018, and meets on a monthly basis. The user group has discussed Logical Observation Identifiers Names and Codes (LOINC) preferred answer lists for disorder interpretations, and disorder specific LOINC submissions among other things. Most recently, the group is tracking gaps in the LOINC panel on a collaborative document that is reviewed with partners at the National Library of Medicine (NLM) and Regenstrief Institute.
Conclusion: The NBS HIT user groups provide an opportunity for open collaboration, sharing and discussion of NBS activities as they relate to Information Management Systems. They provide programs with space to problem solve, approach information management systems challenges uniformly and engage vendors as needed.

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P-006*

Quality Improvement Efforts in Arkansas - A Five Year Update

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Background: Since November 2013, the Arkansas Department of Health Newborn Screening Program has worked with birthing hospitals and Arkansas Children's Hospital to increase the number of specimens received in the lab within 48 hours of collection. Hospitals that do not meet a goal of 80% are considered to be poor performers. The program's nurse educator consults with poor performers to provide education and technical assistance in identifying and overcoming barriers that prevent them from meeting this goal. We later began monitoring specimen delivery processes in the local health units (LHUs) after being awarded a performance improvement grant in November 2015. The Quality Improvement (QI) team monitors timeliness of specimen collection and reporting of results as other QI monitors.

Objectives/Goals: To continue improvement of timely specimen delivery so all facilities meet the goal of 80% and to streamline all processes so time between birth and reporting of results is <168 hours.

Methods: The QI team monitors processes to help identify and overcome barriers to timeliness activities. Monthly QI monitoring determines early on which hospitals may be struggling with their timeliness activities and not reaching the 80% goal. Quarterly timeliness graphs are sent to Hospital Administrators, Lab Managers and NICU/Nursery managers. A summary graph is sent annually that shows hospital placement among peers. Quarterly reports are sent to Maternal and Child Health Specialists at the LHUs for progress updates.

The program reinforces the use of the daily LHU courier service for same day delivery and encourages the use of other overnight delivery services for Saturday and Monday morning delivery.

Educational materials were provided to LHUs and midwives in the form of a newborn screening toolkit thanks to a performance improvement grant received by the NBS program. In-service/training by the nurse educator on newborn screening processes was given in the form of these toolkits and set up as online training for continuing education hours. Though they were provided at an earlier time, toolkits are also available to the hospitals as needed.

Results: Specimen delivery to the NBS lab within 48 hours increased significantly over the last 5 years, from 15% to over 88%. The monthly feedback and quarterly reports to the hospitals have made them aware of the importance of timely specimen delivery. This, in turn, has decreased the average time from birth to reporting results from 218 hours to less than 150 hours and allowed for more rapid identification of possibly affected newborns.

Conclusion: The continued monitoring of QI indicators and education and technical assistance provided has helped the hospitals in Arkansas meet and, in many cases, surpass the timeliness goal of 80%. Meeting the timeliness goal has allowed us to decrease from birth to reporting times and we continue to report results well under the goal of <168 hours.

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P-007

Arkansas' Strategic Planning for Second Screening: Lessons from States that Have Walked this Path

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Background and Problem: Literature review and condition-specific analysis in Arkansas consistently report a net cost savings with early screening, diagnosis, and treatment for rare genetic conditions included in newborn screening panels. The American College of Medical Genetics (ACMG) recommends a uniform panel of conditions to be mandated in state newborn screening (NBS) programs; however, timing and follow-up of screenings are not specified and vary among states.
Methodology: We conducted an online survey to investigate the implementation processes, barriers, costs, and outcomes of states that employ a second newborn screening. We were especially interested in the diagnosis of conditions attributed to second screenings. We used Survey Monkey, an online survey builder, to deliver a 10-question investigator-developed survey to the 14 states that currently collect second newborn screenings. Links to the survey were emailed to state Newborn Screening Program Managers. Significant
Results: All 14 states that collect second newborn screenings responded to our survey. Motivational factors leading to second screening in these states included consultation and recommendations from endocrinology stake holders; high incidence of conditions being diagnosed due to incidental second screens; and trends of early hospital discharge after delivery. Composite survey results from 14 states that collect second screenings include diagnoses of conditions as outcomes solely from second screens: 26% of all congenital hypothyroidism; 14% of all congenital adrenal hyperplasia; 17% of all MSMS diagnoses; 14% of all cystic fibrosis; 6% of all hemoglobinopathies; 9% of all biotinidase; 6% of all galactosemia; 3% of all sickle cell disease; and 6% of all severe combined immune deficiency. Most states use a two-part card for blood spot collection and bill one fee up front which is the major component of their program funding. Timing of the second screen varies from 5-10 days to 2-6 weeks; 10 of 14 states collect second screens between 7 and 16 days. Ninety percent of states that provided statistics for percentage of babies that are followed through with second screen report outcomes of 90% or greater.
Conclusions/Implication: The percentage of conditions diagnosed due to second screens is significant. Economic analysis of NBS has supported positive implications to health services and social welfare and thus supports implementation of second screens.

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P-008

Arkansas Newborn Screening Long-Term Follow-up Database Study – ANGELS Newborn Screening 2017 Annual Report

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Problem/Objectives: Every year approximately 38,000 Arkansas newborns receive a newborn screen for 29 primary core panel metabolic conditions (including Severe Combined Immunodeficiency), hearing loss, and Critical Congenital Heart Disease (CCHD); approximately 80 infants are diagnosed with a

metabolic condition and an average of 50 are diagnosed with hearing loss. 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) Database 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 Database 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 (ACRI). Funding for the Database Study was provided by the ANGELS (Antenatal and Neonatal Guidelines, Education, and Learning System) Project at UAMS. ANGELS is a joint venture between UAMS and the Medicaid Program at the Arkansas Department of Human Services (DHS) to assure the best possible outcomes for high risk pregnancies through tele-consultations, practice guidelines and a 24 hour call center. In 2009, ANGELS was expanded to include a NBS component to address identified gaps in the NBS system, principally database development and analysis for monitoring clinical outcomes on confirmed disorders. 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 Institution Review Board approval in September 2011, the database was implemented in January 2012. Based on projections, the enrollment goal for the Study is a total of 3,000. Calendar Year 2017 marked the sixth full year of implementation of the Database. The 2017 Annual Report provides a summary of the data gathered between 01-01-2017 and 12-31-2017 along with some cumulative data. With 129 new subjects in 2017 representing 130 NBS cases, the Database contained 957 subjects representing 964 NBS cases at the end of 2017.
Conclusions/Implications: The Arkansas NBS LTFU Database Study provides 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.

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P-009

Reliability of a Long-established Newborn Screening Programme for Cystic Firbrosis - Review of Late Diagnoses

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Background: Newborn screening for cystic fibrosis (CF) has been continuous in New Zealand (NZ) since 1983. This has followed a 2-step process, with some changes in methodology. IRT/IRT (immunoreactive trypsin) was used up until 1996 and IRT/limited panel of common genes from 1996-present. A positive CF screen requires an abnormal result in both steps, i.e. raised IRT followed by one or 2 common CF mutations found. All children with CF in the greater Auckland region (population 1.2 million) have full care at Starship Children's Hospital.

Aims: To determine the number of children with a late CF diagnosis and review why they were missed by newborn screening (NBS).
Methods: A retrospective review was performed on all children diagnosed with CF and seen at the Starship Children's Hospital CF clinic over the time period 2003-2017.
Results: Between 2003 and 2017, 110 children with CF were followed through the clinic. 89 children with CF were picked up and diagnosed following a positive newborn screening test and 21 children had a late non-screening diagnosis of CF. Mean age at late CF diagnosis was 32 months (range 6 weeks to 13 years). 8 children had been born abroad; 7 in countries without CF NBS, 1 was picked up by NBS but had an equivocal sweat test. 13 children had been born in NZ; 1 family had refused NBS, 9 were missed as their IRT was not in the top 1%, 2 had high IRT but unusual genes, and 1 was detected by NBS but had an equivocal sweat test.
Conclusion: Even with an established newborn screening programme in place it is important that clinicians remain vigilant about late diagnoses of CF. CF can be missed at all steps of the screening and diagnostic pathway, but the most common reason within our population was having been born in a country without CF NBS.

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P-010*

Newborn Screening for G6PD Deficiency and the Mutational Spectrum in Vietnam
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Objective: Vietnam belongs to a group of countries in Southeast Asia where the potential risk of glucose-6-phosphate dehydrogenase (G6PD) deficiency is associated with a high prevalence of malaria. Study and screening of G6PD deficiency in the Vietnamese population is still scant and fragmented. We present the first nationwide screening study to examine the rate and spectrum of mutations causing G6PD deficiency in Vietnam.
Methods: From January 2018 to July 2018, using the SPOTCHECK® G6PD 50 Hour Reagent Kit, G6PD enzyme activities were measured in dried blood spots of 45,301 neonates, 2-3 days after birth in the Bionet Vietnam Newborn Screening Center. In order to confirm a G6PD deficiency, genetic analysis of the G6PD gene was performed on the remaining dried blood spot samples of 1,083 positive screening results through allele-specific multiplex PCR to detect 8 kinds of mutations reported to be common among Asian populations (871G > A, 1376G > T, 1388G > A, 1360C > T, 592C > T, 487G > A, 563C > T, 383T > C).
Results: Of 45,301 screened neonates (24,205 males, 21,096 females), 44,218 (97.61%) neonates were normal, 1,083 neonates (807 males, 276 females) were deficient for G6PD for an overall prevalence of G6PD deficiency of 2.39% with a male:female ratio of 2.92:1. Among the 1,083 neonates with positive screening results, we found no mutation detected in 431 neonates (39.8%, 294 males, 137 females). 652 neonates (60.2%, 513 males, 139 females) were found to have 6 of the 8 kinds of point mutation of G6PD gene to be present in the Vietnamese population. The frequencies of the mutant alleles in Vietnam were determined to be 17.17% (871G > A), 15.7% (1376G > T), 13.76% (1388G > A), 11.27% (1360C > T), 0.83% (592C > T), 0.18% (487G > A), 0.46% (1360C > T, 1388G > A), 0.28% (1376G > T, 1388G > A), 0.28% (1376G > T, 871G > A), 0.18% (1360C > T, 871G > A) and 0.09% (1388G > A, 871G > A). Two mutation types (563C > T, 383T > C) were not found in the Vietnamese population.

Conclusions: The overall prevalence of G6PD deficiency emphasizes the importance of neonatal screening for early detection and prevention together with proper intervention and genetic counseling in the National Newborn Screening Program. Our study may enrich the molecular diagnosis of G6PD deficiency in Vietnam.

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P-011

Novel Multiplex Method for High Throughput Screening of Pompe, MPS I and X-ALD Disorders

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Background: NBS for Pompe disease and MPS I is commonly performed by measurement of enzymatic activity of acid α-glucosidase (GAA) and α-iduronidase (IDUA), respectively, in newborn dried blood spots (DBS). C26:0 lysophosphatidylcholine (C26:0-LPC) is used as a marker in screening for X-ALD. In California, the NBS program began X-ALD screening in 2016 and Pompe disease and MPS I screening in August 2018. In other newborn screening laboratories, the vast majority of NBS test procedures use an 18-hour incubation and extraction process for routine LSD testing, whereas we report a simplified flow injection analysis (FIA)-MS/MS method that is able to detect GAA, IDUA and C26:0-LPC simultaneously with a short 3-hour incubation time.
Methods: In this method, a 3.2 mm disc of newborn DBS is punched and incubated for 3 hours in an assay cocktail containing GAA and IDUA substrates and internal standards. For X-ALD, blood spots are extracted and incubated for 1 hour in organic solution containing the internal standard d4-26:0-LPC. The extract is transferred and evaporated. Residues are reconstituted into a flow injection solvent. The final extracts are analyzed for GAA, IDUA and C26:0-LPC by FIA-MS/MS in MRM mode with a short run time of 1.5 min per sample.
Results: This method is linear at a wide range of activities (20 levels): GAA=0-22.14 µmol/L/h; IDUA=0-37.59 µmol/L/h; C26:0-LPC (5 Levels)=0-5 µmol/L. Mean recoveries of GAA, IDUA and C26:0-LPC were 80.80%-115.65%, 89.56%-108.50%, and 95.67%-122.33% respectively. The coefficient of variation (CV) was 7.81%-14.91% for GAA; 5.83%-11.20% for IDUA; and 12.36%-16.95% for C26:0-LPC. In our comparison study, we tested 3,000 newborn specimens (normal, borderline and confirmed positives) in three different formats: C26:0-LPC, 2-plex (GAA and IDUA) and 3-plex (GAA, IDUA and C26:0-LPC), with no significant difference observed in the values of GAA, IDUA and C26:0-LPC. Our results agreed 100% with other NBS programs when compared on the same DBS specimens (normal and abnormal). The patient results median observed was 11.0 µmol/L/h for GAA, 9.0 µmol/L/h for IDUA and 0.26 µmol/L for C26:0-LPC. The GAA and IDUA cutoff was based on 18% of the daily median values of GAA and IDUA. The confirmed positive value ranged from 0.51-0.64 µmol/L/h for GAA and 0.31-0.38 µmol/L/h for IDUA. Our 3-plex method has identified all confirmed positives and normal patient results correctly.
Conclusion: This simplified FIA-MS/MS method significantly reduced the sample preparation time and the number of instruments needed for Pompe disease and MPS I analysis. This allowed us to perform simultaneous multiplex testing using a single analysis of Pompe, MPS I, and X-ALD disease on DBS with a short run time of 1.5 min per sample. The improved efficiency of this method resulted in lower cost and high throughput (up to 2000 specimens per day), which is suitable for California's large newborn population.

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P-012

Novel Specimen Card Design and Accession Process for Newborn Screening Test Request Forms (TRF) with Newly-launched Pompe Disease

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The Genetic Disease Screening Program (GDSP) within the California Department of Public Health (CDPH) screens approximately 500,000 newborn per year for more than 80 genetic and congenital disorders, which requires a vast and coordinated system of specimen collection, analysis and reporting of results. Since early treatment of Infantile-Onset Pompe Disease (IOPD) is critical for neonates with cardiomyopathy, decreasing the screening turnaround time by just a few days would result in earlier identification and could affect patient outcomes in newborns with IOPD. In this study, we describe the efforts of the California GDSP to significantly reduce screening turnaround time for IOPD.

Before the launch of newborn screening for Pompe disease in the GDSP central laboratory in August 2018, newborn specimens were first sent to a variety of contracted laboratories located across the state for screening tests; then sent by the regional laboratories to CDPH's biobank. The process usually took around 5-8 days. To expedite screening for Pompe disease, CDPH re-designed the California Newborn Screening Test Request Form (TRF) with six collection spots segregated into two groups of three spots, as a replacement for the previous version that had one group of six spots. The new specimen card design allows the blood spots to be separated and simultaneously tested at different laboratories in separate locations. One extra digit is added to the end of each accession number to differentiate the split cards from the same blood collection, which also serves as an identifier for storage location information purpose.

Based on early results, the new TRF design and accession process has greatly reduced the screening turnaround time for IOPD to only 1-3 days.

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P-013

Newborn Screening for SCID Using Neonatal TREC Kits in California

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Introduction: About 1.47 million newborns were screened for Severe Combined Immunodeficiency (SCID) in California from May 28, 2015 through June 30, 2018 using EnLite™ Neonatal TREC kits. TREC stands for T-cell Receptor Excision Circle, the biomarker that was quantified to screen for SCID and other immune disorders in babies using newborn dried blood spots (DBSs). We used 16 lots of this TREC kit during this period. Minor changes in the reagent components, instability of temperature-sensitive

components and damage to the reagent during transport can contribute to lot-to-lot variation. Hence, we performed reagent verification to ensure lot-to-lot consistency before we used new lots.
Methods: The performance of new lots of the kit was evaluated by comparing against the current lot before depletion. In-house and manufacturer-supplied reference materials and newborn specimens that are positive and negative for SCID were tested. Extended evaluation of the performance of each kit lot was accomplished by comparing performance indicators such as the TREC population distribution, percentage of failed runs, percentage of retests and percentage of infants referred for flow cytometry periodically.
Results: Depending on the size of the kit lot, reagents used in the screening were depleted within 1 to 3 months. There was a considerable lot-to-lot variation in the patient TREC distribution at the 50th percentile. However, at the 1.5th percentile and below, the region of interest, the variation was within one standard deviation from the mean. In the 37 months of screening, the percentage of failed runs was within the laboratory limit of 5% except for two instances. The retest rate varied from 0.30 to 2.25% depending on the TREC cutoff (32, 22 or 18 copies/microliter) used. Specimens were retested when the initial TREC results were ≤ cutoff. In all the kit lots used, more than 70% of these retested specimens overall showed normal results (TREC copies > cutoff) after retesting. In the same cutoff grouping, monthly plots of incomplete and presumptive positive rates showed that rates were mostly within one standard deviation of the mean. Incomplete determinations (0.095%) require collection of a second DBS for a second round of TREC testing. All presumptive positives (0.030%) were referred for flow cytometry testing. Of the 1.47 million babies screened using EnLite™ kits, 21 classical SCID, 2 leaky SCID and 113 other immune disorders were identified. One case of late-onset leaky SCID was missed.
Conclusion: Verifying the performance of a new lot to an existing lot before use and evaluating its performance relative to historical performance of the assay were essential in ensuring reliability of patient results.

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P-014

Overview of California ALD Screening - A Thirty Month Experience

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In 2016, California was the third state in the country to launch newborn screening for X-linked adrenoleukodystrophy (ALD). The ALD biomarker, C26:0-lysophosphatidylcholine (C26:0-LPC), is elevated in affected newborn babies. Our laboratory has developed an efficient tier 1 screening method for analyzing approximately 2,000 specimens per day using a flow injection analysis-tandem mass spectrometry (FIA-MS/MS). A second tier LC-MSMS method followed by DNA testing is required for identification of positive cases. We report on outcomes thus far using a 2 tier system of testing. In the first two and half years of testing about 1.18 million specimens were analyzed using Waters Xevo-TQD MSMS fitted with LC system and sample organizer for tier 1. A 3.2mm disc is punched from each dried blood spot (DBS) and extracted with 100µL solution of MeOH:H_2O (95:5) containing 0.05µmol/L C26:0-LPC internal standard, d4- C26:0-LPC. The DBS is incubated in a shaker incubator for 45 min at 30°C. The specimens are analyzed using FIA-MSMS with a run time of 1.18 min. The cutoff for tier 1 was set at 0.42µmol/L. This cutoff has resulted in about 45,000 tier 1 positives. The patient median observed was

0.26µmol/L.The Waters Xevo-TQS coupled with Acquity UPLC is used for second tier testing. A gradient method using MeOH and H_2O containing 1% formic acid and 2mM ammonium acetate mobile phase is used for analysis, and the run time for each specimen is 2.5min. The tier 2 cutoff for C26:0-LPC was initially set at 0.15µmol/L. The second tier cutoff for C26:0-LPC increased during this period to reduce reporting of false positives and was changed to 0.22µmol/L after analysis of about 22 months' worth of specimens. We studied the sequencing results and case resolutions in order to examine how changing the second tier cutoff impacted the performance of ALD screening. 880,681 specimens were screened for ALD before the cutoff change, and 302,963 specimens were screened after. The referral rate was nearly halved from 25 to 14 per 100,000 screened. The proportion of ABCD1 sequencing results which were found to have variants of uncertain significance (VUS) decreased from 46% to 33% of cases. The positive predictive value increased from 61% to 75%. Changing the second tier C26 cutoff effectively improved the performance of the ALD NBS test, increasing the positive predictive value, decreasing referrals, and reducing the proportion of cases with a VUS. With poor genotype-phenotype correlation and symptom onset after two years of age or older, our positive predictive values will be inflated until clinicians have time to monitor patients and rule out the disorder during long-term follow-up. Furthermore, false negatives cannot be ascertained until our screening population is old enough to exhibit clinical signs and symptoms.

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P-015

Optimizing Newborn Screening for Congenital Adrenal Hyperplasia (CAH) in California

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Objective: Early diagnosis of Congenital Adrenal Hyperplasia (CAH) based on newborn screening before the development of symptoms allows proper treatment, correct sex assignment, and reduced mortality rates. However, the high rate of false-positive results (FPRs) remains an important issue.
Methods: In this study, we assessed the screening performance of CAH screening over the last 11 years in California. To improve screening outcomes, we studied the impact of several parameters including birth weight, gestational age (GA), and continuously adjusted cutoffs of both first-tier and second-tier levels of 17OHP and several other steroids and their ratios. Our goal was to achieve the maximum positive predictive value (PPV) while keeping the sensitivity close to 100%. In order to find the best cutoff on the first tier 17OHP level, a cohort containing more than 250,000 cases (which included 96 cases of different forms of CAH) was studied. Similarly, we studied birth weight and gestational age cutoffs, as well as differentiating markers of second-tier analytes on a cohort that included 57 cases with different forms of CAH and over 2000 non-CAH cases.
Results: During the last 11 years of approximately five million newborns screened, 355 were diagnosed with CAH. The incidence rate of CAH in the state was 1:14,917; 73.8% were identified as classic salt-wasting CAH and 10.4% were virilizing CAH. The PPV of the initial presumptive positive cases after first-tier screening was ~3%. The overall PPV of both first-tier and second-tier screening was 2.6%. The overall FPRs was 97%. The number of FPRs was significantly higher among newborns with birth weight less than 2500g and/or with GA less than 35 weeks. The top 99.9 and 99 percentiles for both direct analyte values and GA and birth weight-adjusted cutoffs were compared.

Conclusion: By adjusting first-tier and second-tier analyte cutoffs and ratios, we would be able to decrease the number of referrals and the FPRs significantly (~50%) with minimum loss of sensitivity. Using birth weight-specific and 99.9 percentile of first tier 17OHP cutoffs, we would increase the PPV to 35%.By utilizing a combination of birth weight-specific cutoffs for 17OHP and other steroids and ratios for both first and second-tier results, we would improve the overall PPV for CAH screening with no loss of sensitivity.

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P-016

Classic Galactosemia in California: GALT Activity and Common Mutations

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Background: Deficiency in galactose-1-phosphate uridyl transferase (GALT) results in an inherited metabolic disorder: classic galactosemia. Common mutations/variants of this enzyme are well identified: IVS2-2A>G, S135L, T138M, Q188R, L195P, Y209C, L218L, K285N, and N314D. The California Newborn Screening Program uses newborn dried blood spots to screen for GALT deficiency. Follow-up testing is conducted for presumptive positive cases and identifies common mutations in the GALT gene. Prior studies have reported a high frequency of some mutations, such as Q188R in select populations. The IVS2-2G mutation is known to be more common in the Hispanic population. We studied the association between GALT activity levels and galactosemia phenotypes using data from eight years of newborn screening in California. In addition, we described the frequency of the most common GALT mutations in the racially and ethnically diverse California population where almost 50% of births are to Hispanic women.

Design: Each year, approximately half a million newborn dried blood spots are tested for GALT deficiency using the API 300 Analyzer. Presumptive positive cases are followed up by DNA testing in order to identify common GALT gene mutations. We studied the association between GALT activity levels and galactosemia phenotypes along with the frequency of seven mutations and two variants in newborns tested from July 2008 through June 2016.

Results: Out of nearly 4 million babies screened in California during the 8-year period, 409 newborns were identified as presumptive positive based on GALT activity ≤50 µM. The final case resolution of the presumptive positives was: classic galactosemia, n=51 (12.5%); Duarte galactosemia (D/G), n=164 (40.1%); carrier, n=24 (5.9%); and no disorder, n=160 (39.1%). The GALT activity during the study period was significantly lower for newborns confirmed as having classic galactosemia (GALT activity median = 18 µM), while newborns with D/G or carrier conditions did not show significant differences compared to screen-positive newborns identified with no disorder. The frequency of common mutations/variants was as follows: Q188R mutation (66.40%) and the variant N314D (66.40%) had the highest allele frequency, followed by IVS2-2G (7.69%), S135L (6.88%), K285N (2.43%), L195P (2.02%), Y209C (0.81%), and L218L (0.4%).

Conclusion: To the best of our knowledge, this is the largest study describing the association between GALT activity levels in newborn dried blood spots and galactosemia phenotypes and the frequency of GALT mutations in a racially and ethnicity diverse population. In concordance with a study from Texas, another state with high percentage of Hispanic births, Q188R was the mutation with the highest prevalence and the IVS2-2G had a relatively high frequency.

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P-017*

Short-Term Follow-Up for Newborns with an ABCD1 Variant of Uncertain Significance

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Objective: The California Newborn Screening (NBS) Program began screening for X-linked adrenoleukodystrophy (ALD) in 2016. Using a 3-tiered screening approach, all newborns with elevated C26 at the first and second tiers of screening receive ABCD1 sequencing analysis and are referred for follow-up. Since a large proportion of specimens are found to have a variant of uncertain significance (VUS), and given the ambiguous nature of VUS sequencing results, we sought to examine how the follow-up service utilization necessary to provide patients with a diagnosis compares for patients with a VUS versus patients who have either a pathogenic variant or no mutation.
Study Design: 242 newborn specimens were screened and received ABCD1 sequencing results since ALD NBS screening began in California. 212 cases that were sequenced had complete follow-up data reported, including specialists involved in care, contact mode, tests ordered, and date of diagnostic decision. We compared the short-term follow-up service characteristics of patients with a VUS to those of patients with a pathogenic variant or no mutation.
Results: Of the 212 patients who had ABCD1 sequencing results and complete follow-up data, a majority (44%) of them had a VUS, while 33% had no mutation, and 23% had a pathogenic variant. The proportion of patients who had a face-to-face contact at a specialty care center was similar for those with a VUS compared to those with a pathogenic variant (64% vs. 65%), and higher than those with no mutation (41% ($X2(1)=9.0$, $p<0.05$)). The number of providers that were involved in short-term follow-up was similar across all three groups, averaging 2.7 providers. More tests were ordered for patients with a VUS compared to those with a pathogenic variant (1.5 vs. 1 ($F(1,141)=6.8$, $p<0.05$)), but less than for those with no mutation (1.8 ($F(1,162)=5.12$, $p<0.05$)). The median number of days to make a diagnosis was more than three times greater for patients with a VUS (250 days) compared to those with a pathogenic variant (76 days) and almost twice as great as those with no mutation (131 days).
Conclusion: We found that patients with a VUS utilized at least as many, if not more, services than patients with a pathogenic variant. Patients who have a VUS generally have a similar number of providers involved in the follow-up care and a similar proportion of face-to-face contacts as patients with a pathogenic variant, but they have more tests ordered and more than a threefold increase in the time that it takes to make a diagnostic decision. States who begin to add ALD to their newborn screening panel should consider the increase in demand on specialty care centers, especially early on when cutoffs are often set conservatively and VUS results might be more common.

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P-018

An Accelerated Pilot Proficiency Testing Program to Measure SMA Screening Assay Performance in Newborn Screening Laboratories

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Spinal muscular atrophy (SMA) is the leading genetic cause of infant death in the United States US). It is a neurodegenerative, autosomal recessive condition affecting approximately 1:10,000 live births. With rare exceptions, SMA is associated with deficient production of the survival motor neuron (SMN) protein that causes loss of motor neurons followed by progressive muscle atrophy and weakness. Approximately 95% of SMA cases are caused by changes to the SMN1 locus on chromosome 5q, such as homozygous deletions surrounding exon 7 of the SMN1 gene or ectopic gene-conversion with a paralogous gene. An SMA treatment was recently approved by the FDA that arrests neurodegeneration, alleviates symptoms, and is most effective when administered pre-symptomatically. With an approved treatment and the availability of validated SMA molecular screening assays to detect SMN1 exon 7 deletions, the US Secretary of HHS approved the addition of SMA to the Recommended Uniform Screening Panel for newborn screening in the US in July 2018. Newborn screening labs across the US are starting to implement SMA screening, and many use a multiplex assay developed at CDC. In addition to providing technical support and qualitative assurance materials for assay development, we started offering an accelerated pilot proficiency testing (PT) program to newborn screening labs to monitor their assay performance. Beginning June 2018, this program provides a panel of de-identified, reference dried blood spots prepared at CDC to participating labs every two months. We analyze the resulting data submitted by all participants and generate a report that identifies the samples and summarizes the consensus results for each sample across participating labs. This feedback allows contributing labs to measure the quality of their assay and accuracy in their data interpretation in comparison to other participants. Currently, thirteen labs at various stages of assay development/implementation are enrolled in this pilot PT program. After our first two rounds of data collection, 100% of the labs correctly identified the SMA-positive samples in the panels. Data from several labs deviated from the general consensus for additional targets that are multiplexed with their SMA screening assays, allowing us to provide constructive feedback to improve their overall assay performance. This pilot PT program allows CDC to further evaluate our SMA reference materials. It also helps us determine what kind of data is most valuable to participating labs and optimize an effective reporting format. By collecting data from different labs using the same sample set, we can also better evaluate different mechanisms that harmonize data between labs, which we will propose to the newborn screening community. We plan to transition this mutually beneficial pilot PT program to the CDC's Newborn Screening Quality Assurance Program within a year.

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P-019

Performance of Multi-Level Testing Algorithms for Sickle Cell and Other Hemoglobinopathies Proficiency Testing

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Background: The Newborn Screening Quality Assurance Program (NSQAP) at the Centers for Disease Control and Prevention operates a proficiency testing (PT) program for newborn screening (NBS) laboratories that test for sickle cell disease and other hemoglobinopathies (HbPT) in dried blood spots (DBS). Three times per year we distribute panels of PT specimens to 76 laboratories in 12 countries. NSQAP is in the unique position to track testing practices and algorithms over time, in addition to developing methods for the preparation of PT specimens that are fit for testing and appropriately challenge participants.
Methods: DBS panels of five blind-coded specimens were prepared from purchased umbilical cord blood containing normal or abnormal hemoglobins. Participants reported the presumptive hemoglobin phenotype, clinical assessment, and methods used in their testing algorithm. NSQAP provided event summary reports, which included compiled error frequencies for each method algorithm reported.
Results: Error frequency data for the reported algorithms was summarized for 2014 to 2018. Phenotype error and clinical assessment error percentages were calculated based on the number of samples assayed by each method algorithm reported. The average percentage for both phenotype and clinical assessment errors was determined over this time period. The algorithms showing the highest phenotype % error were Isoelectrofocusing (IEF) : IEF (6%) and IEF-only (4%). The average clinical assessment error rates for these algorithms were 9% and 4% respectively. Algorithms that relied solely on high performance liquid chromatography (HPLC) had an average error rate of 2% for both phenotype and clinical assessment.
Discussion: NSQAP tracks participant and method performance for hemoglobinopathy screening through the HbPT program. We logged 13 types of testing algorithms used by participants over a 5-year period. We found algorithms that rely solely on IEF, or IEF for both primary and secondary methods, had the highest phenotype and clinical assessment error rates. Phenotype detection was improved when both IEF and HPLC were used in the testing algorithm. Clinical assessment errors often followed an incorrect phenotype designation or could be attributed to using the wrong interpretation code.

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P-020*

Critical Congenital Heart Disease Screening in the United States

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Background: Critical congenital heart disease (CCHD) was added to the Recommended Uniform Screening Panel in the U.S. on September 21, 2011. By July 2018, all states had requirements in place for CCHD screening. While the impact of the pulse oximetry screening itself is unknown, the adoption of regulations for CCHD newborn screening (NBS) is associated with decreased mortality due to CCHD in

the U.S. (Abouk, JAMA 2017). We sought to understand the outcomes of newborns screened for CCHD in the United States.
Methods: The HRSA funded NewSTEPs Data Repository (Ojodu, IJNS 2017) was queried for cases reported between 2012-2018, including CCHDs, non-critical CHDs, and cases identified after the NBS period. Data entry into NewSTEPs is governed by a Memorandum of Understanding between the state newborn screening program (NSP) and the Association of Public Health Laboratories. Three NSPs who were unable to enter data into the repository provided aggregate reports. Frequencies and descriptive statistics are presented.
Results: Ten NSPs provided case data on 145 infants with a CCHD and 81 infants with a non-critical CHD. Aggregate reports from 3 NSPs provided data on 31 infants with CCHD and 51 with non-critical CHD, with one infant identified after NBS. NSPs who were unable to provide data cited no requirement to report to public health, no data reported despite mandate, difficulty in extracting data from electronic system, or constraints of MOU for CCHD data.Of 145 infants reported with CCHDs with case level data, 105 were identified by NBS (22 Total Anomalous Pulmonary Venous Connection, 18 Tetralogy Of Fallot, 16 Coarctation Of Aorta, 14 Transposition of Great Arteries, 10 Pulmonary Atresia, 8 Hypoplastic Left Heart, 7 Interrupted Aortic Arch, 3 Ebstein Anomaly, 2 Double Outlet Right Ventricle, 2 Tricuspid Atresia, 2 Truncus Arteriosus, 1 Aortic Valve). The median age at diagnosis was 2 days (IQR 1-2 days). Three cases identified after NBS were missed due to screening errors, 36 had screening results in normal range, 1 was unknown. Most cases diagnosed later in infancy had coarctation of aorta (21/40) or Tetralogy of Fallot (11/40). The rate of CCHD cases identified by NBS ranged from <1 to 12/100,000 live births and was not associated with size or the percent of rural births in the state. Three NSPs provided the number of newborns identified by NBS compared to total CCHD cases reported to birth defects registries (22/230, 8/561 and 7/169).
Conclusions: CCHD NBS is required in all 50 states and is successfully identifying infants in the first days of life. Data collection for CCHD NBS remains challenging at the hospital, state, and national levels, further limiting the ability to assess the impact of pulse oximetry screening and implement quality improvement initiatives. Further investments need to be made to improve the data infrastructure and data accessibility at all levels in order to continue to strengthen CCHD NBS.

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P-021

Assessing the Performance of Multiple Dried Blood Spot DNA Extraction Methods using Two NGS Methods

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Objective: Assess the performance of CDC's Cystic Fibrosis (CF) Molecular Proficiency Testing dried blood spot (DBS) specimens using two next generation sequencing assays with commonly used DNA extraction methods. Background: An increasing number of newborn screening laboratories are moving towards Next Generation Sequencing based typing technology to screen for CF, making it essential to ensure that the samples from CDC's CF Molecular DBS Repository perform well with these technologies. In an effort to provide comprehensive quality assurance and technical assistance to laboratories moving towards incorporating Next Generation sequencing assays, CDC designed a study to evaluate various commercial and home brew DNA extraction methods from a number of samples with different disease

causing variants using two commercially available Next Generation sequencing assays to sequence the coding regions of the CFTR gene.
Methods: DNA was extracted from one 3.2 mm DBS punch from selected samples in the CF Molecular DBS Repository using eight commercial and laboratory developed DNA extraction methods including a silica column, variations on three commercial boil prep reagents, and three in house boil preps including two Triton-MgCl2 based reagents. The DNA yield was measured for each DNA extract to determine the DNA input for the sequencing assays. DNA extractions were characterized using two commercially available Next Generation sequencing library preps for CFTR from Illumina and Swift Biosciences. Results were checked for concordance using Sanger sequencing of coding regions, intron-exon splice junctions and noncoding regions known to contain CF causing variants. FASTQ files were run through an in-house developed Bioinformatics pipeline as well as the manufacturers' Bioinformatics pipeline when available to determine any differences from the reference sequence as well as coverage.
Results/Conclusion: The performance of the DNA extracts from each sample was determined using freeware bioinformatics tools. The overall specimen coverage as well as the coverage for disease and non-disease causing variants were compared along with the input DNA amount. Preliminary results found that sequencing data from each of the extraction methods was able to identify the disease causing variant(s) when the DNA concentration was in the normal range. Coverage and other quality metrics did suggest that data from the cruder extraction methods may not be as reliable. The complete results should provide insights on the performance of each of the DNA extraction methods with select commercial Next Generation sequencing methods for CFTR, which should give some indication of performance in other assays with these technologies.

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P-022

Development of a New Generation of Proficiency Testing Dried Blood Spots Materials for Mass Spectrometry-based Newborn Screening

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Background: The Newborn Screening Quality Assurance Program at The Centers for Disease Control and Prevention (CDC) provides dried blood spot (DBS) proficiency testing (PT) specimens for disorders of amino acid and fatty acid metabolism to approximately 700 newborn screening laboratories worldwide. Currently, CDC DBS PT specimens are prepared by enriching blood with disease biomarkers at elevated concentrations (10%-50% percentile of disease). However, PT specimens may not closely reflect the metabolic profiles for each disorder, and one PT specimen may contain analyte concentrations that are representative of multiple diseases. Thus, current PT samples may not be compatible with all interpretive algorithms and do not take into account second-tier screening procedures. In order to accommodate the shift to multiple analyte algorithms as well as the adoption of second-tier screening we developed condition-specific PT specimens, which mimic analyte concentrations found in a single disorder using high-accuracy techniques. Furthermore, the target concentrations for each analyte were similar to analyte concentrations from confirmed cases data obtained from NBS laboratories to more closely resemble patient specimens. Finally, this new generation of PT materials are enriched with second-tier screening analytes.
Methods/Results: High accuracy enrichment was achieved by using a two-step approach. Washed and delipidated blood was enriched at five different concentrations points for each of the analytes of

interest. Those included the targeted concentration as well as ±25% and ±50% of the targeted concentration creating calibration curves that dictated the exact volume of each analyte stock solution that needed to be added to achieve the desired concentrations. By using this approach, it was possible to create PT specimens in which analytes of interest were enriched in average within 5% of the targeted concentration. Methionine, Arginine and Succinylacetone were the most challenging analytes to enrich accurately as they tend to degrade rapidly.
Conclusions: A new generation of PT materials was developed which mimic very closely analyte concentrations found in DBS specimens from babies that were diagnosed with a NBS disorder.

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P-023

A Systematic Study of Hydrolytic Degradation of Acylcarnitines During Sample Preparation and Analysis In Newborn Screening using Tandem Mass Spectrometry

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Background: Amino acids and acylcarnitines can be analyzed by mass spectrometry in their underivitized form or after derivitization to form butyl esters. Although derivitization with butyl esters enhances the sensitivity of the assay and can resolve some isobars it partially hydrolyzes acylcarnitines to carnitine, interfering with carnitine's accurate quantitation. In this study, we systematically investigate the hydrolytic degradation of each acylcarnitine to free carnitine during sample preparation and analysis. Different parameters influencing the hydrolysis were also investigated such as reaction time and temperature.
Methods/Results: Unlabeled acylcarnitine standards were prepared at equimolar concentrations. Each acylcarnitine was derivitized in separate wells in triplicate under different derivitization conditions. Hydrolysis of acetylcarnitine was at least 2.5 times higher than higher homologues. Furthermore, derivitization reaction time and temperature had a significant effect on hydrolysis yield, while other analytical parameters had minimal to no effect.
Conclusions: Acetylcarnitine was by far the main contributor of sample preparation induced free carnitine due to higher hydrolysis yields and inherently higher concentrations in blood.

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P-024

Creating Quality Control Dried Blood Spots Representing Rare Genetic Conditions for Molecular Testing using Synthetic Double-stranded Gene Fragments

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Newborn screening assays targeting DNA sequences have become more prevalent in recent years. When producing dried blood spots (DBS) for quality controls (QC), actual clinical materials collected from affected infants is not an option due to the quantity of blood needed. We previously developed

QC materials with immortalized cell lines, either derived from patients or modified in the laboratory, that carry the DNA variants of interest (APHL NBSGTS 2017). While DBS made from cultured cells work, the technique cannot be duplicated within newborn screening labs without tissue culture facilities. We have most recently developed a novel approach by using synthetic double-stranded DNA fragments to mimic key genetic sequences detected in different heritable disorders. These 100-3000 base pair synthetic gene fragments can be custom-made to contain the exact gene sequences of interest together with an internal reference gene sequence used in the assay. To produce QC materials for a multiplex SMA-SCID-reference gene real-time PCR assay, we designed gene fragments containing SMN1 and SMN2 genes (covering the intron 6 to exon 8 region), the RPP30 region of RNase P gene, and the TREC sequence. The various fragments were combined in the correct ratios, and appropriate amounts were then spiked into leukocyte-depleted blood to prepare DBS. The "normal" QC contained a gene fragment pool that comprised SMN1, SMN2, RPP30, and TREC sequences. The "SMA" QC contained SMN2, RPP30, and TREC, while the "SCID" QC had SMN1, SMN2 and RPP30 gene fragments.

The various QC prepared from gene fragments were compared to DBS prepared from human blood in the multiplex real-time PCR assay for SMA and SCID. Results indicated that the gene fragment QC materials performed in a very similar manner to human blood QC, including the proportion lost to washing, proportion remaining for the in situ assay, or proportion recoverable in DNA extraction. We sent out the gene fragment QC to be evaluated by multiple state NBS labs that had established the SMA assay. Every lab was able to correctly identify the SMA positive and negative samples.

In conclusion, we have demonstrated that QC materials prepared with synthetic double-stranded gene fragments can perform well in newborn screening assays. All necessary components are readily available at a very modest cost from commercial sources, so QC materials can be prepared locally within NBS labs with ease. More importantly, even genetic variants that are very rare and difficult to find from clinical materials or cell lines can be readily constructed with gene fragments. This new approach can potentially expand our ability to prepare a wide variety of QC samples that were previously unavailable.

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P-025

Pilot Molecular Galactosemia Program: The 2 Year Experience

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Classic galactosemia is a disorder in which the body is not able to process galactose. Failure to promptly identify and treat with the removal of galactose in diet results in lethargy, failure to thrive, jaundice, liver damage, and further progression to sepsis and shock. Since 2004, all state newborn screening (NBS) programs in the U.S. have screened newborns for galactosemia using an indirect activity assay for galactose-1-phosphate uridylyltransferase and some programs also measure total galactose. Currently seven state laboratories have developed an assay that can be used as a second tier to provide physicians with timely information about GALT variant status. The variant panels differ between newborn screening programs with the following variants included in one or more panels: Q188R (c.563A>G), S135L (c.404C>T), N314D (c.940A>G), K285N (c.855G>T), L195P (c.584T>C), F171S (c.512T>C), Y209C (c.626A>G), T138M (c.413C>T) and c.253-2A>G (IVS2-2A>G).Over the past two years, CDC has piloted a

GALT variant detection program using laboratory created dried blood spots made from EBV transduced galactosemia patient cell lines with rare GALT variants. Recently, CDC's GALT variant repository was expanded to include 78 samples representing 56 unique galactosemia causing variants which were a generous gift from Judith Friedovich-Kiel from Emory University. NBS programs that use a second tier GALT variant detection assay have assessed the performance and suitability of many of these samples using three different assays: florescent probe hybridization, amplification refractory mutation system (ARMS) and Allele Specific Primer Extension. During the pilot testing phase, all seven labs provided correct results based on their variant panels. There were several interesting situations that arose particularly surrounding the evaluations of samples with a deleted GALT gene. Since the gene deletion is not routinely tested, it was not uncommon for a laboratory to incorrectly identify the genotype of a quality assurance sample, but still score 100% satisfactory. A similar situation was seen in the identification of the Q188R variant when a Q188P variant was present. In addition, traditional CDC proficiency testing programs are graded on the correct identification of the analyte or genotype and the clinical assessment. However, variant detection for galactosemia is considered supplemental information to assist physicians in directing timely treatment of the infant and does not impact the clinical assessment, thus requiring a unique approach to proficiency testing. While some of these issues are specific to the GALT variant detection program, others will also affect any molecular detection program, so a consistent approach will need to be developed.

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P-026

Development of High Resolution Mass Spectrometry Applications in Newborn Screening for Determination of Amino Acids and Acylcarnitines from Dried Blood Spots

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Introduction: Amino acid and acylcarnitine first-tier screening typically employs derivatized or non-derivatized sample preparation methods and flow injection analysis (FIA) coupled to triple quadrupole (TQ) tandem mass spectrometry (MS/MS). TQ instruments feature low mass resolving power resulting in difficulties separating nominal isobaric compounds, especially those with identical fragmentation patterns such as malonylcarnitine (C3DC) and 4-hydroxybutylcarnitine (C4OH). Sample derivitization utilizes mass shifts to distinguish C3DC and C4OH, however, this reaction increases sample preparation time, requires corrosive chemicals, and creates other isobars particularly with acylcarnitines such as acetylcarnitine and glutamic acid. The objective of our study was to develop and assess methodologies employing High Resolution (HR) MS for newborn screening assays to overcome these challenges.
Methods: The Centers for Disease Control and Prevention's (CDC) Newborn Screening Quality Assurance Program (NSQAP) quality control dried blood spot cards were analyzed in this study. Samples were extracted following the non-derivatized protocol as previously described [1], and were resuspended and analyzed by FIA in 50:50 acetonitrile:water with 1% formic acid. HR MS analysis was performed using a Q-Exactive Plus equipped with a HESI-II source, using selected ion monitoring (SIM) at mass resolutions 17 000, 35 000, 70 000, and 140 000, and parallel reaction monitoring (PRM) for MS/MS at mass resolution 17 000. Quantitative results were processed using TraceFinder 4.1 and these data were compared to results obtained on our TQ.
Results: HR SIM and PRM analysis produced similar quantitative results to those obtained on TQ platforms. The separation of C3DC (m/z 248.1134) and C4OH (m/z 248.1498) was achieved using a single

SIM at mass resolution 17 000. In HR SIM analyses, most analytes had similar quantitative results across mass resolutions, however, for several analytes quantitation was only accurate at mass resolution 140 000. Quantitative issues with these analytes observed at HR SIM mass resolutions < 140 000, was due to isobaric interference by contaminants with m/z's similar to several commonly utilized internal standards. These issues were mitigated by combining both HR SIM and PRM into a single MS method which yielded accurate quantitation. Furthermore, HR SIM/PRM hybrid methods have been developed to include HR full scan analyses, to combine sensitive targeted quantitative screening with untargeted metabolomic profiling in a single analysis.
Conclusions: The increased specificity achieved utilizing HR MS allowed the resolution and quantitation of individual isobaric analytes, while producing similar quantitative results to TQ MS/MS platforms for other analytes.

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P-027

Development of a CYP21A2 variant assay for 2nd-tier molecular Congenital Adrenal Hyperplasia Newborn Screening

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Objective: Develop a Congenital Adrenal Hyperplasia (CAH) molecular second-tier method appropriate for newborn screening to reduce false-positive and false-negative screening results.
Background: CAH, due to 21α-hydroxylase (21-OH) deficiency, is caused by pathogenic variation in the CYP21A2 gene. Different subtypes of CAH include the two severe (classic) forms of salt-wasting (SW) CAH, which can result in adrenal crisis, and simple virilizing (SV) CAH, which can result in ambiguous genitalia in females; and the milder non-class (NC) form. Screening for CAH involves a fluoroimmunoassay to measure 17α-hydroxyprogesterone (17-OHP) in dried blood spots (DBS) collected 24-48 hours after birth. 17-OHP is a metabolite that accumulates with 21-OH deficiency. CAH screening is challenging due to elevations of 17-OHP in cases of traumatic birth, illness, or prematurity, leading to high rates of false-positive screening results. In addition, delayed accumulation of 17-OHP following birth can lead to false-negative results, especially in one-screen specimen programs that do not collect a routine second DBS specimen. In Minnesota, a significant portion of true CAH cases were missed by the newborn screen despite efforts to improve assay sensitivity and specificity through birth-weight adjusted 17-OHP cutoffs and 2nd-tier 17-OHP biochemical analysis. To address these challenges, a collaboration between the Centers for Disease Control and Prevention, the Minnesota Department of Health (MDH), and the University of Minnesota was formed to develop and pilot test a molecular-based second-tier CAH screening assay to enhance specificity.
Method: A multiplex 21-variant Allele Specific Primer Extension (ASPE) assay was developed to detect pathogenic variants identified in 103 Minnesota families (249 participants) with one or more children with CAH. The CYP21A2 gene was fully characterized and DBS samples were created for method development and validation. The ASPE assay and a long-range PCR test to detect 30kb deletion alleles was transferred to MDH for use in a one-year retrospective pilot study of anonymized DBS samples. Of

the 4,092 retrospective specimens, 381 samples containing at least one variant were sent to CDC for confirmation of the deletion assay and full CYP21A2 sequencing.
Results and Conclusions: Comparison of CYP21A2 sequencing and the CAH molecular method highlighted several challenges of molecular screening. A sequence variant in Exon 6 interfered with five samples in the ASPE assay and another variant in Intron 9 in two samples interfered with sequencing. An alternate variant of an allele required adjustments of test ratios, and sequencing detected one predicted SW/SV allele and four NC alleles not currently on the panel. Furthermore, a higher than expected frequency of multiple variant samples, similar to the carrier family samples, indicates a possible role for phasing.

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P-028

Second-tier Newborn Screening Assays for Detection of Spinal Muscular Atrophy
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Spinal muscular atrophy (SMA) is an autosomal recessive condition, affecting an estimated 1:10,000 live births and is the leading genetic cause of infant mortality. It is caused by the loss of functional Survival Motor Neuron (SMN) protein, which, in most cases, is attributed to a genetic mutation that results in the absence of exon 7 in the SMN1 gene. SMN protein deficiency causes degeneration of the motor neurons and eventually the loss of voluntary muscle control. The clinical phenotype is classified into different subtypes, ranging from Type I (severe) to Type III (mild). A paralog gene, SMN2, produces SMN protein at 10% of SMN1 levels. The number of copies of SMN2 varies between individuals and has been shown to correlate inversely with disease severity. Recently FDA approved an antisense oligonucleotide (ASO) drug that is able to modify mRNA splicing in SMN2 to increase SMN protein production, reducing the symptoms and severity of disease, with the greatest success being in those treated pre-symptomatically. The current recommendation is that SMA infants with three or fewer copies of SMN2 be treated immediately after diagnosis. Currently, newborn screening laboratories are using a real-time PCR assay to detect the absence of SMN1 exon 7 from dried blood spots (DBS) as a first tier test to identify SMA infants. Since ASO therapy is most beneficial when given pre-symptomatically, deciding whether to treat often depends on the genotype alone, without the usual references, such as disease phenotype. There is a need for second tier assays to test for biomarkers that can confirm the screening results and to provide prognosis on disease severity. We are currently developing two second tier assays that can utilize DBS samples to address these issues. In the first assay, we measure full length SMN1 mRNA levels by RT-qPCR. An SMA affected newborn produces mostly truncated mRNA for the SMN protein. Unaffected individuals, or individuals with a non-pathogenic nucleotide change that may potentially yield false positive results in the first tier assay, are expected to produce normal mRNA. The second assay will determine SMN2 copy number using digital droplet PCR (ddPCR). In the absence of a clinical phenotype, SMN2 copy number is the best available marker to determine treatment options. Based on Poisson distribution modeling, ddPCR can provide precision usually superior to other methods. We will evaluate its reliability compared to other methods commonly used in diagnostic laboratories. Precision in the range of 3-6 SMN2 copies per cell, in particular, has the most impact on the treatment decision. Results of these assays will be presented. We expect that these second tier assays will be efficient, low-cost options for state labs to utilize to reduce the number of false positive results, thus

defining and improving the positive predictive value for SMA in NBS, and to allow for better characterization of disease severity.

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P-029

Implementation of State-wide Newborn Screening for Spinal Muscular Atrophy in Public Health Laboratories – An Overview

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Background: Type 1 spinal muscular atrophy (SMA), a neuro-degenerative disease, is the leading genetic cause of mortality in infants. Most SMA cases are caused by variants of the SMN1 gene resulting in SMN protein (SMNP) deficiency. A paralogous SMN2 gene also produces some SMNP and can modify severity. FDA approved a drug to treat SMA in 2016. The HHS secretary added SMA to the Recommended Uniform Screening Panel in 2018. The implementation of effective laboratory testing is a critical component of newborn screening (NBS) for new conditions. Here we discuss the required elements for laboratory adoption of SMA testing and CDC's role to support State programs. First tier assays: Laboratory test for SMA relies on detecting the absence of SMN1 exon 7 region. The presence of SMN2 exon 7, which differs from SMN1 by a single nucleotide, necessitate maximum assay specificity to avoid false negatives. We have developed SMN1 tests based on locked nucleic acid modified probe, which can be multiplexed into the TREC qPCR assay currently used by many NBS labs. These tests have been validated and employed by three state NBS labs. Over 100,000 newborns have been screened and 6 new SMA cases identified thus far. Assays using minor groove-binding probe or alternative technology are other options. Second tier assays: Since therapy may be initiated based on genotype alone in pre-symptomatic stage, second tier assays for biomarkers to confirm positive screening results, and to provide a prognosis on disease severity are needed. NBS labs may choose to establish these tests in-house, or refer to clinical labs. An assay to measure SMNP in whole blood with electrochemiluminescence was reported recently. We are developing a confirmatory assay based on mRNA for the SMNP in dried blood spots (DBS). SMA affected newborns have only the SMN2 gene to produce mRNA, which mostly lacks exon 7 due to erroneous splicing. For prognosis, we have developed a laboratory-developed test for SMN2 copy number quantification in DBS using digital droplet PCR. Test kits for SMN1 and SMN2 copy number are also available from commercial sources. Quality control (QC) and proficiency testing (PT): We have produced SMA QC samples that performed well in state NBS labs, using cell lines derived from SMA patients. We are also exploring the use of synthetic double stranded gene fragments, which would allow state NBS labs to produce QC samples locally. We have started a pilot PT program for SMA in June, 2018.Other considerations: Since the multiplex assay for SMA and SCID can replace the current TREC assay for many NBS labs, no extra lab staff or equipment is needed. With the birth prevalence of 1:10,000, large NBS programs may need additional follow-up staff.
Conclusion: All the essential components for laboratory implement of SMA screening are already accessible, as shown by the success of several state programs. Scientists at CDC are available for advice and technical support.

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P-030*

Harmonizing Domestic and International Newborn Screening Labs MS/MS Analyte Results and Cutoffs using the CDC NSQAP Reference Materials

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Introduction: Newborn screening laboratories cannot accurately compare mass spectrometry-derived analyte results and cutoff values due to differences in testing methodologies (i.e., derivatized vs. non-derivatized methods). The Center for Disease Control and Prevention's (CDC) Newborn Screening Quality Assurance Program (NSQAP) provides newborn screening labs with quality control (QC) materials which contain endogenous and three enriched levels of amino acid and acylcarnitines, as well as proficiency testing (PT) materials that mimic analyte concentrations of newborns with metabolic disorders. The objective of this study was to harmonize domestic and international newborn screening laboratory results and cutoffs using the NSQAP QC samples, and validate harmonization using the PT specimen results.

Methods: NSQAP QC and PT data reported from 256 laboratories in Q3-2016 were used in this study. QC materials were provided as dried blood spot cards which included a base pool and the base pool spiked with specific concentrations of metabolites in a linear range. For each metabolite, a laboratory's quantified QC values were regressed on the CDC NSAQP QC quantified values using linear regression; variables were logarithmic transformed prior to regression. Using this approach, a laboratory's regression parameters were used to scale their reported PT value and cutoff for each metabolite using the CDC as a reference laboratory. The %RSD was calculated for metabolites raw and harmonized PT results for comparison across laboratories.

Results: Regression parameters were calculated for 17 acylcarnitines and 8 amino acids, and bias plots were created to visualize method differences between laboratory results and cut-offs before and after harmonization. All laboratory reported PT metabolite values had decreased %RSD post-harmonization, decreasing 1.18 to 2.79-fold. The largest method associated decrease in %RSD was succinylacetone, malonylcarnitine (C3DC), 3-hydroxyisovalerylcarnitine (C5DC), and arginine, which had %RSD decreases from 145.8% to 52.2%, 53.7% to 23.5%, 35.5% to 14.7%, and 35.6% to 20.3%, respectively. For many analytes, the inter-laboratory variation in raw PT values was associated with method bias, and this method bias was eliminated in harmonized PT values.

Conclusions: The results of this study could assist newborn screening labs compare analytical results, cutoffs, and healthy population range differences to facilitate uniformity.

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P-031

Development of Sanger Sequencing Assay to Characterize the IDUA Gene to Screen for Mucopolysaccharidosis Type I Disease

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Objective: To develop and validate a second tier Sanger sequencing assay for the IDUA gene associated with Mucopolysaccharidosis type I (MPS I) disease for newborn screening.
Background: MPS I, an autosomal recessive condition and one of the lysosomal storage disorders (LSDs), was added to the Recommended Uniform Screening Panel in 2016. As with other LSDs, MPS I has both a severe and attenuated form and has a number of pseudodeficieny alleles which result in the apparent absence of IDUA enzyme activity, but are not associated with a pathological phenotype. One way newborn screening laboratories are decreasing the false positive rates due to the pseudodeficieny alleles is to add second tier molecular testing. Since many of the disease causing variants are private, a second tier genotyping approach would not be feasible. Thus, programs employing a second tier molecular approach must utilize gene sequencing. Due to the small number of samples that require gene sequencing, most labs are electing to use Sanger sequencing over Next Generation Sequencing. This will significantly decrease the turnaround time, which is critical for the severe forms of this disorder. The gene involve with MPS I is α-L-iduronidase (IDUA; MIM# 252800) and maps to chromosome 4p16.3 and contains 14 exons that span ~19kb.
Method: DNA was extracted from whole blood using a commercially available silica column method and from a 3mm DBS punch using both a commercially available and in-house boil prep. A Sanger sequencing method was developed at CDC to include the IDUA 5'-regulatory regions, promotor region and 14 exons with flanking intronic regions and tested on MPS I positive samples from Coriell. The amplification primers were designed to genomic sequences with limited known variability and were tested for cross hybridization within the genome using BLAST. The 15 PCR amplifications were optimized using the same master mix and cycling conditions and the PCR primers contained M13 sequences to streamline the cycle sequencing amplification.
Results/Conclusion: Sanger sequencing of IDUA performed reliably using column purified DNA from whole blood as well as DNA extracted from the DBS. The commercially available and in house boil preps performed similarly well indicating both extraction methods are suitable for extracting DNA from DBS for use with second tier Sanger sequencing for MPS I screening. This sequencing method will also be used by CDC to characterize samples collected in support of the creation of an MPS I variant repository for future quality assurance.

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P-032

How Low Can You Go? Determining the Lymphocytes Transduction Success Isolated from Low Volumes of Blood

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CDC's Newborn Screening Quality Assurance Program provides quality assurance materials to newborn screening laboratories worldwide to ensure the quality and accuracy of newborn screening results. The

adoption of second-tier molecular testing by newborn screening laboratories is becoming increasingly common in order to decrease the false positive rate inherent in some primary biochemical assays or to provide just in time supplemental information for time critical conditions. As a result, there is a more urgent need for quality assurance materials in the dried blood spot (DBS) that are suitable for molecular testing. The first molecular repository created by CDC contained blood samples donated from patients or family members containing cystic fibrosis causing variants. Initially this repository contained DBS made from blood, however as the need increased both in domestic and global programs, it became clear a more sustainable source of material was required. Thus, the repository was expanded to include cryopreserved white blood cells which could then be immortalized for their use in the creation of quality assurance materials. Typically white blood cells are isolated from 8-10mL of blood collected in a CPT tube yielding around 2 to 3 cryovials of 4-5e6 cells each. One vial is then infected with Epstein Barr Virus and Phytohemagluttin-M to create immortalized cell lines. Public health laboratories are now exploring the addition of a second tier multi-gene panel to detect the disease causing variants of Severe Combined Immunodeficiency (SCID), which poses a new challenge for creating quality assurance materials. The blood collection from SCID patients must happen prior to bone marrow transplant requiring blood collection from babies less than 3 months of age. The maximum volume of blood able to be collected from a baby less than 3 months is 3mLs and often only 1-2mLs. To determine the feasibility of successful transduction from low volumes, CDC tested this process using blood volumes as low as 0.5 mLs and up to 2 mLs from 3 cord blood donors (live cell totals between 7e5 to 9.5e6; average 3.75e6 cells). White blood cells were isolated and mixed with EBV and PHA-M and observed under the microscope after 7, 14, 16, 19, and 22 days for signs of transduction (clumps). Media color was also observed as a measure of cell growth. After 1 week, all cell lines contained clumps of growing cells as well as a media color change with the exception of one 0.5 mL cord blood sample. This trend continued through day 14, and the total live cell count for all samples including the slow growing 0.5ml sample varied from 9.24e5 to 1.23e7 (average 7.74e6 cells). While there are differences in growth rates, it appears that all samples have been successfully transduced regardless of starting volumes between 0.5mLs and 2mLs, suggesting that the expected volumes from babies with SCID will be sufficient for creating a SCID variant repository at CDC.

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P-033

A Performance Evaluation of the iPLEX Pro CFTR Assay using Dried Blood Spots from the CDC Cystic Fibrosis Dried Blood Spot Repository

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For newborn screening of cystic fibrosis (CF) there are many different assays for second-tier molecular testing of infants with elevated immunoreactive trypsinogen. These assays are used to identify the presence or absence of one or more pathogenic variants in the CFTR gene. The American College of Medical Genetics and Genomics recommends genotyping of at least 23 variants known to cause CF. However, this panel alone is often insufficient for population based screening; particularly among diverse populations. As a result, many state newborn screening labs have considered implementing or have already adopted commercial methods that offer a more expansive genotyping panel. The majority of U.S. labs performing second-tier CF screening are currently using one of the FDA-approved Luminex

xTAG Cystic Fibrosis kits (39 or 60 variant panel). A comparable option, currently being used by the Illinois Department of Public Health for CF newborn screening is the iPLEX Pro CFTR Panel, which uses MassARRAY technology to interrogate 72 CFTR variants. The vendor has characterized the assay using highly purified, Coriell DNA from CF affected individuals with known CFTR variants and synthetic controls. However, they have not yet validated the assay for use with DNA extracted from dried blood spots (DBS). To test its utility as an application for newborn screening of CF, we evaluated the assay's reliability with DNA extracted from CF repository DBS using commercially available and home-brew boil prep reagents. The results from the DBS DNA were compared to those using a highly purified, whole blood DNA extraction from the same CF repository sample. Preliminary data suggest that the iPLEX assay performs well using commercially available DBS extraction reagents. In addition, the initial set of CF samples tested gave variant calls that are concordant with sequence confirmed genotypes. Further testing of the assay will be performed to evaluate the accuracy and reproducibility of the iPLEX assay using CF repository DBS to investigate 50 of the 72 CFTR variants on the iPLEX CFTR panel. We will also determine the lower limit of DNA required to obtain sufficient results and compare results using the original and newer MassArray systems.

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P-034

Separation of Isobaric Analytes Utilizing Microfluidic Chip-based Capillary Electrophoresis in Newborn Screening Assays

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Introduction: First-tier newborn screening of amino acids and acylcarnitines are typically performed using flow injection analysis (FIA) coupled to triple quadrupole mass spectrometers (MS) in order to achieve the desired throughput of < 2 min per specimen. The absence of chromatographic separation before mass spectrometry analysis leads to isobaric (<0.1 Da difference in molecular mass, different molecular formula) interferences such as malanoylcarnitine (C3DC) and 4-hydroxybutylcarnitine (C4OH), as well as isomer (same molecular mass and formula, different chemical structure) leucine (LEU), isoleucine (ILE), and alloisoleucine (ALE). Capillary zone electrophoresis (CE) separates compounds based on their hydrodynamic radius and charge, where separation may be modified by altering pH, background electrolyte viscosity, and electrical field strength. Therefore, separation of the aforementioned isobars/isomers should be possible by CE.
Methods: The Centers for Disease Control and Prevention's (CDC) Newborn Screening Quality Assurance Program (NSQAP) quality control dried blood spot cards were analyzed in this study. Samples were extracted following the non-derivatized protocol as previously described [1], and were resuspended in 50:50 acetonitrile:water with 1% formic acid and 100mM ammonium formate. The background electrolyte used in this study was 50:50 acetonitrile:water with 1% formic acid. Separation was performed using a 908 Devices ZipChip, a chip-based CE nanospray platform, utilizing a 5nL injection and the peptide analysis kit viscosity and field strength settings. Analysis was conducted on the 908 Devices ZipChip interface coupled to Q-Exactive Plus MS operating at 17,000 mass resolution.
Results: CE-MS separated numerous analytes, including isobars/isomers. More specifically, we report peak separation of C3DC, C4OH, LEU, ILE, and ALE in < 2 min analysis time. Co-eluted analytes that were not isomers/isobars could be distinguished thanks to the specificity of high resolution MS. In order to accommodate the very narrow eluting CE peaks (i.e., 3 sec), analytes were grouped into large acquisition

selected ion monitoring windows. Using this method we were able to achieve similar results to FIA methods on triple quadrupole platforms.
Conclusions: CE-MS is a promising technology for newborn screening, which could be utilized to combine first and second tier screening assays, decrease ion-suppression inherent to FIA-MS, increase the number of analytes analyzed in a single run, while maintaining a throughput of < 2 min acquisition time. In addition, CE coupled to high resolution MS also appears to be a potential application for combining targeted newborn screening with metabolomics-based profiling.

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P-035

Quality Control Data Used to Normalize Proficiency Testing Results for TSH, T4, 17OHP, and TGal

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Introduction: Three times per year, the CDC's Newborn Screening Quality Assurance Program (NSQAP) distributes a five-specimen dried blood spot (DBS) proficiency testing (PT) panel for thyroid-stimulating hormone (TSH), thyroxine (T4), 17-α-hydroxy progesterone (17OHP), and total galactose (TGal). Participants report a quantitative result, clinical assessment, and cutoff for each specimen. Quality control (QC) DBS for these analytes are sent twice per year. Participants report duplicate results for each analyte from 5 independent runs. We used reported QC values for the second half of 2017 to construct an individual normalization curve for each participant. The curves were used to calculate normalized values for positive PT specimens included in the PT panel using data from quarter 4 of 2017.
Methods: Each laboratory's results for QC materials were plotted against our internally generated data for the same materials and analytes. We then used the slope and intercept to normalize the PT value reported by that laboratory. All data were log-transformed before regression. We excluded laboratories that did not use the same analytic method to obtain both sets of data as well as any laboratory whose normalized PT value fell outside of 4 mean absolute deviations. Several laboratories reported values of zero for the lowest T4 QC pool, preventing log transformation of their data and these results were excluded.
Results: For each analyte, we calculated relative standard deviation (RSD) for both pre- and post-normalized data for all methods combined and for individual method means (compared to the overall mean). Methods with fewer than 3 users were included in the "other" method category. For TSH, overall (N = 172) RSD decreased from 16% to 11% and, across method categories (N = 6), RSD went from 6% to 5% of the overall mean. For T4, the overall (N = 38) RSD went from 24% to 21% and the method-specific (N = 4) RSD dropped from 22% to 10%. The RSD for 17OHP (N = 135) fell from 21% to 14% and, between methods (N = 7), from 20% to 11%. For TGal, the overall (N = 79) RSD dropped from 27% to 16% and, between method categories (N = 10), from 24% to 10%.
Discussion: Given the variety of analytic methods in use, equivalency of measurement between newborn screening laboratories is difficult to attain. Using data collected from participants, NSQAP is in the unique position to assist participants with normalizing their results to other newborn screening laboratories.

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P-036

A Universal Second-tier Screening Assay for the Detection of Maple Syrup Urine Disease, Methylmalonic Acidemia, Propionic Acidemia, Homocystinuria, and Guanidinomethyltransferase Deficiency Via Derivatized LC-MS/MS

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Introduction: Routine screening of amino acids and acylcarnitines (AAAC) via flow injection analysis tandem mass spectrometry (FIA-MS/MS) is commonly used for the detection of inborn errors of metabolism. The Recommended Uniform Screening Panel (RUSP) lists 42 metabolic disorders that are detectable by primary FIA-MS/MS screening. Second-tier liquid chromatography tandem mass spectrometry (LC-MS/MS) can be used to confirm the results of the primary screen, increasing specificity, or to differentiate between disorders that have the same primary marker such as propionic acidemia and methylmalonic acidemia. Because of the diversity of analytes used in second-tier screening, a different LC-MS/MS assay typically must be used for each disorder, increasing the cost and complexity of state labs performing their own second-tier screening in house. Multiplexing these discrete second-tier assays makes in-house second-tier screening more feasible on the State lab level, lowering turn-around times in screening and improving patient outcomes. The described derivatized assay simultaneously screens for five disorders that require second-tier testing.
Methods: Stable isotope labeled standards were obtained from Cambridge Isotopes Laboratories, Sigma Aldrich, and CDN isotopes. Dried bloodspot specimens from the CDC Newborn Screening Quality Assurance Program were used to evaluate the assay. A Waters BEH C18 column was used on an Agilent 1290 LC system connected to a Sciex API-4500 MS/MS.
Results: 11 analytes were quantified in unenriched, low, medium, and high calibrator pools using the new combined second-tier assay. The recoveries, linearity, and variance were similar between the combined assay and three standalone second-tier assays
Conclusion: With a 2 hour sample prep and 6.5 minute run time this assay performs similarly to standalone assays for the detection of Maple Syrup Urine Disease (MSUD), Methylmalonic Acidemia (MMA), Propionic Acidemia (PROP), Homocystinuria (HCY), and Guanidinomethyltransferase (GAMT) deficiency.

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P-037

Effects of Accelerated Drying on Dried Blood Spot Quality Assurance Materials

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Background: The Newborn Screening Quality Assurance Program (NSQAP) manufactures and distributes dried blood spot (DBS) quality assurance materials to nearly 700 domestic and international laboratories four times per year. Our laboratory consistently investigates opportunities for quality improvement to

the DBS production process. Loss of biotinidase activity in quality assurance materials has been a challenge in the past. In this study a dehydrator is used to examine the effect of accelerated drying on biotinidase activity and various other newborn screening analytes during production of quality assurance DBS materials as a possible method of analyte stabilization and improved recovery.
Methods: Blood units from three different donors were individually adjusted to 50% hematocrit and dispensed in 75uL aliquots on Whatman grade 903 filter paper. After drying at room temperature for five minutes, half of the cards were placed in a dehydrator and allowed to dry for 30 minutes while the rest of the cards dried at room temperature. After 30 minutes, cards were removed from the dehydrator and continued to dry at room temperature. Three hours later, all cards were packed with desiccant and humidity indicator cards and stored at -20°C. DBS from each of the three donors, dried under both conditions, were evaluated for various newborn screening analytes.
Results: DBS that underwent accelerated drying yielded 10% higher amino acid and acylcartnitine recovery on average, with a range of 2- 20%, compared to blood spots dried at room temperature only. DBS evaluated for galactose-1-phosphate uridyltransferase (GALT) activity yielded nearly 10% lower activity than specimens dried without acceleration. Those tested for glucose-6-phosphate dehydrogenase (G6PD) activity showed a 20% increase after accelerated drying and DBSs evaluated for biotinidase activity assayed nearly 40% higher than typically dried spots.
Conclusions: Analyte degradation or loss of enzymatic activity during the drying process is an ongoing concern when preparing quality assurance materials. Accelerated drying may offer an effective way to mitigate these problems.

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P-038

Production of Quality Control Material for Adenosine Deaminase Severe Combined Immunodeficiency Newborn Screening using Mirror-image Nucleosides

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Introduction: Adenosine deaminase severe combined immunodeficiency (ADA-SCID) is an autosomal recessive disorder in which the build-up of adenosine and deoxyadenosine prevents the maturation of T- and B-cells. ADA-SCID accounts for 15% to 20% of all SCID cases, with an incidence of 1: 375,000 to 660,000 live births. Some infants with the disorder are not detected in the pre-infection period and the diagnosis of ADA-SCID occurs after an infection has escalated. Damage caused by severe infections may be irreversible or fatal, therefore, early detection of the disease through newborn screening is beneficial to patients and their families. Preparation of dried blood spot (DBS) quality control material was challenging because of ADA activity in the human blood matrix. The addition of an ADA inhibitor, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), has been previously reported as a means to prepare QC DBS. However, this adds additional expense and complexity to the preparation of quality control material.
Methods: DBS were prepared by enriching liquid blood with 0, 1, 5, and 10 µM L-adenosine and L-deoxyadenosine. Samples were analyzed using FIA-ESI-MS/MS with a triple quadrupole mass spectrometer (1st tier) and HPLC-MS/MS with triple quadrupole mass spectrometer in positive ion mode (2nd tier).

Results: The QC material enriched with L- enantiomers showed recovery > 90% for L-dAdo and >80% for L-Ado, a significant increase when compared to the DBS enriched with the D-nucleosides treated with EHNA. Analysis with the second tier HPLC-MS/MS gave quantitative results similar to the FIA method with baseline resolution of all analytes.
Conclusions: We have shown that the use of L-nucleosides (L-Ade and L-dAde) is a simple and effective method for QC DBS enrichment. Future pilot studies will determine the performance of this QC material in different laboratories performing ADA-SCID NBS using MS/MS.

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P-039

Improvement of LC-MS/MS Quantification Accuracy When using Separate Internal Standards for C20-26 Lysophosphatidylcholines

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Introduction: Newborn screening for X-linked adrenoleukodystrophy (X-ALD) currently relies on quantitation of very long-chain lysophosphatidylcholines (C26:0- and C24:0-LPCs), which are elevated outside normal limits in X-ALD. Some laboratories also measure C20:0- and C22:0-LPC, which are not elevated in X-ALD, and calculate molar ratios. It was observed when analyzing dried blood spots (DBS) enriched with equimolar quantities of these 4 LPCs using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) that using a single internal standard (D4-C26:0-LPC) gave accurate quantitation of C26:0-LPC but not the other 3 chain lengths. Depending upon ionization mode and chromatographic conditions the inaccuracy of C20:0-, C22:0-, and C24:0-LPC quantitation showed both high and low biases. However, using a mixture of 4 internal standards (all D4-labeled) to extract the DBS gave quantitative results closer to target enrichments for the 3 shorter chain lengths of LPCs.
Methods: Human blood (A+) adjusted to 50% hematocrit was enriched with 0, 1, and 5 micromolar C20:0-, C22:0-, C24:0-, and C26:0-LPCs using methanol stock solutions. The blood was stirred and spotted onto Whatman 903 newborn screening filter paper cards (100 microliter spots). After drying overnight the cards were stored at -20 C with desiccant. Sample preparation of a 1/8" (3.1 mm) punch used either 0.4 micromolar D4-C26:0-LPC in methanol, or a mixture of all four D4-labeled standards (each 0.4 micromolar) in methanol. A negative ion mode isocratic HPLC-MS/MS analysis was performed on 20 different days.
Results: Using only the D4-C26:0-LPC internal standard, the C26:0-LPC apparent recovery was satisfactory in the 1 and 5 micromolar enriched quality control DBS (1.15 and 5.36 micromolar, respectively) but the C20:0- and C22:0-LPC apparent recoveries were not satisfactory (approximately 40% and 50% of enrichment, respectively). The C24:0-LPC apparent recovery was ~90% using the D4-C26:0-LPC internal standard. In contrast, when using all 4 internal standards the C20:0-, C22:0-, and C24:0-LPC apparent recoveries improved to approximately 100%, 96%, and 103%, respectively. We are currently investigating whether the use of separate internal LPCs standards vs. only using D4-C26:0-LPC would increase the quantification accuracy for C20, C22, C24 and C26 LPCs under FIA-MS/MS conditions.
Conclusion: Multiple internal standards are required to accurately quantitate lysophosphatidylcholines in dried blood spots using high-performance liquid chromatography-tandem mass spectrometry.

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P-040

Development of Quality Control Materials for Cerebrotendinous Xanthomatosis Newborn Screening

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Introduction: Cerebrotendinous Xanthomatosis (CTX) is a progressive metabolic leukodystrophy. It is an inborn error of bile acid synthesis with autosomal recessive inheritance. CTX was nominated in 2018 for inclusion on the Recommended Uniform Screening Panel and may be part of future newborn screening analyses of dried blood spots. Quality control materials for this disorder should include enriched biomarkers indicating CTX, such as tetrol glucuronide (5β-cholestane-3α, 7α, 12α, 25-tetrol-3-O-β-D-glucuronide). A previous publication by Vaz et al. shows that a molar ratio between tetrol glucuronide and tauro-chenodeoxycholic acid (which is low in CTX) is the most appropriate ratio/marker to screen for CTX in newborn dried blood spots.
Methods: Human blood (A+) adjusted to 50% hematocrit was enriched with tetrol glucuronide using methanol / water stock solutions. The blood was stirred and spotted onto Whatman 903 newborn screening filter paper cards (100 microliter spots). After drying overnight the cards were stored at -20 C with desiccant. Sample preparation of a 1/8" (3.1 mm) punch used stable isotope-labeled tetrol glucuronide in methanol. A novel gradient HPLC-MS/MS analysis (Phenomenex Luna C8 column, water / methanol / butanol solvents) was developed on a Sciex 4500 triple quadrupole instrument (with ion mode polarity switching) in order to characterize the materials. Chromatography (< 5 min) provided the advantages of resolving isobaric bile acids and associating a retention time with each analyte of interest.
Results: In this study, we discuss early results on the development of dried blood spot based quality assurance materials for CTX, including preliminary assessment of the materials using LC-MS/MS, preliminary (4 months) stability studies as well as future plans for the creation of proficiency testing materials.

Reference:

1. Vaz, F. M., A. H. Bootsma, W. Kulik, A. Verrips, R. A. Wevers, P. C. Schielen, A. E. DeBarber, and H. H. Huidekoper. 2017. A newborn screening method for cerebrotendinous xanthomatosis using bile alcohol glucuronides and metabolite ratios. J Lipid Res 58: 1002-1007.

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P-041

Stability of Markers in Dried Blood Spots at Variable Temperature and Humidity Parameters for Recommended Newborn Screening Diseases in the United States

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Background: The United States' Recommended Uniform Screening Panel (RUSP) consists of 35 core and 26 secondary targeted diseases detectable through newborn screening. The RUSP serves as a helpful guide in designing and implementing statewide newborn screening programs. Under variable temperature and humidity conditions, we performed single, normalized stability studies for markers of

RUSP conditions. Understanding the environmental impact on marker stability and the controls needed for optimal marker recovery is critical in sustaining effective operations of newborn screening testing and associated external quality assurance (QA) programs using dried blood spot (DBS) specimens.
Methods: We individually measured the effect of heat and humidity changes in the assayed levels of 47 markers in DBS specimens. We stored paired sets of DBSs at -20 °C, 4°C, ambient temperature and 37°C for predetermined time intervals with and without desiccant for 30 days, 6 months, and 1 year. The recovered levels of analytes were determined by testing each set in a single analytic run.
Results: The recovery of markers for hormone, amino acid, organic acid and acylcarnitine diseases remained stable throughout the 30-day, 6-month, and 1-year time intervals at -20 °C and 4°C at low humidity. We observed degradation in these markers when DBS were stored at higher temperatures and high humidity. The sharpest declines in recovery at all temperature and humidity conditions were observed in the enzymes galactose-1-phosphate uridyltransferase (67%), biotinidase (50%), and glucose-6 phosphate dehydrogenase (46%), when stored at 37°C and high humidity over a 30-day period. Eight markers lost 70% - 90% of initial levels within one year when stored at 37 °C and high humidity.
Conclusions: Understanding susceptibilities of DBS markers to heat- and moisture-related degradation is important for achieving high quality measurements. The Newborn Screening Quality Assurance Program uses a courier service to ship DBS worldwide to minimize transit times. Minimizing humidity and transit time during shipment ensures specimen integrity upon receipt. Prior to shipment, QA materials are stored at -20 °C with desiccant to protect against degradation during long-term storage. These precautions enable the storage of large inventories of DBS specimens that are fit for both proficiency testing (short term) and quality control (long term) activities.

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P-042

Implementation of an ISO/IEC 17043 Compliant Quality Management System for the Newborn Screening Molecular and Biochemical Branch (NSMBB)

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Background: Quality assurance (QA) is a critically important laboratory system and proficiency testing (PT) is a valuable measure to assess and verify the accuracy and reliability of measurements within testing and calibration laboratories. Satisfactory laboratory PT performance is required in order to maintain regulated, accredited and/or certified status. Some regulatory bodies require laboratories to use the services of accredited or certified PT providers. The ISO/IEC 17043 standard (Conformity assessment—general requirements for proficiency testing) can be used to assess and accredit the competency of PT providers. The Newborn Screening and Molecular Biology Branch (NSMBB) at the CDC is a PT provider for domestic and international newborn screening labs covering 33 of 35 primary disorders and 24 of 26 secondary disorders in the US Department of Health and Human Services Recommended Uniform Screening Panel (RUSP). NSMBB achieved accreditation to the administrative and technical requirements of the ISO/IEC 17043 standard in 2017, following three years of implementation efforts. The initial scope of accreditation was limited to the qualitative PT schemes that included 24 of the primary disorders on the RUSP. Here we address the challenges and benefits of implementing the ISO/IEC 17043 standard. We also discuss the cost of quality, the improvements made to the technical requirements for homogeneity and stability and the adoption of quality management system (QMS) tools.

Methods and Results: QMS tools were developed and used to compare pre- and post-implementation phases and monitor ongoing activities, including a cost (dollar and/or time) analysis, where appropriate. Internal audits were conducted to assess effective implementation and track progress. An external audit was conducted by A2LA in May, 2017. Prior to implementation, NSMBB PT documentation was not fully compliant with ISO 17043 standard requirements and provided several opportunities for quality improvement. Significant successes included the development of specialized tools for quality management, enhancements of the technical requirements for homogeneity and stability of quality materials and the implementation of an automated temperature and humidity monitoring system to ensure consistent oversight of environmental conditions of key processes.
Conclusion: A well-functioning QMS requires appropriate assessment tools and is critical for obtaining and maintaining program accreditation. There were significant benefits with prioritizing costs of quality that are associated with preventing, detecting, and remediating potential issues that could impact quality. The ISO 17043 standard allows for flexibility in structuring a QMS to ensure a proper fit for an organization's current and future needs.

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P-043*

Comparison of Electrospray and Impactor (Unispray) Ionization Tandem Mass Spectrometry for the Analysis of Newborn Screening Biomarkers

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Background: The introduction of mass spectrometry as a screening method has significantly increased the number of disorders for which newborns are screened. Mass spectrometry advances such as ionization modes, sensitivity and acquisition speed have further increased the throughput, the number of disorders that can be simultaneously screened as well as simplified sample preparation through the elimination of previously used derivitization steps. While electrospray tandem mass spectrometry allows polar/ionic compounds to be detected with high sensitivity, there are classes of compounds such as steroids, lipids, and other apolar chemical species such as cortisol or androstenedione here, for which screening is important. However sensitivity has been an issue because these compounds do not ionize well under electrospray ionization. This study compares electrospray to a newly introduced ionization technique known as impactor ionization, and commercialized under the name of Unispray for the analysis of newborn screening biomarkers. Impactor ionization is the formation of ions by directing a heated nebulized spray of liquid onto a surface with an applied voltage.
Methods: Standard solutions containing amino acids, acylcarnitines, steroids, lipids and potentially other classes of compounds were dissolved in 50:50 organic:water containing 0.1% formic acid and analyzed using Flow injection analysis or liquid chromatography tadem mass spectrometry. Both electrospray and impactor ionization analysis were conducted using an Acquity UPLC coupled to a Xevo TQ-S micro Triple Quadrupole Mass Spectrometer with Acquity UPLC.
Results and Discussion: Each analyte of interest was optimized to maximize sensitivity under different source conditions and ionization modes. The majority of the analytes investigated had a comparable or greater response using Unispray compared to Electrospray. More hydrophobic/apolar analytes such as steroids and longer chain acylcarnitines had 3-10 times greater areas under the curve using Unispray ionization while polar/ionic compounds had up to 6 times higher response under Electrospray conditions. Other considerations such as ion-suppresion effects are currently under investigation.

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P-044

Withdrawn

P-045*

Does Increasing Birth Weight Interfere with Acylcarnitine and Amino Acid Results on the Newborn Screen?

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It has been well-established that infant treatments, infant conditions, and maternal conditions can adversely affect the validity of newborn screening results resulting in both false positive and false negative results. In fact, APHL has compiled a list of interfering factors along with a nomination process to submit to the Newborn Screening QA/QC subcommittee for review. Maternal obesity, found in nearly 2/3 of all pregnancies, predisposes infants to both low and high birth weights. Low birth weight has been reported as a significant reason for false positive screens. However, there is less known regarding large for gestational age (LGA) infants. Acylcarnitines are longstanding biomarkers of mitochondrial dysfunction. They are elevated in fatty acid oxidation disorders and organic acidemias tested for on the newborn screen. Elevated long chain, medium chain, and dicarboxylic acylcarnitines have been associated with incomplete beta-oxidation, a phenomenon seen in both established obesity as well as in cord blood, plasma, and mesenchymal stem cells from infants of obese mothers. Similarly, elevations of branched chain amino acids (BCAA) and related short chain acylcarnitines (C3 and C5) are found with anaplerotic catabolism of BCAA, and are similarly elevated in obesity and in the plasma of offspring of obese mothers. This abstract will examine both acylcarnitine and amino acid profiles from LGA versus AGA infants over a 3 year period in Colorado. Pearson regression and/or Student t-test analysis will be used to compare tandem mass spectrometry analyte concentrations (uM) versus weight (kg) to determine whether or not birth weight should be considered and nominated to the Analyte Interference List.

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P-046*

A Missed Case of Classic Galactosemia: A Consequence of Too Many False Positives?

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Background: The newborn screening program seeks to identify newborns with classic galactosemia as soon as possible to prevent fatal symptoms. Children with variant forms and carriers are not at risk and

therefore not a primary target, however, they are often incidentally identified and referred for follow-up in our region along with false positives due to heat exposure of the blood spot card. Children's National Genetics serves as a newborn screen referral center for the District of Columbia and parts of Virginia and Maryland. We encountered a true case of classic galactosemia missed by a local Emergency Department (ED) after evaluation for parental concerns of lethargy. An abnormal galactosemia screen was presented including second-tier identification of two copies of the p.K285N variant, diagnostic of classic galactosemia. ED providers described to parents their experience with frequent false positive galactosemia screens each summer. On that basis, they instructed family to continue breast milk feeding and follow-up with their pediatrician. Upon incidental receipt of the result by our Division, the family was urgently contacted to reinitiate galactose-restriction and follow-up.
Methods: In the context of this missed diagnosis and to better capture galactosemia screen burden, a retrospective review of referral and outcome rates from August 2017 - August 2018 was undertaken. We assessed the number of true positives, carriers, variant diagnoses and false positives identified. We also reviewed all initiated referrals from state programs and community providers that were determined to be low risk infants whose follow-up testing was coordinated through a pediatrician without formal Genetics visit. From August 1, 2017 through August 31, 2018 we evaluated 129 newborns in Genetics following routine referral for abnormal newborn screen, of those referrals 19 (15%) were secondary to galactosemia screening. This did not include non-traditional identification of newborns as described above. Of that cohort we diagnosed 0 infants with classic galactosemia, 1 with Galactoepimerase (GALE) deficiency and 5 with Duarte variant galactosemia. The 13 remaining were heterozygous galactosemia or Duarte variant carriers or false positives. Over that period, 90 galactosemia screen referrals were initiated by follow-up programs or pediatricians that did not warrant urgent Genetics evaluation. Of these referrals, 0 children have been diagnosed with classic galactosemia and average quantitative GALT enzyme reported back is 13.8 umol, consistent with carrier status.
Conclusions: In our region, providers report frustration with false positive galactosemia screens which are responsible for the greatest volume of referrals in metabolic follow-up and may result in putting newborns with true disease at risk for inappropriate urgency in identification.

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P-047

Newborn Screen for MPS I (Hurler Syndrome) – The Washington, DC Experience
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Newborn screening for MPS1 (Hurler Syndrome) was recommended by the RUSP in 2016. HSCT has been shown to stabilize neurocognitive function in these patients which makes early identification and treatment essential. Screening was started in the District of Columbia (DC) in January, 2018; whereby patients identified by NBS in DC are referred to a single center for follow-up. Fifteen patients have been identified by NBS through December 2018. All patients were evaluated by laboratory investigations including repeat dried blood spot for enzyme activity (fluorimetric), measurement of glycosaminoglycans (GAGs) by dried blood spot and urine (LC-MS/MS), and in most cases molecular analysis were performed. Five patients had normal IUDA activity on repeat enzyme analysis, 3 were found to be carriers of single non-benign variants in IDUA, and 4 were found to have variants previously reported to be pseudodeficiency alleles. All patients had either normal urine or blood GAGs. However, 4

of the patients had QNS urine samples. In our cohort, only 3/15 patients had mildly abnormal total blood GAGs with normal Dermatan, one with elevated Heparin Sulfate levels and two elevations in Keratin Sulfate. 2 of these patients were confirmed to be IDUA carriers on molecular analysis, the third child has pending molecular testing. Pseudodeficiency alleles have been identified, and there is a known allele which is common in individuals of African American descent. In our sample, 13/15 patients were of African or African-American descent whom either had normal repeat DBA IDUA activity, blood GAGs or were found to carry pseudodeficiency alleles. 49% of the population in Washington, DC is of African American or of African descent, making these results highly likely in our catchment population. Based on our patient cohort as well as our experience, we feel performing DBS GAG measurement would likely reduce time and expense to resolving diagnosis with a positive newborn screen for MPSI.

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P-048

Clinical Utility of Confirmatory Genetic Testing to Differentiate Sickle Cell Trait from Sickle-β+-Thalassemia by Newborn Screening

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Background: Hemoglobinopathies, a complex set of blood disorders, are the most common disorders detected by newborn screening (NBS). Hemoglobinopathies include sickle cell disease, thalassemias, and hemoglobin (Hb) variants. Protein-based testing (e.g., Hb electrophoresis and HPLC) is used most commonly for NBS and confirmatory testing. Protein-based testing cannot accurately detect several hemoglobinopathies in newborns, especially with β+-thalassemia mutations.. A common NBS diagnostic scenario is differentiation of sickle cell trait (HbAS) from sickle-β+-thalassemia (HbSβ+). Newborns with HbAS have a "FAS" pattern, while those with HbSβ+ have an "FSA" pattern; however, some newborns with a "FSA" pattern actually have HbAS(trait)..Objective: To describe a consecutive cohort of newborns identified with HbSβ+ by NBS who actually had HbAS and illustrate clinical utility of genetic testing for correct and timely diagnosis.
Methodology: Newborns with a "FSA" pattern (suspected HbSβ+) on NBS (Ohio Department of Health) who had confirmatory testing performed at Cincinnati Children's Hospital Medical Center from July 2015 to August 2018 were identified. Genetic testing (HBB sequence analysis and copy number variation analysis of the β-globin gene cluster) was performed at the same time as standard, protein-based confirmatory testing. We compared suspected NBS diagnosis of HbSβ+ to diagnosis determined by (1) standard, protein-based confirmatory testing and (2) genetic testing.
Results: 31 newborns identified with suspected diagnosis of HbSβ+ based on NBS. Of these, 30 had protein-based confirmatory testing; 17 had genetic testing. On protein-based confirmatory testing at 2-4 weeks of age, 23/30 had an FAS pattern indicating correct diagnosis of HbAS, and 8/31 still had an FSA pattern suggesting HbSβ+. Of these 8 who still had an FSA pattern, 7 had simultaneous genetic testing that established the correct diagnosis of HbAS; 1 did not have genetic testing but had repeat protein-based testing at 3 months that established the diagnosis of HbAS. All newborns who had HbAS with confirmatory protein-based testing also had genetic testing and the final diagnosis of HbAS confirmed by genetic testing.
Conclusion: In this sample of newborns with a suspected diagnosis of HbSβ+ based on NBS, none actually had HbSβ+. About two-thirds had a correct diagnosis of HbAS established by 2-4 weeks of age by protein-based confirmatory testing; however, the remaining one-third still had an incorrect

diagnosis.. Genetic testing can definitively distinguish between HbAS and HbSβ+ at any age. We perform genetic testing at the time of confirmatory protein-based testing for newborns with FSA. Turn-around time is 4 weeks, so timely diagnosis can be made before prescribing prophylactic antibiotics or seen by a pediatric hematologist for a presumed, yet incorrect, diagnosis of sickle cell disease.

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P-049

Double the Trouble or Twice the Fun? Advantages and Disadvantages of Moving Servers While Upgrading a Laboratory Information Management System

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In September 2016, the Colorado Newborn Screening Program (CONBSP) initiated a project to move the servers for its laboratory information management system from a site in the Laboratory Services Division's (LSD) building to an off-site data warehouse owned and operated by Colorado's Office of Information Technology (OIT). For a variety of reasons, the project would not be completed until June 2018. The data warehouse, called eFORT, offers many advantages including nearly unlimited redundancy of servers due to virtualization of systems, plus 24-7-365 on-site support as the state's official data warehouse. However, CONBSP was concerned that network speed between the LSD building and eFORT might not be sufficient to maintain rapid database connectivity, perhaps leading to delays in processing steps such as punching of specimens in the production environment. To relieve this risk, the LSD elected to install a new dedicated 10Gb fiber optic connection between LSD and eFORT. To strengthen redundancies, the original 100Mb connection to an independent service provider was left in place for the LSD building. In addition to moving the program's servers, the program decided to upgrade a key module of its LIMS simultaneously. The advantages and disadvantages of this decision were discussed internally in advance, and in the end, the decision was to perform both tasks simultaneously. This presentation will review the decision-making process before the event, as well as evaluate the outcomes of the event. Importantly, unlike previous upgrades to the LIMS, the CONBSP took advantage of redundant equipment to build a mini-lab inside its production laboratory the week of the move. By testing functionality in the mini-lab before the transition, the program could work with the LIMS vendor and OIT staff to troubleshoot issues before going live after a weekend switchover. Coordination for the project was handled by a wide range of individuals including the CONBSP's Program Manager and Data Analyst, the head of IT at the Colorado Department of Public Health and Environment, and software engineers from the LIMS vendor. Ultimately, most major issues were discovered within the mini-lab environment, and only a few minor problems occurred after the switchover. In addition to discussing this project, the presentation will also cover the current distribution of IT duties within the CONBSP, as it is important for NBS programs to ensure IT functions are carefully defined and appropriately staffed.

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P-050

Creating a Peer-to-peer Network for the Colorado Newborn Screening System

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Colorado is a two-screen state for newborn screening (NBS) with approximately 800 unique submitters of specimens per year servicing more than 4,000 different physicians. While pediatricians and family physicians play a critical role in a two-screen system, a survey of more than 100 stakeholders revealed low level of awareness about newborn screening rules promulgated by the Colorado Board of Health (BoH). In the case of survey respondents self-identifying as pediatricians, less than 50% were aware of the BoH rules. The current BoH rules contain key details about the ideal time for collection and submission of newborn screening specimens. To address this low level of awareness, the CONBSP held four peer-to-peer networking events across the state of Colorado. Events were held in the cities of Loveland, Denver, Colorado Springs, and Grand Junction, representing northern, central, southern and western regions of Colorado, respectively. Events were advertised using key physician and laboratory contacts, as well as by engaging with local public health agencies and professional organizations such as the Colorado Nurses Association, American Academy of Pediatrics, and American College of Family Physicians. Two events were two-hour evening events that included dinner, while two were all-day events including breakfast and lunch. At evening events, the CONBSP provided a one-hour overview of the CONBS System with contracted follow-up experts attending the event to answer questions during and after the presentation. At all-day events, NBS program staff alternated with contracted follow-up specialists to provide short presentations about important aspects of the newborn screening system including short explanations of next steps in the case of a positive screening result. During and after the events, some attendees offered to act as community resources to share best practices with peers, such as best practices for collecting blood spots. These offers of regional help came in response to requests for help by CONBSP staff, who recognize the limitations of expert knowledge within the department. Post-event surveys demonstrated significant increases in overall understanding of the newborn screening system by attendees, as well as the relative performance of Colorado submitters on various NewSTEPs quality indicators compared to their peers in other states. Current NewSTEPs data demonstrate Colorado providers struggle with submitting complete information on demographic slips accompanying blood spot cards. Attendees were encouraged to sign up for the CONBSP's quarterly newsletter, as well as to receive facility report cards for their facilities. Attendees were also made aware that CONBSP staff are willing to engage in on-site 'educational visits' to promote best practices in newborn screening. Funding for this project was provided by a grant from NewSTEPs 360.

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P-051

Difficulties in Establishing a Comprehensive Sickle Cell Newborn Screening Program in a Developing Country – The Nigerian Experience

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Background: Sickle cell disease (SCD) affects millions of people worldwide and very predominant in sub-Saharan Africa. Nigeria has the highest incidence in the world of infants born annually with SCD. About 80% of infant mortality rate is related to complications of SCD and the World Health Organization has called on countries to address this important global health issue. Some countries have implemented newborn screening programs (NBS) to reduce SCD associated morbidity and mortality but others including Nigeria still lag behind leaving many children to die before five years of age.
Aim: The pilot NBS program for hemoglobinopathy implemented in Nigeria is to reduce the high morbidity and mortality related to SCD.
Methodology: Community based participatory research principles guided this Nigerian and international (public and private) partnership. By invitation, the team visited 10 states that expressed interest in NBS for SCD. Largely supported by the Association of Public Health Laboratories with limited funds from the Center for Disease Control in the USA, infrastructure and capacity assessments were conducted followed by series of stakeholder education sessions, hands on trainings and follow up consultations. Iso Electric Focusing (IEF) testing machines donated by Perkin Elmer of Finland were installed in three of the 5 states that expressed firm commitment to support the establishment of a pilot NBS for SCD. Pilot NBS began in Oyo State (12/2011) and Anambra State (9/2013) with signed memoranda of understanding (MOU).
Results: More than 200 stakeholders in Oyo, Osun, Kaduna and Anambra States have received education and training in SCD, NBS and hemoglobinopathy counseling. Oyo state engaged 4 birthing centers and as of 10/18/12 reported 456 registered samples, screened 356 newborn, 59 trait, 3 confirmed SCD, 5 insufficient samples, 21 samples to repeat and 102 samples yet to run. No follow up reported. Anambra state engaged 7 birthing centers and as of 7/31/17, registered 5530 samples, 4283 newborns screened, 3186 FA, 1078 FAS, and 16 FS. All families of newborns with FS had disease education, connected to the clinic but lost to follow up and no follow up for families with FAS/C/E.
Conclusions: Pilot NBS for SCD has been initiated in Nigeria and the limited data provide a glimpse to the veracity of this significant public health problem and opportunities for new partnerships for improved and sustainable NBS program. Multiple challenges that have stymied the progress include but not limited to disregard for comprehensive care and follow up, unstable state government, prohibitive cost of reagents, dependence on and expectation of international support for public health programs, SCD not a priority public health issue, lack of accountability and misuse of funds and disregard of agreed upon MOU. A new paradigm shift with private (not State government) and international partnership is currently being explored.

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P-052

Treatment Algorithm for Infants Diagnosed with Spinal Muscular Atrophy through Newborn Screening
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Background: Spinal muscular atrophy (SMA) was recently added to the Recommended Uniform Screening Panel in July 2018. As states begin to implement screening, follow up and appropriate care of identified patients is paramount for achievement of maximal therapeutic outcomes. SMA is an autosomal recessive disease characterized by the degeneration of alpha motor neurons in the spinal cord, leading to muscular atrophy. SMA is caused by deletions or mutations in the survival motor neuron 1 gene (SMN1). In humans, a nearly identical copy gene, SMN2, is present. Because SMN2 has been shown to decrease disease severity in a dose-dependent manner, SMN2 copy number is predictive of disease severity. Here we report the development of a treatment algorithm for SMA-positive infants identified through newborn screening based upon SMN2 copy number. To develop treatment guidelines, a working group comprised of 15 SMA experts participated in a modified Delphi process, moderated by a neutral third-party expert.
Results: The overarching recommendation is that all infants with two or three copies of SMN2 should receive immediate treatment (n=13). For those infants in which immediate treatment is not recommended, guidelines were developed that outline the timing and appropriate screens and tests to be used to determine the timing of treatment initiation.
Conclusions: The identification of SMA affected infants via newborn screening presents an unprecedented opportunity for achievement of maximal therapeutic benefit through the administration of treatment pre-symptomatically. The recommendations provided here are intended to help formulate treatment guidelines for infants who test positive during the newborn screening process.

Acknowledgements: SMA NBS Coalition members, Biogen, and AveXis, for funding this project.

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P-053*

From Idea to Impact: A Case Study on Making Change through Education

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Despite newborn screening being demonstrably successful in life-saving detection and intervention, persistent barriers to newborn screening knowledge and practice in the face of new technologies and policies underscore the need for novel, adaptable approaches to educating and engaging families. Even if educational programs and best practices exist to address these gaps, there is very limited tracking and sharing among newborn screening stakeholders to assess their impact on families' knowledge and awareness levels. Unfortunately, many families of infants are still unaware of the specific conditions for which babies receive screening in their state, the types of screening, who to contact for more information, and for those receiving an out-of-range result, why their child may need additional testing. Baby's First Test, the nation's resource center for newborn screening information, helps bridge this gap by bringing together the latest information and resources to help guide parents, families, loved ones, and their healthcare professionals throughout the newborn screening process. All of our information and resources are easily accessible on our website (www.BabysFirstTest.org). As with any program, especially programs with significant online presence and outreach, evaluation is key to understanding your program's value and investment. Despite metrics and tracking mechanisms for understanding our

program's reach, we had very little data on our actual impact. In order to better understand our impact, Baby's First Test partnered with RTI International to answer the following question: to what extent has Baby's First test contributed to increased knowledge, awareness and understanding of newborn screening among parents and healthcare professionals?

Through a rigorous evaluation, including an online, comparative evaluation with 770 parents and usability testing with health professionals, we were able to successfully demonstrate how an evidence-based, health education website can 1) be evaluated and 2) measure impact. In this presentation, the presenter will discuss Baby's First Test's evaluative journey, including lessons learned, and share testing processes and strategies with attendees on how to measure impact and show their program's value. The presenter will also share accessible evaluation tools that can be utilized for quality improvement in education and communications.

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P-054

It's Not "the PKU test": Language Use with Health Professionals

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When newborn screening began in the 1960s, it only included a single condition, Phenylketonuria (PKU). During this time, newborn screening was often called "the PKU test". Today, the term "PKU test" is still used by healthcare practitioners but, there are now more than 70 screenable conditions. Use of this outdated term has the potential to cause a myriad of problems such as misunderstandings with parents or inaccurate follow-up testing.

To address this issue, Baby's First Test put together a workgroup made up of 16 representatives from 13 states, who met once every two months. Together, this workgroup discussed how to communicate the basics of newborn screening to help healthcare practitioners understand the importance of accurate, consistent terminology. Baby's First Test also collected feedback on this idea from other state newborn screening representatives, professional organizations, and families.

Baby's First Test collaborated with the workgroup to develop a one-page document called "Newborn Screening: More than a PKU Screen" highlighting the importance of using the term "newborn screening" instead of the term "PKU test". This document uses stories and visuals to illustrate potential negative impacts on families and discusses the prevalence of the issue in clinical practice. States or national programs can customize the document to fit their audiences.

The "Newborn Screening: More than a PKU Screen" document the Transtheoretical Model of Health Behavior by working to raise awareness of the issue surrounding the term "PKU test". Raising awareness helps move health professionals from the Precontemplation stage to the Contemplation stage of behavior change. Newborn screening programs can use this document and its messaging to raise awareness of this issue, with the ultimate goal of reducing use of the term "PKU test" in practice.

Accurate, consistent terminology is necessary for communication with families and health professionals and can lead to better informed decision making. Standardizing the use of the term "newborn

screening" over the term "PKU test" creates a strong and accurate foundation to streamline the communication and education process.

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P-055*

Newborn Screening Lab Reports: From Read Out to Communications Opportunity

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The "CLIA Program and HIPAA Privacy Rule; Patient's Access to Test Reports; Final Rule" provides individuals with a right to receive their laboratory testing results, as well as the results of any individual for whom they have legal responsibility. In newborn screening, this provides parents or guardians the right to access newborn screening results for their children. As public visibility of genetic testing increases, families are more interested in looking directly at their child's results to learn and take part in clinical discussions of their child's care. Laboratory results can be long, complex, and difficult to understand even for health professionals. Laboratory reports must be designed with families and non-specialist health professionals in mind, providing the information they need to understand the meaning of results, take next steps, and find more information.

Data from the Baby's First Test Ask an Expert module highlights the need for laboratory reports written for public understanding. As of December 31, 2018, 76 out of 407 submitted questions (about 19%) inquired about laboratory testing results or testing accuracy. This data validates the need for reports that better communicate results and their meaning.

To address this issue, Baby's First Test convened a workgroup of 16 representatives from 13 states, who met once every two months. Together, this workgroup looked at examples of different newborn screening laboratory reports and identified areas for improvement as well as methods to address those weaknesses. Baby's First Test collected feedback on the methods and weaknesses from each of the Regional Genetics Networks as well as families themselves.

Baby's First Test collaborated with each group to develop a set of recommendations for creating standardized laboratory reports that may help families understand tests and results. This guideline is called "Recommendations for Plain Language Newborn Screening Results". Incorporating a public health communications view, this guideline provides a set of recommendations for developing report language and design.

Newborn screening laboratory reports must adhere to state or local regulations, which makes changing them difficult. To overcome this barrier, these recommendations should be discussed alongside other regulatory changes such as the addition of conditions or during regular reviews of reporting systems.

As more conditions are added to the Recommended Uniform Screening Panel and state screening panels, laboratory reports continue to swell. With this in mind, proactivity in addressing the results of the communication process is essential to avoid further confusion and under-informing stakeholders.

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P-056

The Incidence of Newborn Bloodspot Screening Disorders in Georgia, 2011-2017

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Background: Georgia's Newborn Screening Program (NBS) screens newborns for 29 genetic, metabolic, endocrine, and select other disorders through the heel-stick bloodspot card. Early identification and intervention is critical to prevent morbidity and mortality outcomes of conditions and disorders on the NBS panel. In this population-based descriptive epidemiologic study, we examined the incidence of disorders captured through the bloodspot screening card among newborns in Georgia during 2011 through 2017.
Methods: NBS screening results were downloaded from the State Electronic Notifiable Disease Surveillance System. Records from the Emory University Department of Human Genetics and Sickle Cell Disease Follow-up Programs were linked with NBS records and electronic birth certificates. Newborns who received at least one screen, regardless of their state of residence, were included in the denominator. The 29 NBS disorders were grouped into five categories: organic acid disorders, fatty acid oxidation disorders, amino acid disorders, hemoglobinopathies, and others. The incidence per 100,000 live births of each disorder category of disorder was calculated. All analyses were performed in SAS 9.4.
Results: From 2011–2017, over 920,000 newborns received screening in Georgia. Among these, more than 2,300 newborns were diagnosed with at least one of the 29 screening disorders. Overall, the incidence of newborn bloodspot screening disorders was 250 per 100,000 live births. The incidence of organic acid disorders was 7 per 100,000 live births; fatty acid disorders occurred in 15 per 100,000 live births; amino acid disorders were diagnosed in 6 per 100,000 live births; hemoglobinopathies occurred in 121 per 100,000 live births; congenital hypothyroidism was diagnosed in 75 per 100,000 live births and cystic fibrosis occurred in 17 per 100,000 live births. Among all classes, the most frequently-diagnosed organic acid, fatty acid, and amino disorders, respectively, were methylmalonic acidemia (MMA); medium chain acyl-CoA dehydrogenase deficiency (MCADD); and phenylketonuria (PKU). Nine of ten infants diagnosed with cystic fibrosis and three of four infants diagnosed with MCADD were White.
Conclusion: In Georgia, one in every 400 newborns were diagnosed with a newborn bloodspot screening disorder. The most-frequently diagnosed conditions were hemoglobin disorders and hypothyroidism. Long-term morbidity/mortality rates, false positive/negative rates, and average time to diagnose condition could not be examined. This is the first population-based descriptive epidemiology study of the 29 newborn bloodspot screening disorders in Georgia. A surveillance system for the newborn screening diagnosis could assist in assessing long-term outcomes, identifying risk factors and analyzing the cost-benefit ratio of screening for these rare conditions.

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P-057

Implementation of Electronic Test Reporting at a Large Hospital System in Georgia

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Introduction: The Georgia Department of Public Health Laboratory (GPHL) implemented electronic test reporting (ELR) for newborn screening in fall 2018 with Piedmont Hospital, a large hospital system in the Atlanta area that includes ten separate locations (and growing). The project involved a partnership with Ruvos, which is a data integration company; PerkinElmer, the GPHL's LIS vendor; the Association of Public Health Laboratories (APHL); and the Center for Disease Control and Prevention (CDC). Funding for the project was provided by the CDC via a grant from APHL.

Method: The project work flow began with the creation of an HL7 file by PerkinElmer, which was placed on a secure shared drive. The file contained demographic data, test results, and result interpretations for all specimens reported by the laboratory in a given period of time (i.e., usually one day). The HL7 file was processed by Ruvos to extract data for specimens submitted by Piedmont Hospital, and configure a result file that could be easily imported into the Piedmont hospital information system (Epic). The result file was securely transported from GPHL to Piedmont via PHINMS and the APHL Informatics Messaging Services (AIMS) Platform. Because the HL7 file created by Perkin Elmer contained data for all specimens, it will be relatively easy to implement ELR with other hospitals in the future. The next phase of the project will be to implement electronic laboratory test ordering (ELO), which will hopefully be completed by spring 2019.

Results: The implementation of ELO and ELR is expected to provide many benefits. The metrics being monitored include the turnaround time from when the specimens were collected until results were received by Piedmont, the number of specimens received at GPHL that were missing critical demographic data, the amount of time spent by Piedmont staff to complete the NBS form and "order" the test, and percent of infants born at Piedmont hospital with missing newborn screening results. Results of the comparison analyses will be presented at the symposium.

Conclusions/Implications: A couple of very valuable lessons were learned during this project. Successful implementation of ELR and ELO require the involvement of multiple entities, each with competing projects, which tends to lengthen project timelines, even when there is universal agreement regarding the desirability of the project. Difficulties were encountered with coordinating schedules of the participants, and "hitting" their open windows. To streamline and reduce the cost of bringing more hospitals online, it is critical to devise import and export processes with a minimum of variability and customization.

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P-058

Follow-up Testing for Infants with an Abnormal Newborn Screen for Mucopolysaccharidosis Type II

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The state of Illinois began screening newborns for Mucopolysaccharidosis type II (MPS II) in 2017. Of the total 93,219 newborns screened, nine (0.01%) were referred for follow-up testing. The Greenwood Genetic Center performed the follow-up testing for eight of these patients, which includes iduronate-2-sulfatase (IDS) enzyme analysis in plasma using a fluorogenic substrate, urine glycosaminoglycan (GAG) analysis via both the DMB binding assay and UPLC-MS/MS, and molecular analysis of the IDS gene, including gene sequencing, allele-specific PCR for the detection of the common IDS/IDS-2 inversion, and MLPA analysis for deletions/duplications, if necessary. One referred patient had IDS activity within the affected range and urine GAG results consistent with MPS II (elevation of total GAGs, heparan sulfate and dermatan sulfate: 2.5-fold, 85-fold & 4-fold, respectively). This patient also has a positive family history of MPS II, and is therefore considered affected. One additional patient had IDS activity within the affected range; however urine GAGs were not consistent with MPS II (only heparan sulfate was slightly elevated: 2.5-fold). Sequencing of the IDS gene identified an unreported missense variant and appropriate familial testing is planned. The remaining six patients that were referred for testing had IDS activity below the normal range (155 – 1082 nmol/4hr/mL plasma) but above the affected range (0 – 15 nmol/4hr/mL plasma), with activities ranging from 30.4 – 100.1 nmol/4hr/mL. One of these patients (IDS activity = 30.4 nmol/4hr/mL) had a persistent 5-fold elevation of urine heparan sulfate, with normal total GAGs and dermatan sulfate, and a different unreported missense variant in the IDS gene. Familial testing revealed that the missense variant was inherited from a phenotypically normal maternal grandfather, who also had reduced IDS enzyme activity and mildly elevated heparan sulfate. The other five patients with reduced IDS activity had normal urine GAGs. IDS molecular analysis has been performed in four of these patients; three patients each had a unique unreported IDS missense variant and one had normal molecular analysis, including allele-specific PCR and MLPA. In comparison with our experience with follow-up testing for MPS I newborn screening, MPS II has a lower screen positive rate. So far, no carrier females have been detected. Similar to MPS I, most infants who screen positive for MPS II have enzyme activity between the normal and affected ranges. However, whereas four recurrent missense changes (pseudodeficiency alleles) are responsible for the majority of the MPS I positive screens, each patient with a positive screen for MPS II has had a unique and previously unreported IDS variant. Also in contrast to MPS I, since MPS II is X-linked, familial testing can sometimes be useful in determining whether a novel missense variant is clinically pathogenic or benign.

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P-059

Using the Agena Bioscience MassARRAY System to Perform Population-based Newborn Screening for Cystic Fibrosis in the State of Illinois

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Cystic fibrosis (CF) is a genetic disorder that mainly affects the respiratory and digestive systems, consequently resulting in a variety of different symptoms, including difficulty breathing, recurrent pulmonary infections and poor growth in children. Fortunately, CF can now be diagnosed early in life through Newborn Screening, allowing for early medical intervention through which patients can avoid most of the clinically severe adverse effects. Most CF Newborn Screening pipelines use immunoreactive trypsinogen (IRT) levels as a first tier laboratory test for CF, as high IRT levels indicate the pancreatic damage commonly associated with the disease. Current second tier molecular testing minimally involves the detection of 23 different disease causing mutations that occur within the CFTR gene, as recommended by the American College of Medical Genetics (ACMG). Various different methods have been used to detect CF mutations, including DNA hybridization and sequencing approaches. This is the first high-throughput study that explores the robustness and reliability of a commercial MALDI-ToF based detection system for CF newborn screening. The current study conducts population-based Newborn Screening of CF in the state of Illinois using the Agena Bioscience MassARRAY instrument and their commercially available CFTR testing kit, which can detect 72 CF-related mutations. We implemented this CF screening approach in March 2018, and have tested >6,000 specimens using DNA extracted from dried blood spots. We have identified several caveats to data interpretation, and have devised a work flow that allows us to test and confirm mutations within our necessary turn-around time, and feel that the Agena MassARRAY instrument provides a cost-effective CF screening assay for Newborn Screening laboratories.

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P-060

Stable Isotope Labeled Internal Standards Considerations in Newborn Screening

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Introduction: It is widely accepted that Stable Isotopic Labeled-Internal Standards (SIL-IS) are considered essential in quantitative assays employing mass spectrometry detection. SIL-IS are compounds that we are interested in quantitating in which several atoms are replaced by their stable isotopes such as 2H, 13C, 15N etc. SIL-IS are meant to correct for variabilities in evaporation, degradation, dilutions, derivatization, adsorption, ion-suppression, recovery and various instrumental parameters such as injection volume. SIL-IS are especially important in mass spectrometry (MS) based newborn screening (NBS) as Flow Injection Analysis (FIA)-MS is most often used. During the FIA-MS mode, the specimen is infused without previous chromatographic separation which lead to the highest possible ion-suppression and interferences from endogenous compounds. Selection of appropriate internal standards in NBS is particularly challenging due to the high number of analytes that need to be simultaneously quantified while hundreds of endogenous compounds are infused at the same time

under FIA-MS conditions. In this communication we present several NBS related cases where the wrong choice of SIL-IS can compromise the quantitation of the analytes of interest.
Methods: A number of different experiments were performed depending on the SIL-IS investigated. Those included but not limited to varying MS parameters such as source temperature and cone voltage, use of different buffers and solvent compositions at different pH, use of low and high resolution mass spectrometers as well as different sample preparation methods (i.e. derivatized vs. non-derivatized).
Results: Several situations were investigated where the choice of inappropriate SIL-IS compromised the quantitation of analytes of interest due to in-source fragmentation, hydrogen-deuterium scrambling, isobaric interferences etc. Stable Isotope versions of Ornithine, Malonic Acid, Glutarylcarnitine, Creatinine and C20-26 lysophosphatidylcholines were among the compounds investigated and found to either compromise the quantitation of their non-labeled version or interfere with the quantitation of other analytes of interest.
Conclusions: The selection of inappropriate SIL-IS can affect the quantitation of analytes of interest.

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P-061

Development of a Universal, Second-tier Newborn Screening LC-MS/MS Method for the Simultaneous Analysis of Underivatized Amino Acids, Acylcarnitines, Organic Acids and Steroids

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Introduction: Advances in mass spectrometry and its adoption by public health programs have led to the expansion of newborn screening test panels from just a few to several dozens. From 1995 to 2005, in average, states increased the number of disorders tested approximately five times (from 5 to 25) [1]. Despite the high sensitivity and specificity of screening with MS/MS, expanded newborn screening led to an increased number of infants with false-positive results, especially for disorders which primary markers lack disease specificity and have poor diagnostic sensitivity due to the overlap of their concentrations in affected and unaffected newborns. Several ways have been proposed to decrease false positives, such as the use of analyte ratios [2] (e.g. use of Phe/Tyr ratio in PKU screening), substituting the primary analyte with a more specific one in primary screening [3] (e.g. use of Succinoacetone instead of Tyrosine for the screening of TYR I) or by performing second-tier screening [4]. Second-tier screening in particular has been shown to significantly decrease the amount of false positives without sacrificing any sensitivity and several LC-MS/MS methods have been developed for different NBS disorders. The drawback currently is that multiple LC-MS/MS methods are needed to cover the spectrum of NBS disorders requiring second-tier screening putting a lot of pressure on scarce laboratory resources. Furthermore, some of the assays do not have the specimen volume required to be run daily making it difficult to justify adopting them. The purpose of this study was to develop a single, multiplex LC-MS/MS second-tier assay capable of screening simultaneously all disorders requiring second-tier screening.
Methods: Standards and CDC's Newborn Screening Quality Assurance Program (NSQAP) quality control dried blood spot cards were used in this study. Quality control specimens were extracted following a previously described non-derivatized protocol [5]. A proprietary HILIC/mixed-mode column were used to separate amino acids, acylcarnitines, organic acids and steroids using an appropriately buffered Water: Acetonitrile gradient. Several critical pairs such as Leu, Ile, Allo-Ile and Hydroxyproline were separated.

Results/Conclusions: The method is able to simultaneously analyze underivatized second-tier biochemical analytes for the following disorders: MSUD, HCY, MMA, PROP, IVA, GA-I, GAMT, CAH, SCAD and Pompe disease. The method can significantly reduce the cost and resources required compared to having different second-tier methods for different disorders.

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P-062*

Use of a Deliberative Community Engagement Model to Obtain Public Recommendations About Adding New Conditions to Iowa's Newborn Screening Panel

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Adding Pompe, MPS1, and X-ALD to the Iowa Newborn Screening Program (INSP) panel requires careful consideration because these conditions have late onset variants and high false positive rates. As a result, INSP decided to get input from community members utilizing a Deliberative Community Engagement (DCE) model to help inform the INSP decision-making process. DCE is a public decision-making event where people learn about issues, share opinions, and influence a law or policy. This proposed session will describe the DCE project, the recommendations put forth from the event, and lessons learned from using the DCE model in newborn screening.

Twenty-six Iowans of diverse backgrounds participated in a two-day event. Recommendations were elicited on the following questions:• What are important factors to consider when planning for future additions or changes to Iowa's Newborn Screening Panel? • How should the Iowa Newborn Screening Program change communication to families, including results reporting?• How can IDPH continue engaging the public to provide ongoing feedback for the Iowa Newborn Screening Program?

The benefit of using the DCE model over survey methods for capturing public opinion about NBS lies in the informed, objective nature of the opinions derived from the review of NBS educational materials, the discussion-based format and the diverse range of participants. This DCE allowed the program to learn the values of those not normally represented in NBS discussions. All participants were actively engaged in the deliberation and provided concrete, applicable recommendations. Participants voted on whether to add each of the three conditions to Iowa's NBS panel. Furthermore, participants stated they had become advocates of the INSP in their communities.

Three themes emerged for considering how to add conditions to the Iowa NBS panel: (1) Parent choice about wanting or not wanting to know if your child has a condition, especially when no treatment is available; (2) Cost of diagnostic testing and treatment for families of children who have abnormal screening results; (3) Quality of life both for the family and the child.

Finally, Newborn screening programs often hear from families, advocates and commercial entities who have a stake in adding conditions to the NBS panel; use of DCE provided the INSP with objective, measured opinions of Iowans who have no recent personal experience or connection to the INSP.

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P-063

A Post-analytic Approach to Minimizing the Time Interval from Report of Positive Newborn Screen for Cystic Fibrosis to Initial Encounter for Sweat Testing

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Problem: National data indicate median age at diagnosis after a positive NBS for cystic fibrosis in the United States is 19 days, with a range from 4 to 45 days. Guidelines from the Cystic Fibrosis Foundation (CFF) recommend confirming diagnosis and beginning treatment by the time the affected infant is 1 month old. The Iowa Newborn Screening Program (INSP) together with the two CFF accredited CF centers in Iowa implemented a collaborative process improvement project aimed at decreasing the interval between when short term follow up (STFU) staff reports the abnormal result to the primary care provider (PCP) and when baby presents for diagnostic testing at a CF center.

Methodology: We collected data on all CF screen positive infants in Iowa for a two year period, before and after implementation of the process improvement project. On January 1, 2018 we initiated updates to our written and verbal recommendations provided to PCP to emphasize completion of sweat testing by 1 month of age at an accredited CF center. The protocol changes included offering assistance with the referral process and re contacting the PCP's office on the next business day to verify that contact with parents had been made, results had been disclosed, and parents were amenable to direct contact from CF center scheduling staff. STFU then emailed the CF center preferred by the family to initiate the referral process. We also published an article in the spring 2018 newsletter for the Iowa Chapter of the American Academy of Pediatrics to inform practitioners across the state of this initiative.

Results: A two sample t-test analysis was done to compare 2017 data to 2018 data. Statistical analysis suggests a moderate decrease in the length of time it took to get infants sweat tested, from a mean of 17 days in 2017 to a mean of 13.5 days in the first half of 2018.

Conclusions: The stated goals for this CF process improvement project were:
(a) to see a substantial, measurable reduction in the interval between PCP notifications and sweat chloride testing, which in turn reduces the length of time to diagnosis and, more importantly, treatment for these children in order to optimize their health outcomes,
(b) to provide an evidence-based rationale for short term follow processes, and
(c) to provide data that will be used to guide future process improvement activities.
Although the reduction in time to initial diagnostic testing was not a large one, we are encouraged it does provide evidence that our first attempt to streamline the referral process for CF testing is on the right path. Further data analysis will help to inform refinements in how this process moves forward. One key component already identified for further study is to analyze the association between process improvement activities and outcomes.

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P-064

Quality Assurance of Newborn Screening Form Completion

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Newborn screening labs receive forms that are incorrectly or partially filled out and samples that are unsatisfactory for reliable testing. In an effort to communicate effectively and efficiently to submitters, Iowa Newborn Screening Laboratory faxes submitters automatically when fields are missing or sample collection is poor quality. The concept has improved and expanded over the years and now faxes are sent daily requesting information or notifying of the need to recollect if poor quality sample. Corrected information is received before testing and final report is complete. This decreases the amount of cases created for follow up and amount of final reports that require corrections. Real time notification of the need to re-collect is made to help decrease the time it takes to receive a repeat screen. Most importantly it reduces incorrect follow up or potential unnecessary repeat screens for the patients. Minimal effort upfront has large impact downstream in the newborn screening process.

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P-065

ZAP70 Deficiency and Newborn Screening for Severe Combined Immunodeficiency (SCID)

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Autosomal recessive zeta-chain associated protein tyrosine kinase of 70 kDa (ZAP70) deficiency is a rare cause of combined immunodeficiency characterized by normal numbers of CD3+ T-cells with markedly decreased CD8+ T-cells and abnormal T-cell function. Given the absence of T-cell lymphopenia the current method of SCID screening using a TREC (T-cell receptor excision circle) assay would not be expected to detect this disorder. We report identical twins with ZAP70 deficiency; one with a presumptive positive SCID NBS, the other with a completely normal TREC assay.

The identical twin males were the product of a 37 4/7 wk gestation, born to consanguineous parents of Pakistani ancestry. Twin A's initial SCID NBS was indeterminate; repeat screen 9 days later was reported as presumptive positive. Follow-up testing showed a normal total lymphocyte count with decreased CD3+ cells, normal CD4+ cells, undetectable CD8+ cells and abnormal T-cell function. He was found to be homozygous for the C39X variant in the ZAP70 gene and heterozygous for a partial duplication of the ATM gene. Twin B had a normal SCID NBS screen however during assessment as a possible donor for his twins transplant, was found to have undetectable CD8+ cells with a normal lymphocyte count, normal CD3+ and CD4+ cells and abnormal T-cell function. Mutational testing identified Twin B as homozygous for the C39X variant in the ZAP70 gene and heterozygous for a partial duplication of the ATM gene.

ZAP70 deficiency represents a type of SCID characterized by circulating T-cells, which would not have been detected by TREC-based screening. As expected the TREC assay did not identify Twin B with

ZAP70 deficiency but unexpectedly identified Twin A. There is one published report of two siblings with ZAP70 deficiency found to have low TRECs on archived dried blood spots (Jilkina et al. 2014). The authors speculated that the degree of suppression of thymopoiesis and TREC generation might be linked to genetic factors. Our patients were identical twins and thus genetic factors would not explain why only one twin had an abnormal SCID NBS. The duplication of uncertain clinical significance in the ATM gene is also unlikely to explain this finding. There were no significant clinical differences in the babies however only Twin A had decreased CD3+ cells and evidence of 1% maternal chimerism. Twin A's CD3 lymphopenia suggests impaired TCR signaling and thympoiesis which likely lead to the abnormal TREC assay, but the discrepancy between the twins NBS results remains unexplained. This report illustrates the importance of communication of twin family history/genetic information in the NBS system, the limitation of TREC-based NBS to detect T+ SCID and the need to further identify the role of ZAP70 in T-cell receptor signaling during thymic development.

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P-066

Promoting Inclusive Educational Efforts: Michigan's Newborn Screening Conference for Midwives

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Background: Compared to the blood spot newborn screening (NBS) rate of 99.4% in the general population of Michigan births, approximately 62% of midwife-attended homebirths receive a blood spot screen, and even fewer receive a hearing screen (25%) or pulse oximetry screen (24%). When a screen is obtained for a homebirth, the screen is more likely to be collected late and slow to arrive in the state laboratory for testing compared to hospital births. Since the time from birth to treatment initiation is critical for several disorders included in the NBS panel, failure to obtain a screen at all or in a timely manner can have devastating consequences. In response to these concerns, NBS Program staff developed a NBS conference specifically for midwives.
Methods: A planning committee was formed with staff from all three components of NBS (blood spot, hearing, and heart), as well as a staff member from the birth registration unit and a midwife who serves on a NBS advisory committee. The committee created a list of crucial and potential topics to cover to fill the day-long agenda. Midwives were surveyed to assess level of interest in the potential topics, when and where to hold a conference. The responses helped guide the final selected list of topics, conference dates, and locations. Registration and conference information was distributed to midwives both electronically and through the mail. A post-conference evaluation was given to all attendees.
Results: A total of three conferences were held around the state. Webinar participation was offered at all three sites, but nursing contact hours were only available for in-person attendees. Approximately 30 people attended in-person and 3 participated via webinar. Topics covered at each conference included detailed overviews of each component of NBS and information on how to register birth certificates for home births. Elective topics varied at each conference and included information on perinatal mood disorders, how to apply for public insurance, and new disorders under consideration for NBS. A total of 19 attendees completed the post-conference evaluation, and the results were very positive.
Conclusions: Through informal conversations with attendees, we found that offering contact hours, which are needed for a new licensure law in Michigan for certified professional midwives, and including a presentation on birth certificate registration bolstered interest. Midwives expressed gratitude for

having a conference that focused on needs and issues unique to the homebirth community and appreciated having face-to-face interactions with NBS staff. Due to the success of this conference, the NBS Program will likely continue holding a conference for midwives every other year to provide updated information and work towards strengthening relationships with the homebirth community to ensure all newborns have the opportunity for early identification through NBS.

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P-067

Standardizing Critical Congenital Heart Disease Follow-up: Getting to the Heart of the Matter

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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 or a reason why the screen was not completed to the NBS Program. Michigan collects individual-level data to alert hospitals of potentially missed screens, determine the outcome of failed screens, and evaluate the CCHD screening algorithm. Due to poor data quality, Michigan's NBS Program had a need for a more standardized process for follow-up of missing records and failed screens to ensure that all newborns born in Michigan were screened for CCHD and to determine the final outcome of failed screens.
Methods: In October 2016, the Michigan NBS Program implemented a standardized follow-up process. A hospital receives a fax asking for information if an infant failed a screen, if the screen was reported as missed for that infant, or if the program has not received any information for that infant. On a monthly basis, the NBS Program staff determine the pulse oximetry screening reporting rate for each hospital. The NBS Program's goal is for each hospital to have a reporting rate of 90% or greater. If at any point a hospital has a reporting rate of less than 90%, technical assistance will be offered. The NBS Program also sends out quarterly quality assurance reports to each birthing hospital. The reports include three metrics: the percent of CCHD screens reported, the percent of infants with CCHD screens reported in a timely manner, and the percent of CCHD screens conducted within the recommended time frame after birth. Each birth hospital is also provided with the number of passes, rescreens, and fails and a list of algorithm compliance errors.
Results: There has been a significant increase in the percent of pulse oximetry screens reported between April 2014 and June 2018 ($P<.001$). Prior to initiation of the standardized follow-up process, 80% of pulse oximetry screens were reported statewide. The standardized follow-up process was implemented in October 2016 and by December 2016 85% of pulse oximetry screening values were reported to the NBS Program. The current reporting rate is approximately 93%. Before standardized follow up, 35 hospitals met the 90% reporting goal, compared to 65 hospitals after.
Conclusion: Our findings indicate that implementing a more standardized follow-up process for pulse oximetry screening, has increased the number of infants with screening results reported to the Michigan NBS Program. It has also resulted in more complete records, which will allow for more accurate studies of the effectiveness of the screening program and evaluation of the screening algorithm.

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P-068

Evaluation of Pulse Oximetry Screening Rates among the Midwife-Attended Out-of-Hospital Birth Community in Michigan

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Introduction: In Michigan, pulse oximetry screening of newborns has been mandated since April 2014, with the aim of detecting cases of critical congenital heart disease (CCHD) before symptoms manifest. The State of Michigan Newborn Screening (NBS) Program receives individual-level pulse oximetry screening data from birthing hospitals and midwives across the state. Pulse oximetry screening rates for CCHD are assessed for birthing hospitals but have not been assessed for the midwife-attended births that occur in the out-of-hospital birth community. The State of Michigan NBS Program operates a pulse oximetry loan program that allows midwives to borrow pulse oximeters. Analysis was needed to determine pulse oximetry screening rates among the midwife-attended out-of-hospital birth community in Michigan overall, screening rates among midwives provided with loaned pulse oximeters from the Michigan Department of Health and Human Services (MDHHS), and whether future intervention is needed to increase pulse oximetry screening rates in the out-of-hospital birth community.
Methods: Birth records obtained from the MDHHS Division for Vital Records and Health Statistics for midwife-attended out-of-hospital births between April 1, 2014 and December 31, 2016 were linked via probabilistic matching with newborn screening (NBS) records. Pulse oximetry screening rates were calculated for the midwife-attended out-of-hospital birth population overall, by midwife, and stratified by receipt of loaned pulse oximeters from MDHHS. Births from midwives who attended five or more non-hospital births during the study period were included in this analysis.
Results: Of the 3,410 midwife-attended out-of-hospital births, 20.8% (n=710) were reported as having received a pulse oximetry screen for CCHD. For births attended by midwives who received pulse oximeters from MDHHS, 50.5% had pulse oximetry screening results reported, compared to 12.7% among births attended by midwives without a loaned pulse oximeter. Of the 78 total midwives, 18% (n=14) reported pulse oximetry screening results on greater than half of the births they attended. Of the 14 midwives who received a pulse oximeter from MDHHS, 50.0% (n=7) reported screening results for greater than half of all births they attended.
Discussion: Our findings indicate that reported CCHD screening rates are low among the midwife-attended out-of-hospital birth community in Michigan. Screening rates were higher among midwives who received a pulse oximeter from MDHHS, but fewer than half of the attended births had a reported pulse oximetry screen overall. The use of the MDHHS pulse oximetry loan program was correlated with higher pulse oximetry screening rates among out-of-hospital births, but further discussions with the midwife-attended out-of-hospital birth community to better understand screening or reporting barriers may be beneficial.

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P-069*

Age at Enrollment in Children's Special Health Care Services (CSHCS) among Children Diagnosed with a Disorder on the Michigan Newborn Screening (NBS) Blood Spot Panel

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Introduction: Children's Special Health Care Services (CSHCS) is a program at the Michigan Department of Health and Human Services that strives to improve health outcomes and quality of life for children with special health care needs. Enrollment in CSHCS is based on qualifying medical conditions and is coordinated through local health departments. All disorders on the Michigan newborn screening (NBS) blood spot panel qualify for CSHCS. Each year in Michigan there are approximately 270 infants diagnosed with a disorder on the NBS blood spot panel who are eligible for CSHCS.
Methods: All infants born between January 1, 2012 and December 31, 2016 who were diagnosed with a disorder on the NBS blood spot panel were included in the study. Diagnosed NBS cases were linked to electronic birth certificate (EBC) records and then EBC records were linked to the ID within the Michigan public insurance claims data warehouse to retrieve the CSHCS data of enrollment and qualifying diagnosis. Using the child's birth date and date of enrollment, age of enrollment was categorized as before 3 months, between 3-6 months, between 9-12 months, and after a year of age.
Results: Between 2012 and 2016, 1,339 infants were diagnosed with a condition on the NBS blood spot panel; five infants were diagnosed with more than one disorder. Almost all the diagnosed cases were linked to Michigan EBC records (99.4%). Nearly half of the diagnosed population with an EBC enrolled in CSHCS (49.6%). Children diagnosed with cystic fibrosis, sickle cell disease, and severe combined immunodeficiency (SCID) were most likely to enroll in CSHCS (81.7%, 70.0%, and 63.5%, respectively). Infants diagnosed with hemoglobin H, galactosemia, and biotinidase were least likely to enroll in CSHCS (14.3%, 20.0%, and 23.4%, respectively). Among the children enrolled in CSHCS, the majority enrolled by three months of age (60.6%); another 12.4% enrolled between three and six months. Only 5.9% enrolled between six and nine months, and 4.6% enrolled between nine months and a year of age. The remainder of the children enrolled after one year of age (16.5%). Infants diagnosed with sickle cell disease were least likely to enroll by three months of age (35.4%), which makes sense based on the confirmatory testing being performed at a later age for this group, and infants diagnosed with SCID were most likely to enroll by three months of age (92.6%).
Conclusion: Our findings indicate that almost half of the infants diagnosed with a condition on the NBS blood spot panel enroll in CSHCS. The percent of children enrolled and the time to enrollment differ by disorder. Future work includes stratifying by county of birth to identify local health departments associated with delays in enrolling families and comparing qualifying diagnosis to NBS diagnosis. This study will help the NBS Program target CSHCS educational efforts regarding low or delayed enrollment

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P-070

Implementation of an Automated Liquid Handler for Post-Amplification Sample Processing for the Detection of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Variants using the Luminex xTAG® CF60v2 Kit

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The Michigan (MI) Newborn Screening (NBS) Laboratory implemented the Luminex xTAG® CF60v2 kit in March of 2016 following the Hologic CF InPlex® recall for the detection of mutations/variants in the CFTR gene. The Luminex xTAG® CF60v2 assay detects 60 mutations/variants that are divided into two panels. The need for using automated liquid handling pipetting became evident during the validation of the Luminex xTAG® CF60v2 kit due to the nature of the extensive post-processing steps. With each specimen being processed in two reaction wells, an automated liquid pipetting system was incorporated during this transition to allow for high-throughput sample processing in a 96-well plate format. The Eppendorf epMotion® 5075 TMX liquid handler was the automated liquid pipetting system of choice for this task as it was first introduced to the MI NBS lab in October of 2011 for the first primary molecular screening assay, enumeration of T cell receptor excision circles (TRECs) for the detection of primary immune deficiency syndromes (PIDS).During the validation of the Luminex xTAG® CF60v2 kit, the epMotion® 5075 TMX was incorporated into the Cystic Fibrosis assay for detecting CFTR variants for use with pre- and post-amplification sample processing. The post-amplification steps of the Luminex xTAG® CF60v2 assay modified for use with the epMotion® 5075 TMX include Amplicon Treatment, Allele-Specific Primer Extension and Bead Hybridization. The use of automated liquid handling minimizes risk of human-pipetting errors, increases time-efficiency and allows for precise and accurate results. Michigan was one of the first states to implement an automated method for post-amplification sample processing for the Luminex xTAG® CF60v2 kit.

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P-071

The Data Behind the Decision: Clinical Outcomes for Michigan's Short-Chain Acyl-CoA Dehydrogenase Deficiency and Isobutryl -CoA Dehydrogenase Deficiency Cohort

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Background: Michigan's Newborn Screening (NBS) Program removed short-chain acyl-CoA dehydrogenase deficiency (SCAD) and isobutyryl-CoA dehydrogenase deficiency (IBD) from its screening panel in October 2017. Prior to formal removal of these conditions, clinical outcome data of all patients referred for isolated elevations of C4 acylcarnitine (C4AC) on NBS were reviewed to ascertain if these conditions were associated with medically significant co-morbidities among the identified NBS cohort. **Methods:** In Michigan, all newborns who screen positive for an isolated elevation of C4AC were referred to a single metabolic center for confirmatory testing and treatment. The metabolic specialist at that center reviewed the confirmatory outcomes and the clinical data of NBS-identified SCAD/IBD patients for several outcomes, including genotype-phenotype correlation, laboratory testing values, number of emergency department visits/hospitalizations, developmental outcomes, and parental compliance with

short term and long term follow-up recommendations. In addition, an extensive review of SCAD/IBD patient cohorts from other states and countries was completed and compared to the Michigan experience.
Results: From 2005-2017, 167 referrals led to a total of 98 Michigan newborns diagnosed with SCAD or variant SCAD and 15 with IBD. The detailed clinical review of the Michigan cohort included 77 SCAD patients and 13 IBD cases. None of the SCAD or IBD patients had hypotonia, myopathy, hypoglycemia, acidosis, seizures, or other evidence of clinically significant metabolic sequelae. Risk of secondary carnitine deficiency based on this cohort was deemed to be very low for IBD patients and very low to none for SCAD patients. Risk of developmental outcomes among both disease groups were generally comparable to those in the general population and in those found to have developmental concerns the delay was mild. There was no consistent medical or dietary intervention prescribed across the cohort outside of general precautions regarding avoiding prolonged fasting. No adverse long-term consequences were observed in the studied cohort during the follow-up period that could be attributable to the disorders in question. Therefore, Michigan findings were comparable to evidence found in the literature and other recently reported cohorts: SCAD and IBD patients identified prospectively through newborn screening are generally doing well without need for significant medical or dietary intervention with no particularly significant morbidity or mortality.
Conclusions: The clinical data review of Michigan's NBS SCAD/IBD cohort supports growing available evidence that these conditions represent largely benign disorders of metabolism and identified no clear risk of adverse clinical outcomes to warrant mandated identification of these cases though the state's newborn screening panel.

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P-072

Withdrawn

P-073

Validation and Implementation of MPS II Newborn Screening in Missouri using a Fluorimetric Assay
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Mucopolysaccharidosis Type II (MPS II) is a lysosomal storage disorder (LSD) caused by a deficiency of the lysosomal enzyme iduronate-2-sulfatase (IDS). MPS II satisfies all criteria defined by the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) for inclusion in the Recommended Uniform Screening Panel (RUSP) for newborn screening (NBS), with the exception that minimal prospective population screening data is available. The Missouri State Public Health Laboratory (MSPHL) validated a microtiter plate-based fluorimetric assay for measurement of IDS activity in newborn dried blood spot (DBS) specimens. This assay requires minimal hands-on time and an incubation time of two hours. The analytical validation of this assay included precision, linearity, analytical sensitivity, and carry over testing. Results for all analytical tests were comparable to existing NBS assays.

Additionally, clinical validation was completed using deidentified presumptive normal NBS specimens as well as diagnostic specimens from patients known to be affected with MPS II. More than 5,000 presumptive normal specimens were tested; the population was determined to be lognormally distributed with a median value of 89.63 umol/L/h and a minimum presumptive normal value of 29.38 umol/L/h. Seven diagnostic DBS samples from patients affected with MPS II were also tested; the median affected activity was 3.15 umol/L/h, with a maximum affected value of 7.00 umol/L/h. Based on the reproducibility of the assay in the affected range, the separation between the highest affected value and lowest normal value was more than 25 times the standard deviation. The results of the analytical and clinical validations demonstrate robust analytical performance as well as significant separation between the affected and presumptive normal populations.

MSPHL will begin full population pilot screening for MPS II on 11/1/2018. We will present data on the results of prospective screening through early 2019, which will include results for at least 25,000 specimens. During screening, MSPHL will utilize molecular 2nd tier sequencing for all presumptive positive samples. These results will help to provide context to future consideration by the ACHDNC for addition to the RUSP and will provide guidance for states considering testing for MPS II in the near future.

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P-074*

Long Term Follow-up with Families that Have a Child with Critical Congenital Heart Disease (CCHD): Findings from Local Public Health (LPH) Nursing Assessments

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Problem/Objectives: The Minnesota Department of Health (MDH) contracts with Local Public Health (LPH) agencies to contact families of children identified with a hearing loss or a birth defect to provide education and a connection to local resources. The objective of this analysis was to identify the resource needs and interventions provided to families with a child with a CCHD from the MDH Birth Defects Information System. The twelve CCHDs targeted by pulse oximetry screening were the focus of this analysis.

Methodology: LPH nurses contact families and through a nursing assessment, they identify family needs. The results of the assessment are documented using the Omaha System, a research-based standardized taxonomy for documentation, which uses a client assessment, interventions, and evaluation to standardize data collection for individuals, families, and communities. Starting in 2017, all assessments completed by LPH used the Omaha System to document five problem areas. The two areas of focus of this analysis are Caretaking/Parenting and Growth/Development. The interventions provided by LPH in these areas were analyzed to determine the needs of families with a child with a CCHD and what interventions were most commonly provided to them.

Results: From 01/01/17 through 03/31/18, MDH requested follow-up from LPH for 150 families with a child with a CCHD. Of those, 74% (n=103) received a nursing assessment. When Growth/Development was assessed, nearly one third of the children were identified as having inadequate achievement/maintenance of developmental tasks. Overall, 158 interventions were provided in this area, including referral to education/developmental resources such as Early Intervention (EI), Early Childhood Family Education, or the Follow Along Program. This type of referral was provided to 62% of

the families. Of the 103 children, 39 were enrolled in EI and 16 were referred at time of assessment. The most common reason for not referring was that a parent had declined. When Caretaking/Parenting was assessed, few signs/symptoms were identified. However, 85% of families received at least one intervention. In total, 171 interventions were provided, of which 72 were in the teaching domain and 78 in surveillance.
Conclusion: The use of a standard documentation tool of a LPH nursing assessment for families of children with a hearing loss or birth defect, including those with a CCHD, has provided a framework for assessment and data collection. The use of this tool has shown that interventions are provided to families to help meet the needs of their child, regardless if specific signs/symptoms are identified at the time of the assessment. The next steps are to conduct additional analyses to determine further resources that could benefit families.

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P-075

Helping Minnesota Hospitals Improve Quality through Site Visits
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Objectives: Conduct non-regulatory site visits at hospitals and walk through their processes to improve quality and timeliness in newborn screening. We created a small team of subject matters experts in our operations and short-term follow-up units who were interested in outreach to hospitals to help them improve quality and timeliness, and also to discover if there were ways in which our program could be providing more assistance to them.
Method: Initially we reached out to a handful of hospitals who needed improvements based on their most recent quality assurance (QA) report. We described the purpose of our visit and explained that it was a non-regulatory visit. We asked them to walk us through their process, from the time a baby is born to the time the specimen card leaves their facility. We mostly focused on "a day in the life of a blood spot card" but also discussed hearing and critical congenital heart disease (CCHD) screening. We did not have a formal agenda, but did have some targeted questions we would ask if the conversation didn't naturally go where we expected it to. Upon completion of the site visit, we typed up notes in presentation form using PowerPoint and shared them with the hospital. This report included what we learned on the visit and our recommendations and areas for potential improvement of both quality and timeliness.
Results: Site visits have been conducted at five hospitals and several more are planned. The visits have been very well received and hospital staff and management have been thankful that we've taken the time to visit. We have observed something at each hospital that would not have been discovered without an in person visit. For example, we've seen more than one hospital drying specimens vertically on a rack. This is not something that would have likely been discussed during a phone call or email, but really was just something we observed as we walked by the area. We've been able to make recommendations and build relationships with staff which has opened up lines of communication regarding other issues. Staff from those hospitals have contacted us with other questions regarding screening. We will measure the timeliness and quality for the hospitals we have visited and then share with them how they have improved by implementing changes

Conclusion: Site visits have been a beneficial initiative for both the hospitals and the newborn screening program. It is important to build relationships with hospital staff, especially as new initiatives are moved forward. We will share performance metrics showing changes in hospital QA data such as time from collection of a specimen to receipt at the lab and unsatisfactory specimens due to poor quality before and after a site visit was conducted.

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P-076*

Improvements in Pre- and Post-Analytical Efficiencies - What Does Operations Mean for Newborn Screening?

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Objectives: The term Operations typically leads people to think of manufacturing, not newborn screening. We will demonstrate how the creation of an Operations (Ops) unit in your newborn screening (NBS) program is a benefit to pre- and post-analytical processes. In this day and age of doing more work with less money and fewer staff, an Ops unit can handle tasks in an efficient way so that laboratory and follow-up staff can focus on screening, new method development, and abnormal result notification.
Method: The Public Health Laboratory (PHL) at the Minnesota Department of Health (MDH) had an Environmental Lab Operations Unit for many years that was successful in tackling complicated administrative issues so that lab scientists could focus on lab work. The PHL set out on a mission to create an Infectious Disease Ops Unit and a Newborn Screening Ops Unit. In April of 2014 the NBS Ops Unit was created. An experienced lab analyst was chosen to supervise this unit because multiple duties of the new position were already being performed by this individual. Having a lab background, specifically in newborn screening, is important for this role.
Results: The Ops unit has made improvements to several aspects of the newborn screening program. Tasks that fall under this unit include budget management and forecasting, distribution of NBS cards, invoicing for NBS cards received, grant administration, contract administration, purchasing, health information technology (HIT), project management, demographic data entry and verification, amendments, unsatisfactory specimen callouts, mailing of result reports, technical support, quality improvement, data analytics, and health education. The Ops unit continues to grow and take on more duties.
Conclusion: The creation of an Ops unit in the Minnesota NBS program has been extremely successful. Laboratory and Short-term follow-up staff have a group to go to for guidance with any of the tasks listed above. The Ops unit has also provided backup support and knowledge during times of staffing shortages for both pre- and post-analytical duties. Since the inception of the MN NBS Ops unit, we have successfully completed several projects, such as a transition to an invoicing model, onboarding of a new laboratory information management system (LIMS), and several HIT initiatives focusing on interoperability. These projects will be highlighted and further described. Operations has given lab and follow-up staff the time and focus needed in order to accomplish multiple program goals.

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P-077

CYP21A2 Variant Panel as a 2nd Tier for CAH Newborn Screening Challenges for Nationwide Implementation Based on the Minnesota Experience

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Objective: To transfer a Centers for Disease Control and Prevention (CDC) developed CYP21A2 variant assay and implement and develop an operating paradigm for a 2nd tier molecular screen for CAH at the Minnesota Newborn Screening Program. Background: Despite decades of screening for CAH, state newborn screening programs, like Minnesota's, still struggle to achieve false positive and false negative rates commiserate with other screened disorders. Newborn screening for CAH currently involves the use of a fluoroimmunoassay to measure 17-hydroxyprogesterone (17-OHP). Unfortunately, the measurement of 17-OHP in the newborn period has several limitations that result in an increase in both false positive and false negative results. Numerous attempts have been made to reduce false positive and false negative results, including adjusting cut-offs for various characteristics, employing a low birth weight serial screening protocol, adding additional tiered testing, or collecting routine second specimens on all infants. While these methods do result in a reduction of false positive results, false negative cases still remain an issue. This project, in conjunction with the CDC and the University of Minnesota, set out to examine whether molecular testing could be incorporated into the CAH newborn screening process in order to reduce both false positive and false negative screening results.
Methods: Validation and modifications of the CAH assay for implementation in a high-throughput environment was completed. Additionally, the 2nd tier molecular assay was employed in a one-year retrospective analysis of 4092 specimens with elevated 17-OHP. Full Sanger sequencing was also performed on the 381 specimens that had one or more pathologic variants detected by the assay in order to confirm the findings and detect any other variants not included on the CYP21A2 variant panel.
Results and Conclusions: There were several lessons learned during the validation and retrospective analysis that spanned assay implementation, turn-around time, result reporting for complex molecular results, and follow-up. Although the molecular assay was able to detect the one true positive and three known false negatives included in the retrospective analysis, the number of infants with single or multiple mutations was higher than expected. As such, 3rd tier sequencing or repeat newborn screens might still be needed to ensure identification of all CAH cases. Outcomes of this project illustrate the potential utility of a CYP21A2 variant panel in improving screening metrics for CAH, particularly in improving detection rates. However, the complex nature of the CAH gene, combined with several other factors encountered by this project, warrant thoughtful discussion in order to devise the most appropriate screening approach for each state program.

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P-078*

Incorporating Race/Ethnic-specific Physiological Variation into Newborn Screening Algorithms: The Case of Total Galactose

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Background: The Minnesota Newborn Screening Program screens for galactosemia, galctokinase, and galactose epimerase deficiency through a screening algorithm that analyzes galactose-1-phosphate uridylyltransferase (GALT) enzyme activity in parallel with total galactose (TGAL). The theory behind this approach is that measurement of the enzyme in conjunction with the product can provide programs with two distinct pieces of information that can help point clinicians to a likely diagnosis. For example, extremely elevated total galactose in the presence of normal GALT activity might lead clinicians down a path of kinase or epimerase deficiency rather than classic galactosemia. The Hmong are a Laotian tribal group that emigrated to the US during the late 1970s. Today, Minnesota is home to one of the largest Hmong communities with over 66,000 Hmong individuals in the state. It was discovered that the Hmong population had a routinely-seen elevation of TGAL. This resulted in a disproportionate number of presumptive positive cases in this population; none of which have resulted in a confirmed case. A separate cut-off was implemented in March of 2009 to improve this disparity. Recently, Minnesota has become home to another refugee population from the same geographical area: the Karen people from Burma. The Karen population is quickly growing within the state with approximately 12,000 individuals. Similar TGAL findings have been observed in this population.
Methods: Infants were identified as Hmong based on their last names and vital records data. Previously, normal and abnormal distributions of both GALT and TGAL were run for 7,049 Hmong infants and 7,049 non-Hmong infants. Unsatisfactory specimens, those with more adult than fetal hemoglobin, and infants weighing less than 500gm were excluded. The 99th percentile between the two groups was assessed to determine the cut-off values for the Hmong population. Similar analyses are currently in process for the Karen population to determine if a separate cut-off for this population can be implemented.
Results and Conclusions: Work accomplished thus far illustrates an average offset of TGAL values between Hmong and non-Hmong infants of 5 mg/dL. Thus, Hmong-specific TGAL cutoffs have been implemented that allow for higher values than non-Hmong infants. GALT cutoffs remain the same between the two groups as no shift was seen for this analyte. This demographic influence has been built into the Lab Information System and false positive results in the Hmong population have since been virtually eliminated with no reports of missed cases. Preliminary analysis of the Karen infants suggests a similar, though less pronounced, shift in TGAL. Our findings illustrate the potential need to account for race and ethnicity in screening algorithms and warrants further research into the cause of the physiologically higher TGAL in populations from the southeast Asian region.

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P-079

QI Project Update: Improving Communication of Normal Newborn Blood Spot Results

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The purpose of newborn screening is to triage at-risk infants into the medical system to aid in early detection and treatment of affected children. Thus, communication of abnormal results is often the primary focus of newborn screening programs and has evolved into a relatively consistent and streamlined process. In addition, public health programs meet regulatory compliance with accurate and timely reporting to specimen submitters but have varying approaches as to how normal newborn screening results are reported to front-line medical providers, which often results in a more haphazard process. A Quality Improvement (QI) project presented previously is now near completion. The goal of this project was to understand and intervene where improvements were needed for the efficient and effective communication of normal results to physicians and families. Based on the information gathered during the initial stages of the QI project, a normal blood spot results fact sheet was created to be utilized by providers to aid in communication of normal results.

The following components of this QI project will be discussed:
• Assessment of how normal newborn screening results are handled at several hospitals and clinics
• Proposed improvement of communication processes based on the information collected and the capacity of a newborn screening program
• Development of a normal blood spot results fact sheet to aid communication of negative results
• Comparison of pre- and post-intervention parental surveys used to determine if families received and understood their normal screening results before and after implementation of the fact sheet
• How the results from this QI project were incorporated into a regional American Academy of Pediatrics Maintenance of Certification (MOC) course
• Identification and recommendation for newborn screening programs to adopt continuous quality improvement measures regarding effective reporting and communication

The ultimate aim of this project was to increase the likelihood that all newborn screen results are successfully communicated to both providers and families, including normal ones. The parental surveys indicate that 68.4% (n=98) of parents at clinics utilizing the fact sheet received and understood their normal results as compared to 44.4% (n=144) at these same two clinics prior to the intervention. The hope is that this QI project and subsequent MOC course will lead to providers and parents with a better understanding of screening results and their limitations.

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P-080

Primary Care Physicians' Perspectives on Positive Newborn Screens for Cystic Fibrosis: A Statewide Survey

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Background: Newborn screening (NBS) for cystic fibrosis (CF) incorporates DNA analysis in the screening methodology. Results for screen-positive infants can be challenging to interpret, especially in states that use advanced next-generation sequencing technology. This survey aimed to provide the first systemic evaluation of primary care physicians' information needs when receiving positive newborn screens for CF in a state utilizing advanced technology.
Methods: A web-survey was distributed to the Wisconsin pediatrician population through the Wisconsin Chapter of the American Academy of Pediatrics. Major question domains addressed preferences for information and communication practices, familiarity with the methodology for screening newborns for CF, and knowledge of risk stratification in screening.
Results: Fifty-four physicians completed the survey (6% response rate). Major findings of this study indicate physicians with a higher familiarity with the methodology for screening for CF had a higher level of comfort communicating results to parents (p-value < 0.001). Respondents indicated the value of the immunoreactive trypsinogen (IRT) measured in the first-tier screen for CF was important to include on the NBS report, as well as the normal range of IRT (N=52, 98%). In addition, respondents indicated statistical information on the likelihood a newborn is affected would be helpful (N=53, 98%). There were no major differences in preferences for information based on the respondent's experience with NBS or years in practice.
Conclusion: The findings of this study indicate the following information included in newborn screen reports is useful: 1) the next steps in the diagnostic process 2) methodology for screening for CF, and 3) information on the genetic basis of the condition. It may be helpful if NBS laboratories also included specific values for IRT and DNA analysis as well as evidence-based estimates of residual risk on reports.

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P-081

Quantification of 11 Enzyme Activities of Lysosomal Storage Disorders using LC-MS

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Introduction: Lysosomal storage disorders (LSDs) are characterized by the accumulation of lipids, glycolipids, oligosaccharides, mucopolysaccharides, and other biological substances because of the pathogenic deficiency of lysosomal enzymes. Such diseases are rare; thus, a multiplex assay for these disorders is effective for the identification of affected individuals during the presymptomatic period. Previous studies have demonstrated that such assays can be performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with multiple reaction monitoring (MRM) detection. Thus, an assay procedure to quantify the activity of 11 enzymes associated with LSDs was examined.
Method: First a validation study was performed using dried blood spot (DBS) samples with 100% and 5% enzyme activity for quality control (QC). Then, enzyme activities in a Japanese population were quantified using LC-MS.

Results: Consistent with the previous data, the enzyme activity exhibited a bell-shaped distribution with a single peak. The averaged enzyme activity for the healthy neonates was as follows: GLA, 3.80 ± 1.6; GAA, 10.6 ± 4.8; IDUA, 6.4 ± 2.3; ABG, 8.6 ± 3.1; ASM, 3.3 ± 1.1; GALC, 2.8 ± 1.3; ID2S, 16.7 ± 6.1; GALN, 1.2 ± 0.5; ARSB, 17.0 ± 8.7; NAGLU, 4.6 ± 1.5; and GUSB, 46.6 ± 19.0 □mol/h/L (mean ± SD, n = 200). In contrast to healthy neonates, the enzyme activity in disease-affected individuals was lower than the minimum enzyme activity in healthy neonates. The analytical range, defined as the ratio of the peak area of the enzyme reaction products from the DBS for QC with 100% enzyme activity to that from the filter paper blank sample, was between 14 for GALN and 4561 for GLA under the assay condition. **Conclusion:** The results demonstrate that the population of disease-affected individuals was distinguished from that of healthy individuals by the use of LC-MS/MS.

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P-082

North Carolina Cystic Fibrosis Newborn Screening Genotype Spectrum

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Enhanced awareness of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) sequence variations in different demographic backgrounds and their association with Cystic Fibrosis (CF) phenotypes is critical for improving screening programs. Due to the low positive predictive value of the first-tier biochemical Immunoreactive Trypsinogen (IRT) screen for CF in newborns, most screening programs have implemented a second-tier DNA screen for CFTR variants. In 2009, North Carolina began utilizing a targeted variant analysis approach (Hologic InPlex assay) to identify CFTR variants. In 2016, the Hologic assay was recalled and the North Carolina State Laboratory of Public Health (SLPH) Newborn Screening (NBS) Unit began sending specimens to the Wisconsin State Laboratory of Hygiene (WSLH) Newborn Screening Unit for CFTR variant analysis using the Illumina MiSeqDxTM CF 139-variant assay. Genotypic data analysis of retrospective specimens screened from 2010-2018 demonstrated that race and clinical assessments (e.g. carriers, CF, and CFTR-related metabolic syndrome) were associated with presence or absence of a variant. This presentation will discuss the CFTR variants identified in the newborns screened in North Carolina.

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P-083

Improving Monitoring for Newborn Screening Quality Assurance in Nebraska

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The timeliness of newborn bloodspot screening can impact the health status of babies in the first few days of their lives. In 2017-2018 the Nebraska Newborn Screening Program (NNSP) implemented changes in how its newborn screening program manages delayed specimens. NNSP has monitored specimen delays between the time of collection and receipt at the PerkinElmer Genetics newborn

screening lab in Pittsburgh, PA. Nebraska's screens are shipped overnight by UPS or FedEx to the screening lab six days of the week. A screen is considered delayed if the specimen is not received at the lab within 3 days from the time of collection. In early 2018 about 10% of all screens were considered delayed. (All delays cannot be eliminated as some are caused by weather and others are caused by holidays when no shipping is available.) The program notifies all birthing facilities of delays and requests them to respond to and work with the NNSP to investigate and prevent future delays. NNSP staff workload impeded implementation of routine weekly monitoring until a third follow-up specialist was hired in 4/2017. The NNSP began reviewing delayed specimens routinely as of 8/2017. The goal was to decrease the number of delayed specimens. The 2017-2018 method changes sought to increase efficiency in tracking specimen delays by differentiating between delays in shipping and delays that occurred prior to shipping from the birthing facility. This enabled us to notify hospitals of only those delays which occurred prior to shipping. Then the birthing facilities would only need to investigate the reasons for delays that were in their control. After six months of routine monitoring, the NNSP established a baseline for the expected number of unidentified delays. Facilities and screening staff worked to decrease delays by reeducating staff on facility procedures, by improving the processes from collection to shipping, by working with the screening lab and UPS to create UPS pick up points, and by reviewing and adapting procedures to avoid missed specimens. Between late 2017 and mid 2018 the number of delayed specimens from birthing facilities decreased from 38 to 6 per quarter. This corresponds to a 25% decrease in birthing facility delayed specimens. This decrease reduced the total number of delays in Nebraska by almost 2%. As a result of the program changes, the NNSP is now able to efficiently determine when and where delays occur from the collection time to receipt at the lab. The NNSP then follows-up to assist facilities with changing their procedures and addressing any gaps as necessary. Since the change the total number of incidents has decreased for facilities to review. The increased ability of NNSP staff to report screening results in a shorter period of time allows more timely interventions for Nebraska's newborns who screen positive for one of the 32 conditions on Nebraska's newborn screening panel.

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P-084*

The BabySeq Project: Genomic Findings and Preliminary Survey and Economic Results

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Objectives: The BabySeq Project is a randomized clinical trial assessing the medical, behavioral and economic outcomes of providing genomic sequencing (GS) information from whole exome sequence analysis of a DNA sample collected after birth to a baby's parents and clinicians. With enrollment completed, we summarize our final genomic findings and preliminary follow-up data.
Methods: Well and sick infants are randomized to standard care (= that informed by newborn screen (NBS) results and family history (FH)) or standard care plus GS. GS analysis focused on genes associated either with pediatric onset disorders or with actionable adult onset disorders that ACMG recommends reporting with GS. Pathogenic or likely pathogenic variants were reported if penetrance was moderate or high. Result disclosure was performed in-person by a genetic counselor and study physician.

Results: Disclosed results included monogenic disease risk, carrier status for recessive traits, and pharmacogenomic (PGx) risk. Of 5,002 approached families, 10% agreed to hear about the study in detail and 2/3 of those families (n=325) enrolled. Of the 159 newborns sequenced, 32 were enrolled from ICUs and 127 from well baby nurseries. For carrier status, 310 variants were reported, (range: 0 – 7 variants/subject, average: 2 variants/subject). The 5 genes in which alleles with pathogenic variants were most commonly identified were BTD (15), RBM8A (11), GBJ2 (10), CFTR (6) and MUTYH (6). The most common pathogenic variant reported was p.Asp446His in BTD. No variants were reported in 12% of subjects. Monogenic disease risk was reported in 18 subjects involving KCNQ4, GLMN, ANKRD11, BTD, CYP21A2, TTN (n=4), VCL, MYBPC3, CD46, SLC7A9, G6PD, ELN, BRCA2 (n=2) and MSH2 (disorders associated with variants in bolded genes may be detected by newborn DBS or hearing screening). PGx risk variants in TPMT and DPYD were reported in 4% of sequenced subjects. Follow-up surveys collected three months post-disclosure, when compared to control subjects that did not undergo GS, revealed no increase in maternal perception of child vulnerability, parent-child bond disruption, parental conflict, depression or anxiety, even for those who received monogenic risk results, and preliminary economic analyses suggest a trend towards minimally higher health care expenditures ($200 – 400/family) in GS families.
Conclusions: By our protocol, GS identified genetic variants for monogenic disease risk in 11% of sequenced subjects. On average, carrier status for 2 disorders per subject was reported. While our cohort size is small, GS of newborn infants revealed a surprising array of disorders for which increased risk and carrier status was identified. We have not detected significant negative impacts on either family dynamics or health care costs within the first months after result disclosure. Ongoing follow-up of the cohort continues.

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P-085

Algorithms, Assessments, Anxieties and Adaptations: Massachusetts Experience with SMA Screening
A. Comeau, New England Newborn Screening Program/University of Massachusetts Medical School, Worcester, MA

Background and Objective: We began offering statewide pilot screening for Spinal Muscular Atrophy in January 2018. As the provider of Massachusetts newborn screening services, we provide SMA screening on specimens from infants whose parents have consented (80 percent infants) and screen all specimens for SCID (mandatory test, 100 percent infants). Likewise, as a regional laboratory, we test only for the conditions authorized by each of our client states and SMA has not yet been adopted by states other than Massachusetts; all authorize screening for SCID. We perform one SCID assay for all. Unlike others who have implemented screening for SMA, we are using independent assays for SMA and for SCID until SMA screening is universally required. This presents challenges and opportunities. Strategy, Results and More Strategy. Our chosen assay is a RealTime™ assay to identify "Homozygous Absence of SMN1 Exon 7"; carriers are not identified. For assay development, we worked closely with the CDC, testing a variety of CDC primers and probes within our modified thermocycling conditions (Lan Ji et al submission). We tested sets of de-identified specimens and coded samples donated by SMA families. From the clinical validity data, we developed a two-tiered strategy with low frequency (<0.2%) reflex testing that could be done in the same day.

Intermittently and at intervals of approximately 10,000, we re-evaluated our assays and test algorithms for clinical validity in a high-throughput environment. Retrospective data supporting continuing clinical validity were available, will be presented, but did not assuage our concern for having potentially missed an SMA case. We developed another assay that could be done 'in real time' that would support tiers one and two. Our submission by Dr. Kumar et al describes details of a Sanger sequencing assay for a short amplicon to confirm the presence or absence of SMN1 exon 7.
As of this writing, more than 36,500 Massachusetts infants have been tested and no infants with SMA have been identified. Sequencing data have confirmed the presence of SMN1 exon 7 in all specimens with results that prompted tier two and provide additional insight about copy numbers of SMN1 and 2.
Conclusions: Until SMA screening is universally required in all states for which we provide screening services, we will perform independent assays for SCID and for SMA. In the meantime, data we generate will be useful for methods comparison and harmonization with CDC PT panels and states that are performing multiplex SCID/SMA assays. Additional uses might be made of sequencing capacities for SMA.

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P-086

Withdrawn

P-087

AVA – Automated Variant Annotation Framework

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Background and Objective: In 2018, the New England Newborn Screening Program (NENSP) of the University of Massachusetts Medical School began providing gene sequencing to complete the screening algorithms for three specific conditions. In accordance with guidelines from the American College of Medical Genetics (ACMG), all detected sequence variants are classified and reported as one of the following: pathogenic, likely pathogenic, uncertain significance, likely benign, or benign. For each identified variant, the following types of resources are routinely consulted prior to determination of final classification: disease-specific databases, large clinical-outcome databases, large high-throughput sequencing databases, online functional evaluations, and peer-reviewed publications. Retrieval of specified data from each resource is time-consuming and subject to human error. In our experience we know that one cannot rely upon the final classifications reported by these resources, because conflicting interpretations are common. We set out to streamline the data collection and documentation process with automation.
Strategy and Methodology: Our strategy has been to automate as much of the current NENSP manual process as possible, with an eye to future needs as NGS is implemented. Our first focus was conversion of variant nomenclature reported by the instrument (Applied Biosystems Variant Reporter® Software) into an HGVS standardized nomenclature. Our second focus was on data collection and its documentation, using the NENSP - developed SOP for gathering required data in order to manually classify a variant.

We developed a tool, AVA - Automated Variant Annotation framework, which gleans information from online resources, documents the data and collection in a local knowledge base, complete with curator comments, and presents summary data in a user-friendly web interface. AVA is a one-step, locally installable package, thus preventing the need to upload the sensitive data to third-party servers.
Results: We will present AVA and its validation. AVA provides end-to-end support for variant annotation with minimal human intervention. AVA 1) takes input in either VCF, Annovar or CSV format, 2) generates annotation, 3) captures curator comments for each variant, 4) persists curator confirmed-variants into a Knowledge Base, and 5) allows exploration of variant history. AVA can be used as a standalone package or can be integrated into existing workflows of newborn screening centers across the nation.
Conclusions: Reliable retrieval and documentation of specified data is essential in order to maintain the spirit of the ACMG guidelines. Optimizing and automating the variant data gathering process saves time, reduces errors and expedites the classification of variants for clinical reporting. The AVA knowledge base should facilitate interfaces with national initiatives.

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P-088

Variant Interpretation at the Program Level: A Can-do Review and Some Updates

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Background: Newborn screening algorithms that include a sequencing component are becoming more commonplace, whether the sequencing is performed in house or contracted out. In both cases, a solid understanding of what lies behind the interpretation of any one DNA variant is a knowledge that is key to the provision of high quality screening services. Such knowledge is not only necessary for accuracy in quality reporting, but for our long-term evaluations of the genotype phenotype relationships associated with the rare diseases for which we screen. In preparation for our January 2018 implementation of sequencing services, we developed protocols and instruments that could be used at the program level to streamline and to ensure compliance with ACMG guidelines in variant interpretation. We have presented these at roundtables and webinars and have shared our instruments with public health newborn screening programs through the APHL. Since our implementation of sequencing services, we have successfully maximized the utilities of our program's variant database and have been developing automated processes for the program level that should be compatible with national newborn screening initiatives.
Conclusion: Program-level instruments for variant interpretation are helpful for routine services and additionally for follow up staff to develop a knowledge base. Instruments developed at the program can be automated, significantly increasing workflow and facilitating interactions with national initiatives.

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P-089

Evaluation, Optimization and Validation of Real-Time™ Assays for High-Throughput Newborn Screening for Spinal Muscular Atrophy

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Background and Objective: Recent advances in the treatment of SMA and demonstration of successful population-based SMA screening in Taiwan promoted interest in the development of reliable assays for use in US newborn screening programs. The consensus assay of choice for such is a Real-Time™ assay to detect the homozygous absence of SMN1 Exon 7, which should identify 95% of all SMA patients without identifying carriers. At the time we were preparing for implementation of Massachusetts-wide pilot SMA newborn screening (which requires independent assays for SMA and for SCID due to policy), Lee and Mercer at the CDC were developing new versions of their SMA/SCID multiplex assay that would enhance assay specificity. We modified the multiplex CDC assay and joined forces with colleagues at CDC to evaluate, optimize, and validate a high-throughput assay.
Strategy and Methodology: The major challenge in the use of a Real-Time™ assay to identify homozygous absence of SMN1 Exon 7 is the existence of the SMN1 paralog gene, SMN2, which differs from SMN1 by only five distinct nucleotides. Lee and Mercer were in the process of improving on the use of Locked Nucleic Acids for primers and probes to increase assay specificity. We present data from our evaluation of a series of such primers and probes. Our optimization consisted of titrating the concentration of primers/probe for each target (matrix to be presented) and tests of thermocycler profiles (matrix to be presented). The approach was first validated with 68 coded patient- or obligate SMA carrier- (parents of SMA patients) specimens kindly provided by Biogen from a repository of consented samples. We implemented a multiplex two-tiered testing algorithm that targets SMN1 Exon 7 and RNaseP in Tier One and SMN1 Exon7, SMN1 Intron 7 and RNaseP in Tier Two.
Results: Matrix evaluation data will be presented; these yielded two sets of conditions with similar performance in clinical validation. We also present data from the application of these conditions to sets of approximately 3,500 de-identified NBS samples that had been collected prior to March of 2015, and our projections for use of different primer sets in a two-tiered algorithm. Finally, for quality assurance, patient and control data that have been generated since implementation will be reviewed.
Conclusions: The data we generated will be useful for methods comparison and harmonization with CDC PT panels and states that are performing multiplex SCID/SMA assays.

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P-090

Development of Streamlined Workflow of DNA Sequencing for Newborn Screening for Lysosomal Storage Disorders and X-linked-Adrenoleukodystrophy

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Background: Newborn screening programs screen for rare genetic disorders to ensure that developmental needs are met for those affected children. DNA sequencing for the screening of lysosomal storage disorders (LSD) including MPS I and Pompe disease, and X linked-

adrenoleukodystrophy (X-ALD) has been successfully implemented in other state newborn screening programs as a second tier test. In January 2018, these disorders were added to the Massachusetts statewide newborn screening pilot. DNA sequencing protocols for these three diseases were designed to cover all exon and exon-intron boundaries to discover the variants related to the disease, and help establish genotype-phenotype relationship. These assays were optimized and validated for clinical use.
Material and Methods: The major challenge in the assay development was to design the primer sets for amplicons to cover all the exons, exon-intron splice junctions, and deep intronic regions of the genes. We took the advantage of BigDye Direct Sequence Kit technology which allows sequencing up to 800bp. The assay development also consisted of optimization of the instrument's variant analysis software where multiple layers of the reference genes (IDUA, GAA, ABCD1) were generated for mapping as well as nomenclature of the variant. The approach was validated with three independent coded specimens for each gene along with control samples (either obtained from the New York newborn screening program or Coriell).
Results: Bidirectional sequencing was performed for each assay. Initial validations passed for all three genes. The robust algorithms of the variant analysis software in combination with well-defined layers of interest, precisely called all the SNPs, variants, insertions, deletions, and heterozygous insertions/deletions for the data generated for these three genes. For quality assurance, DNA with a known variant was run with each assay.
Conclusion: We have developed a streamlined workflow for DNA sequencing from assay development to analysis and review of the variant, and believe our data will be useful for methods comparison with other newborn screening programs.

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P-091

Withdrawn

P-092*

Hitting the "Ct"-Spot: A Three-Year Retrospective Analysis of SCID Screening using Ct Values in New Jersey

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The New Jersey Newborn Screening (NJ NBS) Laboratory screens for severe combined immunodeficiency (SCID) through the detection of T-cell Receptor Excision Circles (TRECs) in newborn dried blood spots using the on-card quantitative PCR (qPCR) method developed by the Centers for Disease Control and Prevention (CDC). The qPCR instrument reports the number of cycles it takes for the amplification curve to meet the pre-set fluorescence threshold value (Ct value). NJ NBS was one of the first NBS program to use Ct values for its reference range and reporting algorithm, and not transform these values into TREC copy number using calibrators. To do this, a total of 13,331 initial specimens were screened over a 7-week period and population statistics were used to set the cutoffs. These cutoffs were re-evaluated and adjusted once screening began. The primary objective of this retrospective study was to assess the

diagnoses and clinical outcomes of the infants identified through screening with this method. The secondary objectives were to identify the incidence of SCID in New Jersey, characterize the genetic breakdown of SCID, assess other etiologies of T-cell lymphopenia identified through NJ NBS, and compare these results to those reported from other states. During the first three years of screening (June 30, 2014 – June 30, 2017), around 70 newborns each year were referred for clinical follow up with an immunologist, transplant physician, or infectious disease specialist. Of those, 6 newborns were diagnosed with classic SCID, 3 with a variant form of SCID, and 27 with other T-cell deficiencies. The genetic variants reported to the NJ NBS Follow Up Program were found in the ADA, IL2RG, NHEJ1, CD3D, RAG1 and moesin genes. The other T-cell deficiencies identified included secondary T-cell lymphopenia, idiopathic T-cell lymphopenia, neutropenia, and 5 other syndromes with low T-cells: ataxia telangiectasia, DiGeorge, CHARGE, Micro del 14Q13.3, and Nijmegen Breakage Syndrome. The rate of detection of classic SCID in NJ, approximately 1 in 50,000, is comparable to those seen in other state NBS programs. Results of population screening statistics and case outcomes demonstrate that the Ct value cutoff used in NJ is set at a point where false positive screens are minimized while still allowing for the detection of a variety of other T-cell deficiencies.

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P-093

A Two-Step Approach to Improving Newborn Screening for Cystic Fibrosis in New Jersey

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The New Jersey Newborn Screening (NJ NBS) Laboratory screens for cystic fibrosis (CF) using a two-tiered algorithm. All newborns are screened for immunoreactive trypsinogen (IRT) levels, and any specimen with elevated IRT is reflexed to a second-tier molecular assay. The IRT cutoff was fixed at 90 ng/mL, and the second-tier molecular assay is an endpoint qPCR assay that detects only the most common CFTR mutation, F508del (c.1521_1523delCTT). Feedback from pulmonologists in New Jersey indicated that newborns with CF were being missed by NJ NBS and their diagnoses delayed until clinical presentation. The algorithm lacked sensitivity due to both its high cutoff and minimal mutation screening. In response to these concerns, NJ NBS has taken a two-step approach to improve the sensitivity and specificity of its CF screening algorithm. The first step of this project was to lower the IRT cutoff from 90 ng/mL to 70 ng/mL and change the reporting language for infants with elevated IRT without the F508del mutation to include a recommendation for clinical evaluation by sweat testing. The second step is the implementation of multi-mutation second-tier analysis using the Illumina MiSeqDx Cystic Fibrosis 139 Variant Assay. After each change, data on the number of infants identified and diagnosed with CF through NJ NBS and the time to diagnosis is being collected. Retrospective and prospective data analysis of the CFTR mutations found in the New Jersey newborn population is also being performed. Data collected after 5.5 months of the lower IRT cutoff showed that the number of specimens reflexed to second-tier molecular testing tripled due to the cutoff change. Of the 389 specimens tested for F508del, 11 were heterozygous. 7 of those were only detected due to the new cutoff. No homozygous specimens were detected during this time. Implementation of the 139 Variant Assay is in progress and data on its impact on the NJ CF algorithm will be presented. The two-step approach is effective and beneficial to NJ newborns and allowed NJ NBS to make improvements without overwhelming the Laboratory or Follow Up staff.

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P-094

Results of Blinded Pilot Study for Lysosomal Storage Disorder Screening in New Jersey

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Objective: Newborn screening for six Lysosomal Storage Disorders (Krabbe, Pompe, Gaucher, Fabry, Niemann-Pick and Mucopolysaccharidosis I) is required by legislative mandate in the state of New Jersey. Validation of a six-plex FIA-MS/MS method to measure enzyme activity associated with these disorders using customized reagents from Perkin Elmer is complete and was previously presented. Prior to going live with screening, a blinded pilot study was performed to establish workflow procedures, define the normal distribution of enzyme activities in our patient population and determine screening cutoffs.

Methodology: Over a six-week time period, 8,223 de-identified dried blood spots (DBS) were screened using the validated six-plex assay. Preliminary screening cutoffs for each enzyme were defined as a % of the daily patient median, using data from other NBS programs as a guide. To validate these cutoffs, specimens from diagnosed patients, known carriers and individuals with identified pseudo-deficiency alleles were assayed. Additionally, molecular analysis was performed on specimens with low activity for three of the disorders, Krabbe, Pompe and MPS1. The genes were analyzed using Sanger sequencing and results interpreted according guidelines published by the American College of Medical Genetics and Genomics.

Results: The range of enzyme activities measured using the validated six-plex assay displayed a normal, Gaussian distribution for our population of infants. Using this information in combination with data from other states, we defined preliminary cutoffs for each disorder. For each condition, a percent of the daily median was used. The following are the specific cutoffs used in the pilot study: Gaucher [glucocerebrosidase (ABG)] 20%; Niemann-Pick A/B [acid-sphingomyelinase (ASM)] 20%; Pompe [acid-α-glucosidae (GAA)] 28%; Krabbe, [β-galactocerebrosidase (GALC)] 15%; Fabrey [α-galactosidase A (GLA)] 26% and MPS-1[α-L-iduronidase (IDUA)] 20%. These cutoffs were refined based on information acquired from analyzing specimens from diagnosed patients, known carriers and individuals with identified pseudo-deficiency alleles. Additionally, for four of the disorders (Gaucher, Pompe, Krabbe and MPS-1), we established a repeat cutoff to further refine our screening algorithm. The revised cutoffs are Gaucher (ABG) 15%/12%; Niemann-Pick A/B (ASM) 15%, Pompe (GAA) 15%/10%, Krabbe (GALC) 15%/11%, Fabrey (GLA) 15% and MPS-1(IDUA) 12%/8%.

Conclusions: New Jersey successfully completed a blinded pilot study to prepare for full implementation of screening for six LSDs. Based on results of this pilot, a workflow procedure was established, the normal range of enzyme activity for our population was delineated, and screening cutoffs were defined. Finally, molecular analysis of enzymes (GALC, GAA, and IDUA) from DBS with low enzyme activity supported these refined cutoffs.

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P-095*

Use of LEAN Principles to Address Data Entry Delays and their Impact on Achieving Timely Reporting of Results

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Objective: To improve timeliness in the data entry task, which delayed timely evaluation and reporting of results
Methods: A LEAN consultant trained Newborn Screening Program staff on LEAN principles and staff participated in a Kaizen event. Data was collected and improvements were implemented throughout the Program. Data entry delays were determined to be a major contributing factor to delays in reporting results. Subsequent data entry workflow changes included a three-step data entry process, an all-hands on deck (AHOD) approach to data entry, electronic mailer review and an Excel-based generation of reports for important missing demographic information.
Results: From January 1, 2018 to March 31, 2018, data entry was behind 44 out of 62 days. With the combination of three-step data entry process, AHOD data entry, revised data entry procedures for collection of missing information and electronic mailer review, data entry was behind 2 days from August 1-August 31, 2018. Electronic mailer review and the automation of reports to collect missing information was extrapolated to be equal to 1.0 FTE in the data entry unit.
Conclusions: Delays in data entry can impact the timely reporting of results, but LEAN principles can be applied to improve timeliness even in light of the inability to maintain full staffing levels.

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P-096

Developing a New Process for Handling Special Health Concerns

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The New York State Newborn Screening Program (NSP) frequently receives expedited result disclosure requests from providers for babies at-risk for conditions on the newborn screening panel. Our previous process for handling these was paper-based, requiring significant staff time, and no limitations as to what could be requested. We tracked these 'special health concern' (SHC) requests for approximately 6 months in 2018, analyzed the reasons for the requests, and created a protocol for handling future requests using a shared Excel file and email notifications to appropriate staff. During the tracking period, we received 78 requests from providers, an average of 3.25 per week. These impacted all laboratory sections, but the majority (N = 42) were for the MS/MS Laboratory. The most common reasons for the requests were: clinical concern/symptoms (N = 39), one or both parents were a carrier of a condition (N = 20), or there was a family history of a condition (N = 18). The most common clinical concerns/symptoms were high bilirubin (N = 9), unclear or vague concern (N = 6), hypoglycemia (N = 4), lactic acidosis (N = 4), meconium ileus (N = 4), and ambiguous genitalia (N = 3). Sixteen (20.5%) of the SHCs (N=78) had positive results: of these, ten were clinical concerns/symptoms, two had parent(s) who were known carriers, two had family histories, and two were cases in which the baby was diagnosed prenatally with a condition. This process has been established and a Standard Operating Procedure

(SOP) is being developed which will include approved reasons for a SHC (i.e. baby with signs/symptoms of a condition, one or both parents being carriers, family history, and prenatal diagnosis). We no longer accept SHC requests for "everything." Instead the requestor is asked to be as specific as possible. We provide this important service to our state's clinicians, and our revised process minimizes staff time and facilitates expedited tracking of these requests.

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P-097

The Impact of Seasonal Changes on Thyroxine and Thyroid Stimulating Hormone in Newborns in NYS

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Introduction: Newborn screening for congenital hypothyroidism (CH) in the developed world has led to the near elimination of intellectual disability caused by this common endocrine disorder. Screening strategies differ but the main approaches involve 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 effect of seasonal changes on measured T4 and TSH concentrations and on referral and false positive rates.

Methods: From 2008 to 2017, the NYS NBSP screened approximately 2.4 million babies for CH. T4 concentration in dried blood spots was measured using the AutoDELFIA Neonatal thyroxine kit (Perkin Elmer, Turku, Finland). Approximately 344,000 infants were then screened for TSH using the AutoDELFIA Neonatal hTSH kit (Perkin Elmer). Infants with abnormal results were referred for diagnostic testing or if a borderline result was obtained, a repeat specimen was requested. The daily mean values for T4 and TSH were recorded.

Results: NYS has a humid continental climate with a large seasonal temperature difference. The summer months are humid and hot and winter months are cold, sometimes severely so. Seasonal temperature changes did not appear to affect daily mean TSH values. Overall the trendline indicated a slight increase in daily mean TSH values over the 10-year period. A significant seasonal variation is observed in daily mean T4 values. During colder months the daily mean T4 values were higher (average of 18.08 µg/dL in 2014) and during warmer months the daily mean T4 values were lower (average of 16.43 µg/dL in 2014). This pattern was clear from 2008-14 and in 2016 but was not observed in 2015 or 2017. Plots of daily mean T4 values and the daily mean temperature in NYC were constructed for each year from 2008-17. The trend of the plots for each year looked similar with mean T4 values decreasing with increasing temperature. The positive predictive value (PPV) for CH testing was calculated for January-March and July-September of each year from 2008-17 and found to be lower (14.5%) in winter than summer (19.4%).

Conclusion: In NYS, seasonal temperature variations affect measured concentrations of T4 but not TSH. Daily mean T4 is lower in the summer and higher in the winter. The observed cyclical pattern observed due to seasonal variation was disrupted in 2015 and 2017. This could be due to two T4 manufacturer kit lot changes during these 2 years leading to higher values of daily mean T4, although the manufacturer

did not report any significant changes in the kits. Using our algorithm, the elevated T4 values in the cold months led to higher referral and false positive numbers and consequently lower PPV for CH testing.

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P-098

Moving Data Using Flash Drives? Beware of Malware

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Objective: To describe an event of malware impacting instrument computers due to transmission by flash drives

Background: In January 2018, an automated scan detected network traffic and signature indicative of WannaCry(pt) worm at 4:15 AM. The PC with the virus was immediately removed from the network. The PC was also physically disconnected. By 9:30 AM, additional infections were detected and all instruments program-wide were physically disconnected. The source was determined to be an imager, which was not on the network. It was determined the infection was spread to Autodelfia computers via use of USB flash drives, which are routinely used to move data from instruments. Vendors also use flash drives to install updates and trouble shoot instrument. In total, over 100 flash drives were collected from staff and scanned for the worm. In total, 18 flash drives were infected.

Response: All instrument PCs were configured to receive patches and anti-virus updates. All PCs were networked to avoid the need for flash drives. PCs that could not receive anti-virus updates were quarantined and placed on a special network. New policies were implemented requiring service technicians to provide any USB drives for scanning prior to use on an instrument PC. The use of flash drives was also prohibited by staff to transfer data.

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P-099

Ontario's Evaluative Framework for a Phased Approach to Cytomegalovirus Newborn Screening

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The Infant Hearing Program (IHP) in collaboration with Newborn Screening Ontario (NSO) developed the Expanded Hearing Screen to broaden the detection of infants who have or are at risk for congenital and non-congenital permanent hearing loss (PHL). The Expanded Hearing Screen involves, with parental consent, audiometric testing and screening for hearing loss risk factors from the dried blood sample collected for routine newborn screening. Hearing loss risk factors include cytomegalovirus (CMV) and mutations in genes associated with PHL. Audiometric testing alone remains available.

The introduction of expanded hearing screening is anticipated to identify an increased number of children who could benefit from early intervention and decrease the number requiring surveillance for the risk of early childhood PHL. Despite these potential benefits, universal screening for CMV remains controversial. Namely, the majority of infants with CMV detected at birth are asymptomatic and identification of this cohort could lead to unnecessary medicalization. Acknowledging the debate about universal CMV screening, it was recommended by the province's advisory group on newborn screening that the initiative undergo thorough evaluation, allowing for decisions regarding its continuance or modification.

Expanded hearing screening is being implemented through a two phase approach in Ontario. Phase I involves targeted CMV screening. Infants are eligible if there has been a refer result on audiometric testing. Phase II involves universal screening for CMV and genetic mutations in infants regardless of the results of audiometric testing.

To determine whether expanded hearing screening enhances the existing screening paradigm, three areas for evaluation have been identified: 1) educational and consent content and approach, 2) the family experience related to monitoring screen positive, asymptomatic children, and 3) short and long-term health and system-relevant outcomes. In Phase I, we are surveying parents who consented to and those who declined expanded hearing screening to assess awareness and understanding. Semi-structured interviews with caregivers of screen positive infants are also planned to gather preliminary insight about the diagnostic journey and initial surveillance experience. Finally, process and short-term outcome metrics related to Phase I will be presented.

With Ontario poised to be the first jurisdiction in the world to offer universal CMV screening, Phase I represents an opportunity to optimize consent strategies and educational materials, and ensure health services are equipped to manage the larger number of infants requiring follow-up care in Phase II. Phase I findings will also guide programmatic decisions on how to maximize the benefit and minimize the risks associated with this innovative screening paradigm.

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P-100

Ontario's Clinical Care Implementation for Targeted Cytomegalovirus Newborn Screening

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A collaboration between Ontario's Infant Hearing Program (IHP) and Newborn Screening Ontario (NSO) was established to offer Expanded Hearing Screen. The Expanded Hearing Screen includes both the audiometric hearing screen and the hearing loss risk factor blood spot screen. The blood spot screen is performed by NSO and uses the same dried blood spot taken for the newborn blood spot screening program.

Expanded Hearing Screening in phase 1 involves targeted cytomegalovirus (CMV) screening which will primarily be used to identify a potential etiology of hearing loss. The hearing loss risk factor blood spot screen will be performed with parental/guardian consent when there has been an IHP refer result on the newborn hearing screen. Parental/legal guardians consent is required and can be restricted to the audiometric hearing screen alone or to the expanded hearing screen. Phase 2, which will launch approximately a year after phase 1, will include CMV screening and genetic testing for mutations associated with congenital permanent hearing loss, and will be universally available to all infants with consent, regardless of the hearing screen outcome. Phase 2 will be used to identify infants who are at risk for hearing loss at birth or later in childhood.

A clinical committee began meeting in January 2018 to delineate appropriate clinical care pathways and program evaluation prior to initiation of phase 1. The phased approach to the expanded hearing screen and the involvement of many of the specialists involved in the care of these infants will allow for assessment of areas that need improvement prior to and during the implementation of phase 2. The clinical committee consisted of NSO staff (project coordinator, clinical content specialists, and medical director), IHP audiologists and project coordinator, infectious disease specialists, otolaryngologists, and an IHP training specialist. For phase 1 the primary objectives of the committee were to: 1) establish a referral pathway, 2) develop clinical care guidelines for CMV screen positive infants, 3) create a report form for the collection of clinical data on screen positive infants, 4) produce educational materials for families and health care providers, and 5) develop communication strategies for health care providers in the province who would be involved in the care of CMV screen positive cases.

All of these objectives were accomplished prior to the first screen positive case of phase 1. The clinical committee has discussed successes and areas that need improvement as each screen positive case has moved through the clinical care pathway. As in any new initiative, there are adjustments that have had to be made as phase 1 has progressed and ideas that have been brought forth which will need to be considered for phase 2. The work of the clinical committee is ongoing.

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P-101

Collection and Quality Assurance of Critical Congenital Heart Disease Screening Results in Ontario

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Newborn Screening Ontario (NSO) is the provincial screening program of Ontario and oversees the Critical Congenital Heart Disease (CCHD) screening program. Standardized pulse oximetry screening for CCHD started in the province in February 2017. The program was rolled out across the province in stages, and has been fully implemented since May 2018.

CCHD screening results, including oxygen saturation values and evaluations (pass, repeat, refer), are collected by NSO on every baby screened in Ontario. This information is documented by the screener on an additional page of the newborn screening card and is submitted by mail to NSO. NSO enters screening results into an information system. An overall quality result is assigned to each card based on an automatic check in the information system, indicating whether the algorithm was followed and if not, what type of error was made.

As part of their role in program quality assurance, NSO follows up on cases where the screening algorithm was not followed and the screen may be incomplete or not appropriately managed. These are referred to as "Unsatisfactory CCHD Screens". Follow-up for unsatisfactory screens is initiated by a clinical specialist at NSO on the day of entry of the screening result into the information system (when the baby is approximately 3-4 days of age). A call is made to the screening site and the results of the screen are confirmed. At that time, the specialist may recommend that the screening site recall the baby to be screened again or that the baby be referred urgently to a physician for physical exam.
To date, NSO has collected 138203 CCHD screens, including 648 unsatisfactory screens that required follow-up. Of the screens that required follow-up, 286 resulted in re-screen recommendations. Two babies have screened positive and had significant diagnoses after unsatisfactory screen follow-up. The unsatisfactory screen rate has decreased from 0.56 % in 2017 to 0.42 % in 2018. The most common error identified has been misinterpretation of the screening algorithm, where a screener evaluates the result to be a 'pass' although the algorithm calls for a repeat test to be done.

By evaluating screening quality at the time of data entry, NSO is able to provide patient-level feedback to hospitals and midwifery practices offering CCHD screening, giving the opportunity to correct screening errors and helping to ensure that babies have been appropriately screened. When trends in unsatisfactory screens are identified, targeted education sessions are offered to screening organizations. In the future, NSO will continue to monitor and follow up on unsatisfactory CCHD screens on a patient level, and will begin reporting back to hospitals and midwifery practices on key quality indicators. NSO will also be working on new methods of pulse oximetry screening data collection, including electronic transmission of screening results from screening site to NSO.

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P-102*

Collection of Dried Blood Spot Samples on Recently Printed Filter Paper Cards Inhibits Biotinidase Activity

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Background: From 2016 to 2017 the annual screen positive rate for biotinidase deficiency in Ontario more than doubled from 2.65x10-4 to 6.57x10-4.
Methods: A data mining approach was used to investigate the role of temperature, filter paper lot, and print run on the screen positive rate for biotinidase deficiency. The laboratory information system was queried for all biotinidase activity results for the period 2014-01-12 to 2018-09-27 (n = 678,354). During the study period biotinidase activity was measured on the SpotCheck Pro platform (Astoria Pacific, Oregon USA.) with results reported in microplate response units (MRU).

Results: Decomposition was used to separate seasonality from an underlying trend in the 4 year time series of biotindase activity results. This analysis revealed a marked seasonal effect (winter = mean + <= 16 MRU, summer = mean - <= 19 MRU) and a profound non-linear negative trend (max = 126.7 MRU, min = MRU). External temperature was correlated with biotinidase results (Pearson's r = 0.79) but not with the observed negative trend (Pearson's r = -0.06).
Time series analysis of biotinidase results grouped by print lot of filter paper revealed that recently printed filter paper cards exhibit a time dependent inhibition of biotinidase activity (observed in seven print lots over four years). This inhibition resolved in approximately 3 months (Lot A: mean result 0-3.5 months = 66.0 MRU, mean result 3-6 months = 90.3 MRU).
Conclusions: In Ontario, lots of filter paper collection cards are generally issued once a year. Due to operational changes in 2017 four filter paper collection cards lots were issued. These new filter paper lots caused a pronounced negative trend in biotinidase activity leading to an increased biotinidase deficiency screen positive rate in 2017.

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Development and Integration of a Punching Module in OMNI-Lab Software for Newborn Screening
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Newborn Screening Ontario (NSO) is the provincially-run program that oversees the timely and comprehensive screening of all newborns in Ontario (140000 annually) for serious but treatable diseases that are not normally apparent in the neonatal period. NSO screens for Metabolic diseases, Endocrine diseases, Sickle Cell disease, Cystic Fibrosis, Severe Combined Immune Deficiency, and Critical Congenital Heart Disease.

In order to address their ever-expanding and changing needs, NSO embarked on a project to rebuild their Laboratory Information and Management System (LIMS). Integrated Software Solutions (ISS), an Australia-based software vendor, offered the OMNI-Lab software package as a viable product that would allow NSO to continue to offer the highest quality testing while taking control of the configuration and maintenance of its LIMS.

Early on in the development discussion between NSO and ISS, it was decided that ISS would create a Punching Module to allow NSO to process the large volume of samples received on a daily basis. ISS's other customers punch blood dots for analysis but use methods and workflows outside the OMNI LIMS framework to do so. The development of such a module in OMNI-Lab was identified as critical to NSO and a worthwhile endeavor for ISS as they sought to expand their software package offerings to current and future customers.

NSO identified a number of unique requirements in sample processing, and more specifically, in blood dot punching. The new module developed by ISS would need to incorporate a streamlined user experience that offered NSO a rapid way to take blood dots from a very large number of samples while also tracking the dots as they moved from preanalytical punch into a 96-well plate to a variety of

different laboratory analyzers. Essentially, the module needed to balance sample volume and patient risk reduction.

Armed with NSO's preanalytical requirements, ISS developers and programmers created the Punching Module as part of their software release to NSO in Fall 2017. This provided a framework for the iterative improvement cycle that has taken place over the past year: NSO functional testing, feedback to ISS, development work at ISS (including bug fixes and improvements) and then software patches/new product releases to NSO.

After multiple rounds of testing and product development, NSO went live with OMNI-Lab Punching Module in December 2018, for its Cytomegalovirus testing workflow, and will go live in 2019 for full screening. The module's evolution has given rise to an invaluable means for NSO to accurately and efficiently prepare samples and ultimately offer the highest quality screening and care to Ontario's newborns.

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Implementing a Newborn Screening Information Management System - Lessons from a Full Program Rebuild

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Information management systems are the central process management, clinical decision and communication hubs for many newborn screening programs. As part of the roll out of expanded newborn screening using MS/MS and related technologies many newborn screening programs implemented batch (sample) centric information systems which are critical to the day to functioning of their labs. However, the shift into new technologies and paradigms for newborn screening including next generation sequencing, point of care technologies such as pulse oximetry, diagnostic testing are pushing the limits of the original software architectures. As the field evolves, a more comprehensive approach to newborn screening information management will be required to support the expanding needs of newborn screening programs. NSO is a fully integrated program that manages all aspects of newborn screening and follow-up activities for children born in the province of Ontario (approximately 140,000 births per year). When NSO was established in 2006 it procured a single LIS solution for overall lab and program management. As the complexity of NSO's mandate has increased it was determined that a broader approach was required to allow NSO to expand into the areas noted above. In late 2014 NSO undertook an end to end assessment of its information management needs which resulted in a project to develop a flexible IS ecosystem and related process changes to enable NSO to better manage its current and future workflows and communication needs. An idealized vision of a Screening Information Management System (SIMS) was developed which was refined into enterprise and functional architectures. This was followed by the development of technical specifications, user requirements, procurement and finally full implementation of a hybrid batch/patient centric SIMS. NSO launched critical congenital heart disease screening using the SIMS in mid-2017 and blood spot CMV hearing ascertainment was moved into the new system in mid-2018. Population blood spot screening and related case management functions are being fully deployed. In undertaking a full product redesign

NSO faced a number of technical, process and change management challenges across the entire program. A number of metrics were developed to measure the benefits and key outcomes of the project across a number of domains including: Configurability, process innovation, ease of addition of new technologies, overall program benefits and ease of data analysis and reporting. The preliminary results of the analysis of these key performance metrics will also be presented.

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Utilizing an Integrated Data System for Newborn Screening Follow-up

K. Holland, Pennsylvania Department of Health, Harrisburg, PA

This poster will demonstrate how Pennsylvania is utilizing an integrated data system to improve newborn screening data and follow-up processes. Since July 2016, Pennsylvania's newborn screening program has been utilizing an internet case management system (iCMS) for housing all newborn screening data, including dried blood spot, hearing, and critical congenital heart defects screening and diagnostic data. iCMS is a web-based software application, developed and supported by Neometrics™, a division of Natus Medical Incorporated. The system includes a data warehouse which allows for reporting of all data in the system. iCMS is being implemented in three phases. In July 2016, phase one of the project went live with dried blood spot results and critical congenital heart defects screening results being sent to iCMS via HL7 messaging from the contracted laboratory and newborn screening staff utilizing the system to manually enter hearing screening results and complete follow-up activities for all abnormal newborn screens. In Fall 2017, the phase two rollout began, which allowed hearing screening providers to submit hearing screening results via manual entry, device upload, or HL7 messages and allowed contracted treatment centers to directly enter diagnostic data in iCMS. All hearing screening providers, except for midwives without internet access, and contracted treatment centers were utilizing the system by June 2018. Phase three, which is in development with a planned completion date of June 2019, will allow non-contracted treatment centers, immunologists, endocrinologists, cardiologists and audiologists to enter diagnostic data in the system and provide primary care physicians view-only access to their patient data. The system is further being advanced by adding other modules including one for vital records match and a neonatal abstinence syndrome (NAS) module. The vital records match module, implemented in November 2018, allows birth records to be matched with data in iCMS to ensure all babies born receive a newborn screen. The module for NAS reporting and follow-up is in the initial planning stages and the goal is to have it completed in the first half of 2019. The move to iCMS has allowed for: more consistent follow-up processes as actions are built into the system based on the type of result received by the system; the receipt of individual level hearing screening data; easier reporting of diagnostic information; and the move to a case management approach to newborn screening follow-up activities. This poster will provide information on iCMS system capabilities, how follow-up activities were streamlined and shifted to a case management approach and quality improvement activities that can be completed with the system's increased and multi-level reporting capabilities.

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P-106

The Duchenne Newborn Screening Consortium: Accelerating the Path to Nationwide Newborn Screening

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Duchenne Muscular Dystrophy (DMD) is one of the most severe and common pediatric genetic diseases and affects an estimated 1 in every 5000 male births worldwide. DMD is a progressive neuromuscular disorder that is 100% fatal disease, but the development and adoption of comprehensive, multi-disciplinary health care guidelines and recent therapy approvals by the U.S. Food and Drug Administration have yielded increased life expectancies. Studies have demonstrated that optimal outcomes result from early identification and intervention to preserve muscle health, and the therapy development pipeline has many promising new treatments including molecularly designed drugs that target specific variations in the DMD gene sequence as well as others that are applicable to the entire Duchenne population. Foreseeing the launch of new treatments and the ability to screen newborns for DMD using laboratory tests, Parent Project Muscular Dystrophy (PPMD), initiated a national DMD NBS effort in 2015 to facilitate the creation of critical NBS infrastructure required to pilot DMD screening with parental consent.

Significant Results: PPMD created the DMDNBS Consortium (Consortium) to provide a unique approach toward conducting a state-based pilot utilizing a pre-competitive partnership between biopharmaceutical industry, patient advocacy and federal agency partners to provide expertise, financial support, and infrastructure to a state pilot. The pilot will utilize tools, resources and expertise at ACMG's Newborn Screening Translational Research Network (NBSTRN), a program funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Implications: This presentation will highlight the unique structure of the Consortium and its decision-making processes and data sharing agreements, as well as the structure of a comprehensive NBS pilot program. We will discuss the unique ethical, legal and social considerations of screening for an X-linked condition, define an approach to identifying the most useful screening and diagnostic algorithms to guide treatment choice, engage parents and families to understand the impact of cascade testing and early identification, and assess both the public health and clinical care impact of DMD NBS. The creation of a data collection system for long-term follow-up and educational materials developed for screening for Duchenne will be described. In addition, preliminary data from the pilot will be presented. The Consortium provides a useful roadmap for other conditions, which may benefit from early identification and treatment through NBS.

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Orotic Aciduria Identification as By-product of Detection of Abnormal Citrulline Levels in Newborn Screening

L. Resto, M. del C.G and S. Rivera, University of Puerto Rico, San Juan, PR

Orotic aciduria is a disorder of pyrimidine metabolism with a variety of clinical presentations. Findings in Newborn Screening of two cases with increased citrulline levels prompted the diagnosis of orotic aciduria after confirmatory tests. Confirmatory testing consisted in quantitative plasma amino acids panel, orotic acid levels in urine, plasma ammonia and quantitative urine organic acids. In one case high citrulline levels were detected in plasma amino acids with high orotic acid levels and mild elevation in plasma ammonia. A second case with persistent high orotic acid was detected but with normal citrulline and plasma ammonia levels in confirmatory tests. Diet was recommended in both cases.

High citrulline levels in a newborn screening sample can be associated to urea cycle disorders as ASA, Citrullinemia, PC, CPS-1 and OTC variants. The differential diagnosis of OA includes the urea cycle disorders and uridine monophosphate synthase (UMPS) deficiency as well as other disorders. In these two cases, orotic aciduria was detected as a secondary finding.

To the best of our knowledge, these cases are the first identified in the pediatric population in Puerto Rico and the newborn screening analysis was important for their detection. Clinical follow-up and further molecular analysis will be performed to better understand this condition in our population.

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SCID Compass: Improving Education and Newborn Screening for Severe Combined Immunodeficiency

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Objective: Severe combined immunodeficiency (SCID) is a collection of genetic disorders that cause profound defects in cellular and humoral immunity. Currently, 47 states have implemented newborn screening for SCID, which covers approximately 95% of births in the United States. Despite this success, significant challenges remain for patients, families, clinicians, and public health professionals. These challenges include communication between the newborn screening community, health care providers, and families; disparities in knowledge and care for patients with SCID in rural and underserved communities; and general awareness and knowledge about SCID and SCID newborn screening for all stakeholders. SCID Compass, a grant recently funded by HRSA, seeks to address these important gaps in the field to improve outcomes for infants with SCID detected through newborn screening.
Methodology: SCID Compass is comprised of a partnership between the Immune Deficiency Foundation (IDF, a patient advocacy group), the Association of Public Health Laboratories (APHL, a professional association responsible for supporting newborn screening programs), and RTI International (a nonprofit research institute). The goals of the project are to (1) increase awareness and knowledge about SCID and newborn screening for SCID among parents, families, health care providers, public health

professionals, and the public; (2) provide education, training, and support for newborn screening programs; (3) educate families with children diagnosed with SCID and link them to clinical and other services, especially those in rural and medically underserved areas; and (4) improve clinical care through education and training for providers caring for individuals with SCID.
Results: This presentation will provide an overview of SCID Compass's core activities, including (1) Develop and disseminate linguistically and culturally appropriate education and awareness resources ad well as online website, (2) Assist remaining states that are not screening for SCID move toward full implementation and address ongoing educational needs for states screening for SCID, (3) Increase use of telehealth to link families in rural and underserved areas with care services, (4) Develop strategies for long-term follow-up.
Implications: SCID Compass is designed to include the perspectives of patients and families, health care providers, and public health professionals. Our hope is this will maximize impact in educating and providing resources to these stakeholders.

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Potential Use of Unmanned Aerial Systems to Transport Newborn Screening Specimens
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Objective: Rapid and efficient transport of newborn screening (NBS) specimens is one of several critical steps in the timely identification of newborns affected with a disorder. NBS programs utilize a variety of methods to transport specimens from collection sites to the screening laboratory; however, regardless of the transportation method employed, challenges exist that can cause delays in specimen transport. To identify potential solutions, NBS specimen transport scenarios were modeled using unmanned aerial systems (drones).
Methodology: A proof-of-concept methodology was employed to evaluate drone use for NBS specimen transport. Reviews of the regulatory barriers and strategies for addressing these barriers were performed; an assessment of the most promising options for safe, reliable, and functional drone technologies for transporting NBS specimens was completed; and models of these solutions and their effectiveness at improving timeliness were developed and evaluated. Cost, availability, and sustainability were assessed. The performance of the model scenarios in improving timeliness was measured through assessment of national timeliness quality indicators, specifically, time from specimen collection to receipt at state newborn screening laboratory.
Results: Federal and state regulations that were reviewed reveal that the most challenging aspects of the use of drones for transporting cargo relate to flight distance, time in flight, and pilot training requirements. Absent of higher-level licensing, flight of drones is currently limited to line-of-sight. However, drone regulations continue to mature in response to new technology and use demands. A review of the off-the-shelf, commercially available aircraft that could immediately be applied to meet NBS sample delivery requirements revealed several potential options at moderate cost; however, the cost of operating drones is still under investigation. Additionally, models of use cases suggest that the time from specimen collection to receipt at lab could be affected by use of drones in select NBS specimen transport scenarios.
Conclusions/Implications: Although challenges exist in the current regulatory environment for the widespread use of drone technology for NBS specimen transport, regulations continue to evolve and

adapt to commercial demands. Likewise, the cost for drones that can effectively and safely transport these critical dried blood spots continues to become more affordable. Models suggest that, in certain use cases, drones could be deployed to successfully transport NBS specimens and have an impact on timeliness quality indicators. Further, use of drones could be considered for emergency response.

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Using the Florida-specific New Disorder Review Framework to Assess New Conditions

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Objective: The Secretary of Health and Human Services' Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) has developed a thorough and rigorous evidence review process to evaluate disorders that are nominated for addition to the Recommended Uniform Screening Panel. The Florida Department of Health contracted with RTI International to develop a similar framework for the addition of conditions to the state's mandated screening panel and to apply this framework to new conditions under consideration by the Florida Genetics and Newborn Screening Advisory Council (GNSAC) for potential recommendation for addition to the state's mandated panel.
Methods: To develop the review framework, the RTI project team evaluated relevant peer-reviewed publications for newborn screening disorder review processes utilized at the national level, as well as publications from states and international newborn screening programs. In addition, an environmental scan was performed to examine scientific presentations at national and international conferences and to evaluate newborn screening program websites for disorder review information. Reviews of mucopolysaccharidosis type-I (MPS-I), Pompe disease, and spinal muscular atrophy (SMA) included a similar analysis of peer-reviewed literature and environmental scans for scientific presentations. In addition, subject matter experts from the Florida newborn screening system were interviewed for their insights on the implications of adding the conditions to state's mandated newborn screening panel.
Results: An initial report proposed review criteria in four categories (Screening Test, Benefits of Early Detection, Public Health System Readiness, and Cost). A subsequent report described the applicability of the ACHDNC framework for each review criterion and detailed additional state-specific analyses that would inform the disorder review process. The disorder reviews for MPS-I, Pompe disease, and SMA included reports that applied the Florida framework to the specific condition under consideration. The reports include data on the impact of each new disorder addition in Florida for the GNSAC to consider when making a recommendation. However, the reports do not contain specific recommendations regarding the feasibility or benefit of adding the condition.
Implications: Florida's GNSAC were in favor of using the proposed criteria and framework to assist with a more uniform evaluation of conditions under their consideration for recommendation. Details from the framework and disorder-specific reports will be presented.

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Voluntary Screening for Spinal Muscular Atrophy in the State of North Carolina through the Early Check Program

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The Eunice Kennedy Shriver National Institute of Child Health and Human Development awarded a contract to RTI International, the University of North Carolina at Chapel Hill, and the North Carolina State Laboratory of Public Health to conduct a newborn screening pilot study for spinal muscular atrophy (SMA). SMA type I is considered the most common lethal autosomal recessive genetic disorder in infants and SMA was recently recommended to the Recommended Uniform Screening Panel (RUSP) in February 2018. We validated a real-time qPCR assay to detect the presence or absence of the SMN1 exon 7 deletion, a method adapted from the previous Taylor et al. publication. We characterized the performance of this method by evaluating assay precision, accuracy, sensitivity, and specificity on the Agilent AriaMX Real-time PCR Instruments. The validation study included dried blood spot (DBS) samples from 10 SMA patients and 20 carriers with known SMN1/SMN2 genotypes and 2,000 de-identified newborn DBS samples. In the validation, no patients confirmed with SMA had amplification of the SMN1 gene and the median SMN1 Cq value in the de-identified newborn DBS samples was 23.45. SMA screening will be included in a voluntary screening program called Early Check, which offers screening to all families state-wide in North Carolina for a panel of conditions. This presentation will discuss how the laboratory implementation experience was different from standard newborn screening, the screening and follow-up algorithm, as well as screening and confirmatory results from the consented North Carolina population. The results from this study support the application of this method for high-throughput newborn screening programs and screening for SMA facilities the early identification of infants receiving clinical services.

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The North Carolina X-linked Adrenoleukodystrophy Pilot Study: Challenges and Lessons Learned

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X-linked adrenoleukodystrophy (X-ALD) is a disorder that leads to demyelination in the brain or spinal cord and adrenal insufficiency. Because population screening methods are now available and treatment options have improved the outcome for patients affected with X ALD, it was added to the Recommended Uniform Screening Panel (RUSP) in 2016. Currently, only a few states have implemented full population screening. The North Carolina team composed of RTI International, the University of North Carolina at Chapel Hill (UNC-CH), Duke University, and the North Carolina State Laboratory of Public Health (NCSLPH) conducted a newborn screening pilot study for X-ALD. The first-tier screening

method was a high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) assay in negative ion mode to measure the C24:0-lysophosphatidylcholine (C24:0-LPC) and C26:0-lysophosphatidylcholine (C26:0-LPC) biomarkers, followed by Sanger sequencing of screen-positive dried blood spot samples at Duke. A total of 52,301 specimens were screened and 12 infants were identified with screen-positive results. Out of the 12 screen-positive samples, 6 had no variants detected, 3 samples had pathogenic or likely pathogenic variants, and 3 samples had variants of unknown significance. All infants with screen-positive results received very long chain fatty acid (VLCFA) confirmatory testing at the Kennedy Krieger Institute and follow-up services at UNC-CH. The screen-positive infants included three males with confirmed X-ALD, two heterozygous females or X-ALD carriers, two newborns with peroxisome biogenesis disorders, one infant with Aicardi-Goutières syndrome, one newborn with possible liver dysfunction, and three false positives. The estimated incidence was 1 in 10,460 births. The positive predictive value for the first-tier assay was 83.3%, with a false-positive rate of 0.004%. A new law was recently enacted in North Carolina that increased the newborn screening fee and added several disorders, including X ALD, to the state's mandated panel. The successful completion of the pilot should help guide the state program's decision makers as they pursue full implementation of screening for this disorder.

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Validation and Population Screening using a Custom FMR1 PCR Assay System in the Early Check Program

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Newborn screening (NBS) is designed for pre-symptomatic identification of serious conditions for which there are effective treatments or interventions that must begin early. NBS policy requires that pre-symptomatic treatment be more effective than treatment after symptoms appear. Additionally, one or more public health laboratories must perform prospective pilot studies using the screening method to test the analytical performance of the assay. Early Check is a longitudinal research endeavor to assess conditions for inclusion in widespread NBS. One of the conditions on the Early Check panel is Fragile X syndrome, a disorder caused by an expansion of CGG repeats in the 5'UTR of the FMR1 gene. We adopted a custom PCR-based assay and analysis software system developed by Asuragen for a high-throughput sample workflow to provide robust detection of FMR1 repeat expansions from dried blood spots (DBS). We established the method by analyzing cell lines and quality control reference material with CGG repeat sizes spanning a range of genotypes, including normal, premutation, and full mutation alleles. We characterized the performance of this method by evaluating assay precision, accuracy, sensitivity, and specificity. The assay consistently performed within 5% of the expected CGG repeat requirements, and proficiency testing results were in 100% concordance with the results from reference laboratories. Full mutation alleles were consistently detectable at 0.5 ng in the male sample and 0.125 ng in the female sample. Premutation alleles were consistently detectable at 0.125 ng in the female sample. Full mutation alleles were detectable in the presence of the normal allele as low as a 2.5% abundance in the female sample. The normal allele in the female sample was detectable as low as 1% abundance. Premutation alleles from the female sample were detectable in the presence of the normal allele as low as 5% abundance. Furthermore, we used a straightforward sample preparation workflow

for the analysis of 963 de-identified newborn DBS samples from the North Carolina Laboratory of Public Health NBS Program to determine preliminary population distributions and to develop a screening algorithm. We found 957 normal, 6 premutation, and 0 full mutation specimens. The validated assay is being used to screen newborns enrolled in Early Check. The results from this study support the application of this system for high-throughput NBS programs and the value of public-private partnerships to address unmet needs for the pre-symptomatic identification of individuals with rare diseases.

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Assessment of Timeliness Activities during the NewSTEPs 360 Project

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Problem: Approximately 88,000 babies are born and screened in the state of Tennessee each year. 2015 data showed that only 31.4% of specimens submitted to the state laboratory arrived < 2 days after collection. The percentage of unsatisfactory specimens was 2.78% and only 87.6% of initial specimens were collected within the target collection age (between 24-48 hours of age). Regarding initial specimens from birth to reporting results, the average turnaround time was 171.5 hours or 7.1 days. 28% of presumed positives for time-critical and 62.5% for non-time critical disorders were reported to follow-up by ≤5 and ≤7 days of life respectively highlighting improvements were needed to meet the national timeliness goals. In 2016 Tennessee began working with the NewSTEPs 360 project as an extension of previous work on a CoIIN (Collaborative Innovation and Improvement Network) project that focused on timeliness in newborn screening. A result of this project was the identification of root causes contributing to delays in reporting, namely specimen receipt time and specimen quality. Additionally, to improve overall timeliness and reach the national goals, focus was placed on reporting time for screening results within 5 days of life for time critical disorders and 7 days of life for non-time critical and other results.

Methodology: Throughout the project period (2016-2018) several interventions were introduced to address the problems identified. Educational materials were distributed to birthing facilities; education was provided through site visits to birthing facilities, midwives, and health departments; consistent quality assurance reporting was enhanced using new and existing reports; evaluations of the laboratory workflow and environment were completed; and, an annual provider survey to assess compliance with recommendations and customer satisfaction was implemented.

Results: Preliminary data from 2018 showed that on average 39.5% of specimens submitted to the state laboratory arrived the same or next day after collection; the average percent of unsatisfactory specimens submitted was 2.56%; and, 92% of initial specimens were collected within the target collection age. The percent of presumed positives for time critical conditions reported by ≤5 days of life for the first two quarters of 2018 was 58.5% and the percent of presumed positives for non-time critical conditions reported by ≤7 days of life was 77%.

Further analysis will be completed at the conclusion of the 2018 calendar year.

Conclusion: Interventions have improved processes significantly for decreasing unsatisfactory specimens, improving birth to collection times and birth to reporting for both time critical and non-time critical disorders. Further improvements are expected with the recent implementation of Sunday testing of time-critical disorders and ongoing efforts to educate birthing facilities.

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Tennessee's Experiences Performing Newborn Screening for Five Lysosomal Disorders

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The Tennessee Department of Health Newborn Screening Laboratory began state-wide screening for five lysosomal disorders (LDs) on July 1, 2017, two years after recommendation by their Genetics Advisory Committee and endorsement by the Commissioner of Health. Testing for Pompe, Mucopolysaccharidosis Type I, Gaucher, Fabry, and Krabbe diseases was performed using tandem mass spectrometry with reagents developed by Perkin-Elmer, Inc. After method validation, 117,816 specimens were tested during the first sixteen months of routine screening. Overall, 152 patients were referred to short-term follow-up, and 31 of these were subsequently confirmed positive. During this time, initially conservative cutoff values were refined to reduce false-positive reporting, and presumptively positive specimens were sent out for second-tier biochemical testing and DNA sequencing to further improve specificity and disease prognosis. Additionally, the Mayo Clinic Collaborative Laboratory Integrated Reports (CLIR) method of analysis (using results for five LDs and other pertinent patient information) was applied to these specimens to see if this evaluation would differentiate between specimens that required further testing and confirmation from those that are negative for the disorder in question. Results of second-tier biochemical testing, sequencing, and CLIR were evaluated with regard to their effects on overall costs, turnaround times, and patient outcomes. Biochemical second-tier tests, available for all the disorders except Fabry, promise to be cost-effective, rapid methods to rule out false-positive results, thereby reducing the time and expense of unnecessary follow-up. While CLIR analysis as applied in Tennessee can reduce the number of specimens with low enzyme activities that are sent for second-tier testing, its intended application is in evaluating the results of all specimens. CLIR analysis is designed for application without cutoffs using the results of enzyme analysis for six LDs – the five described here plus Niemann-Pick A/B and other covariates. Using CLIR as designed promises to reduce additional testing while further improving turnaround times. A reduction in the number of specimens sent for second-tier testing conserves resources that can then be applied to sequencing of high-risk cases during the newborn period – thereby improving health care equity, providing the opportunity for more effective intervention, and eliminating the need for a costly and lengthy diagnostic odyssey.

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Peer Network Resource Center and Proficiency Sample Exchange Highlight Programmatic Differences

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The problem studied and/or objectives: Missouri (MO) serving as a Peer Network Resource Center (PNRC) established through funding from the Association of Public Health Laboratories has proven beneficial in assisting Tennessee (TN) through validation of five Lysosomal Disorders. MO provided samples to TN to help establish accuracy and to aid in setting cutoffs. Later, both states entered into a sample exchange to assist each other in meeting licensure requirements for proficiency testing (PT) since materials are lacking for Gaucher and Fabry. Through these processes, some differences between programs were noted.
Methodology: MO provided TN a panel of 40 samples consisting of true positives and true negatives covering five Lysosomal enzymes. A second panel of 44 samples specifically targeting the detection of Fabry was also shared with TN. Results were compared from both panels. For the PT sample exchange, TN and MO alternate sending five PT challenge samples each quarter for Gaucher and Fabry. A minimum score of 80% is required to pass. Discrepancies, if any, are resolved by retesting the discrepant sample at both laboratories with corrective action reported if there was no resolution.
Significant results, including statistical significance where applicable: The first and second panels during validation showed discrepant results for Fabry. Tennessee called two samples within normal limits (WNL); MO indicated that these displayed A143T and R118C respectively. For the second panel, TN called five samples WNL; two had the A143T mutation, one had the Y207H mutation, and two were reported as inconclusive due to low values across five enzymes. Three proficiency cycles have been completed with 100% passing rate. However, one sample was evaluated more thoroughly as a potential discrepancy for Gaucher. TN called the specimen as WNL however MO determined that this sample was diagnosed as a carrier with one pathogenic mutation and one benign mutation.
Conclusions and/or implications: The PNRC and sample exchange help to facilitate compliance with licensing regulations but does highlight program differences in cutoffs and program objectives for case detection. Nonetheless, engaging in a sample exchange between states provides real cases with confirmed genotypic and phenotypic differences for examination. Additionally, the exchange serves to challenge each laboratory's program thereby contributing to improved quality assurance and a more robust newborn screening system.

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Biotinidase Positive Case Prompts Cutoff Evaluation for Two States

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The problem studied and/or objectives: Cutoffs for newborn screening can be difficult to establish therefore programs aim to balance reporting as few false positives as possible and avoiding false negatives. For Biotinidase, the manufacturer of the Genetic Screening Processor (GSP), Perkin Elmer recommends cutoffs based on percentiles or on calculations at 30% of the normal population mean

since patients with partial Biotinidase Deficiency can have activity between 10 and 30% whereas with complete deficiency patients can have values < 10%. Tennessee has a cutoff of ≤38.93 U/dL with 22 presumed positives reported in 2017; three confirmed as complete deficiency and one as partial deficiency for a PPV of 18.18%. S. Carolina validated the GSP for Biotinidase and began testing with an initial cutoff of <76.5 U/dL. The test was implemented for approximately four months before adjusting the cutoff. In 2017, five cases were confirmed including one carrier, one partial and three complete deficiencies. Since both states use the GSP for screening, they engaged in a sample exchange with hopes of determining whether cutoff refinements were needed that could potentially improve PPV.
Methodology: Tennessee and South Carolina exchanged 11 samples which included false positives, one complete deficiency, one partial deficiency, and normals. Both labs tested the samples on the GSP and compared result using correlation analysis.
Significant results, including statistical significance where applicable: Results correlated between laboratories (r2 = 0.93). S. Carolina identified all samples correctly. Tennessee missed the partial Biotinidase case due to the cutoff placement. The enzyme value for this patient when tested by S. Carolina was 68.29 U/dL whereas it was 39.73 U/dL when tested by Tennessee.
Conclusions and/or implications: Tennessee reevaluated mean enzyme activity for their population and raised the Biotinidase cutoff to 44.64 U/dL which represents 20% of the normal mean activity yielding a projected 27 presumed positives. Placing the cutoff at 30% of normal mean activity would result in 188 projected presumed positives and increase the follow-up burden. In January of 2018, S. Carolina also adjusted their cutoff to ≤ 70 U/dL, thus far resulting in two partial and three complete deficiencies. It is hoped that changes to both programs cutoffs will improve PPV.

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Faster, Better, AND Cheaper Severe Combined Immunodeficiency Screening of Newborn Specimens in Texas

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Objective: To improve the newborn screening method for severe combined immunodeficiency (SCID) at the Texas Department of State Health Services Newborn Screening Program.
Background: The State of Texas began screening all babies for SCID and T-cell related lymphocyte deficiencies in December 2012 using a laboratory-developed, multiplex Real-Time quantitative PCR (RT-qPCR) method to detect the presence of T-cell Receptor Excision Circles (TREC). A referral to immunologists is recommended if the TREC quantity is below the Limit of Blank or if the newborn has repeated non-normal results. Approximately 2.3 million newborns have been screened and 0.05% (1179) of them were referred for diagnostic testing. So far, 49 cases of SCID have been diagnosed through the newborn screening process with a positive predictive value of ~4%. To improve assay specificity and sensitivity, reduce costs, and decrease turnaround time, a number of changes to the testing method were proposed and evaluated.
Methodology: The dried blood spot is screened for SCID through an automated DNA extraction method followed by a RT-qPCR assay to detect the presence of TRECs, the primary marker, and RNaseP, the reference gene. Performance of various master mix, standard curve source material, and extraction solutions and protocols were evaluated and different concentrations of RNaseP reagent kit component

were assessed to determine the optimal screening method. A validation study was conducted and data was reviewed to determine the best cutoff algorithm.
Results and Conclusion: The new SCID screening method was validated and implemented in September 2018. Turnaround time for SCID screening results was reduced by one day with an anticipated cost savings of $500,000 annually. The accuracy, precision, analytical sensitivity and specificity, reportable range, and reference range of the new method were determined and will be presented. In addition, cutoff algorithms using Multiple of Median, TREC quantities, and cycle threshold (Ct) values will be compared. Factors such as transfusion, age at collection, and birth weight will be discussed. Our observations and experiences in improving assay performance, cost, and timeliness will be shared to hopefully help other programs to better their SCID screening process.

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11 Year look at CAH Screening in Texas
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Objective: The objective is to review numbers and characteristics of cases identified on first and second newborn screens for severe congenital adrenal hyperplasia (CAH) in Texas over the past 11 years, and report on findings.
Methodology: While published studies indicate that two newborn screens will improve identification of CAH cases, Texas, as a large two screen state is also able to review this data. Texas will pull retrospective data from 2007 to 2017 on all diagnosed CAH cases by classical salt-wasting, classical simple virilizing, and non-classical cases. The report will include types of (CAH) cases detected on the first versus the second newborn screen, including evaluating case characteristics.
Results: Preliminary data of 678 cases shows approximately 15% of classical salt-wasting CAH, 82% of classical simple virilizing, and 88% non-classical CAH cases are normal on the first screen. Although data is not yet available, results will include demographic data and elements such as hours of age at collection to look for any relationships.
Conclusions: Approximately 60% of CAH case in Texas are identified on the second screen. Other conclusions will be presented once tabulated.

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Withdrawn

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Decreased Concentration of Isovaleryl-Carnitine in Patients with Maple Syrup Urine Disease (MSUD)

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Background: Newborn screening (NBS) for MSUD is a special challenge since patients can metabolically decompensate rapidly without adequate treatment within the first two weeks of life. The sum of the isobaric amino acids leucine, isoleucine, hydroxyproline (Xle), and Val are used as primary markers. For the confirmation of a positive NBS for MSUD a second-tier UPLC method for the separation of allo-isoleucine is usually used.
Results: From 491 samples of MSUD patients under treatment, we additionally measured the concentration of isovaleryl-carnitine (C5) in dried blood spots (DBS). C5 in MSUD patients was 0.02 ± 0.01 µmol/l (mean ± sd), reference range in healthy newborns 0.04 – 0.49 µmol/L (0.1st – 99.9th centile). C5 in 9 NBS samples from patients with confirmed MSUD was in the range of 0.01 – 0.06 µmol/l. From these samples we calculated additionally the ratios of Xle/Alanin/C5, and Val/Alanin/C5. The ratio Xle/Ala/C5 proofed to be the best indicator for MSUD, with the lowest value of the tp's being 7.3 times higher than the highest value of 168 fn's. Val/Ala/C5 was the second best indicator, followed by Xle/Ala and Val/Ala, with the lowest tp's being 4.1, 3.0, and 1.4 times higher than the highest values from fn's, respectively. As a proof of concept we are retrospectively evaluating 5 MSUD cases from Ireland and 12 from Bavaria.

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Highly Specific Amino Acid and Acylcarnitine Profile in Dried Blood Spots of Patients with Adenosine Kinase Deficiency

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Background: Adenosine kinase deficiency is a disorder which affects the metabolism of methionine and adenosine. In addition increased concentrations of adenosyl homocysteine inhibits most methylation reactions. Patients with adenosine kinase deficiency have a severe phenotype with neurological symptems, dysmorphism, and hepatic involvement. Biochemical findings are hypermethioninemia, and increased concentrations of S-adenosylmethionine, and S-adenosylhomocysteine in plasma, and increased adenosine in urine.
Results: We have investigated amino acids and acylcarnitines from 6 dried blood spots samples of a patient with adenosine kinase deficiency over a period of 4 months. The first sample was from the newborn screening, the last 2 under methionine restricted diet. With a multiparameter analysis we could establish a factor, which could separate all 6 samples from the patient with adenosine kinase deficiency, from 1.000 unselected samples form selective screening. We calculate the factor of total carnitine (TC) multiplied by the sum of tyrosine and methionine (tyr + met): TC * (tyr + met). All 6 samples had values of > 40.000, while most other samples had values < 10.000. We only had only sample from a child with possible tyrosinaemia type 2 or type 3 which had also a value > 40.000.

Conclusion: The introduction of the factor TC * (tyr + met) provides a specific marker for adenosine kinase deficiency. Early detection in newborn screening and early implementation of a diet low in methionine could possibly improve the outcome of patients with adenosine kinase deficiency.

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Premature Infants have Higher Dried Blood Concentrations of (iso) Valerylcarnitine

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Introduction: Isovaleric aciduria (IVA), an autosomal recessive inborn error of metabolism, is a defect in leucine catabolism due to isovaleryl-CoA dehydrogenase dysfunction. The clinical presentation of IVA is highly variable, ranging from severely affected to asymptomatic. It can present either in the neonatal period as an acute episode of fulminant metabolic acidosis, which can lead to coma or death, or it can manifest later with developmental delay, with or without recurrent acidotic episodes during periods of stress and infections [1]. IVA is included in the expanded German newborn screening panel since 2002. (Iso)valeryl (C5)-carnitine is the primary marker for the detection of IVA in newborn screening by tandem mass spectrometry.
Methods: In our newborn screening center, we have statistically evaluated C5-carnitine concentrations during a 3 year-period (2015-2017) with a total of 135 670 samples. Results refer to the following groups: A) gestational age > 32 weeks, sampling time 36-100 hours; B) gestational age > 32 weeks, sampling time 1-35 hours, and C) gestational age ≤ 31 weeks. Statistical analysis has been performed using Roche Diagnostics IT Solution GmbH Berlin (Version 16.5.2017), Swisslab program.
Results: All newborns that showed an elevation of C5-carnitine above our cut-off value of 0.4 umol/L were included in the evaluation. Approximately 10 % of newborns in group C) (premature newborns) showed an elevated C5-carnitine concentration. The average concentration of C5-carnitine was 0.48 umol/L. In contrast, the percentage of newborns with elevated C5-carnitine concentration among the other two groups was below 0.4 %. It is noteworthy that pivalic acid derivatives are not used in antibiotic regimens in Germany.
Conclusion: Premature newborns, below the 32nd gestational week, show higher C5-carnitine concentrations in comparison to the other two groups. This may reflect immaturity of the enzyme system involved in the metabolism of branched-chain amino acids. As a consequence, the application of statistical cut-offs results in higher recall rates among premature infants. Therefore, it is important to adjust cut-offs according to gestational age and to compare them to results of affected patients of different gestational age groups.

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Targeted Second-Tier Confirmatory Next Generation Sequencing Newborn Screening Pipeline

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The Utah Newborn Screening (NBS) Program is implementing a second-tier exome-based Next Generation Sequencing (NGS) pipeline with a priori analytic restriction to candidate genes associated with respective newborn screening abnormalities.

As a second-tier screening tool, this technology provides a "gene-agnostic" solution, alleviates limitations associated with amplicon-based gene panel testing, and provides economies of scale as this tool is not limited to a narrow set of predetermined genes and can be used for other screening applications outside the scope of NBS. This solution will negate the diagnostic odysseys for patients with rare mutations in genes not commonly associated with the disorder.

Whole-exome sequencing (WES) will be performed on genomic DNA extracted from dried blood spots (DBS) of newborns with two abnormal screening results. Actual sequence analyses will be restricted to the gene/s associated with the NBS disorder. Focus on known genes associated with the disorder will avoid or limit incidental findings.

The sequencing analysis pipeline is based on of the Genome Analysis Toolkit's (GATK) Best Practices pipeline. Because we are restricting our analysis to a gene or genes associated with a newborn screening condition, this limits the number of potential variants we discover. Since the GATK algorithm relies on large datasets we are implementing the hard filter recommendations for analyses with low numbers of variants. For the process of variant interpretation, we have created a local database containing data from resources such as ClinVar, dbSNP, HGMD and disease specific databases. Variants will be annotated with SnpEff and VEP and pathogenicity will be predicted querying these variants against publicly available variant data. Variants, especially variants of unknown significance (VUS), will be re-evaluated every six months to determine if pathogenicity designations have changed.

Pipeline validation will proceed in three phases (1) gene/disease-specific validation, (2) integrated laboratory and analytical pipeline validation and (3) validation with third-party data. Gene/disease-specific validation will utilize data from gene specific sequencing. The entire laboratory and analytic process will be validated with DBS material curated for specific disorders. Laboratory processes will be optimized to achieve sufficient read coverage (analytic sensitivity and specificity. Data from other NBS programs will be used to ensure the validity of the pipeline with external data. The pipeline will be shared and distributed as both a command-line tool and in a graphical user interface format.
This project has been selected as a Sync 4 Genes Phase 2 Pilot Site, testing the consent-based sharing of sequence data with clinical experts if common causal molecular variant/s are not associated with the respective NBS disorder.

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A Complete Newborn Screening Patient Record: Utah's IT Infrastructure and Health Information Exchange

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The Utah Newborn Screening (NBS) Program at the Utah Department of Health (UDOH) is establishing a complete NBS patient record that will be integrated in the laboratory information management system (LIMS) and electronic health records (EHRs) used by birth hospitals, primary care providers, and clinical specialists. In this system anyone with justifiable need requirements and clearance can access the newborn record. The newborn record includes the following patient and process information:
1. Real-time and dynamic data regarding status and location of the newborn patient's specimens establishing complete chain-of-custody; 2. Secondary operational data regarding individual and aggregate process timeliness; 3. Complete demographic data, screening data and summaries, clinical diagnostic data, and potential treatment data for the newborn patient;

Establishing a complete patient record requires interoperability between disparate electronic systems. Disparity challenges can be overcome by leveraging health information exchange (HIE).

The proposed solution in Utah leverages an existing community resource, the Clinical Health Information Exchange (CHIE), which possesses the infrastructure to connect the Utah NBS Program to EHRs throughout the state using electronic messaging. More specifically, bidirectional interoperability will be established with EHRs, including both Laboratory Orders Interface (LOI) and Laboratory Results Interface (LRI) implementation. Through this partnership, the CHIE's infrastructure will be further augmented to achieve the necessary interoperability requirements to establish a complete NBS patient record.

Phase one of this project will focus on establishing a rolling admission, discharge, and transfer (ADT) message feed for all birth hospitals in Utah and complete implementation of LOI and LRI with the largest healthcare network in Utah (≈ 55% of births in Utah). Following implementation of the IT infrastructure, operational improvements and implementation costs will be assessed and documented. The bidirectional flow of data with the provider EHR is anticipated to eliminate onsite data entry requirements, offsetting implementation 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. This IT infrastructure will also enable long-term follow-up of patients with abnormal NBS results. This system further allows the Utah NBS Program to establish disease cohorts to assess outcome based, cost-benefit and cost-utility analyses of NBS specific conditions.

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Withdrawn

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Timeliness and the Victorian Newborn Screening Program: A 7 Year Review
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Background: Victoria has the second largest newborn screening (NBS) program in Australia, screening babies for 52 years with the annual current birth rate at approximately 80,000. A written consent program began August 2011, it was observed while monitoring consent that NSB cards were arriving between 7 and 14 days of life, however guidelines state sample collection between 48 and 72 hours of life. We therefore initiated a two year audit to assess the timeliness of sample collection and the impact of changes to the national postal service on sample transit. Following this initial audit opportunities were identified for improvement regarding timeliness. Our objective was then to determine the effectiveness of our implemented improvement initiatives.
Methods: Two audits were conducted for samples received for screening from 105 maternity providers. The first audit was conducted over two years commencing in June 2011, which led to improvement initiatives released by the Victorian Department of Health and Human Services in 2015. The second audit incorporated all NBS samples received between June 2011 and May 2018. The data extracted for these audits included birthday, sample collection, postal marks and laboratory receipt dates and times. This data was compared to the Human Genetic Society of Australasia's recommended key performance indicators of 1) time of collection - 95% of samples collected between 48 and 72 hours post birth; and 2) transit time - 95% of samples reached the laboratory for analysis within 4 days of collection.
Results: Between June 2011 and May 2018, the proportion of NBS samples collected between 48 and 72 hours after birth increased from 89.4% to 97.3%. The proportion of samples were received within 4 days of collection increased from 73.6% to 93.6%. In 2011 only 73% of samples were arriving within 4 days of sample collection post birth, with the majority sent via the postal service. By May 2018 83% of all maternity providers used either a courier service or express post (next day). The other 17% used priority mail (guaranteed within 4 days), which represented predominately private midwifes due to the cost of the alternatives.
Conclusions: This review demonstrated the importance of ongoing monitoring of collection and transit times and the need for ongoing education, increased communication and extensive follow up to ensure all relevant parties appreciate the role they play in all facets of this lifesaving program.

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Utilization of Bioinformatics Resources to Improve the Timeliness of Variant Interpretation for Lysosomal Storage Disorders in Virginia
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In 2018, The Virginia General Assembly mandated the addition of Lysosomal Storage Disorders (LSDs,) Pompe and MPS1, to the Virginia core panel of newborn screening testing. In response to evidence showing a high yield of false-positives from first-tier biochemical testing due to the presence of pseudodeficiency alleles, the Virginia Genetics Advisory Committee, and more specifically the pediatric specialists participating on the statewide Pompe/MPS1 working group, requested second-tier

confirmatory testing in the form of targeted resequencing. Implementation of sequencing and the associated data analytics pose significant obstacles for many state screening laboratories, resulting in many states opting to contract out and seek assistance with the sequencing component. The analysis, annotation, interpretation, and management of the resulting variant data is both time-intensive and prone to human error and misinterpretation. Additionally, the resources needed to support an interpretation are widely dispersed and continuously evolving at varying levels of curation. To overcome these obstacles, Virginia Division of Consolidated Laboratory Services is working to utilize bioinformatics resources and open-source tools executed in a Linux operating system in parallel with a relational database. The resulting tool minimizes the manual steps required for decision making by automatically retrieving, cleaning, normalizing, and aggregating the respective data sources into one local resource. It expects each data source to change continuously, and thus, performs routine updates with simultaneous comparisons to past patient records, reporting changes related to our population. The tool reduces a task that once took hours down to minutes. It ensures the quality and continuity of our reported variant data and creates an avenue for identifying potentially clinically significant changes to a previous patient's record. Virginia's experience implementing LSDs demonstrates how bioinformatics can be utilized to improve the timeliness of sequencing-based newborn screening. Large-scale bioinformatics tools can be applicable to small throughput use-cases with the added benefit of future scalability to support multigene analyses as the panel of sequenced based screening increases.

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From Brochure to Booklet: Redesigning the Parent Guide to Newborn Screening in Washington State

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Background: The Washington State Newborn Screening Program (NBS) has dreamed of having a better brochure for parents since a critical evaluation by a Seattle University student in 2014; the current trifold brochure is text-heavy and in need of a redesign and content update. The brochure is provided to parents at the time of testing and is kitted with the NBS specimen card and mailing envelope. NBS received a grant from APHL to help implement screening for three additional conditions which included redesigning educational materials, such as the parent brochure. As subject matter experts, NBS created the written content for the updated and expanded booklet and partnered with our agency's Health Promotion and Communication Section (HPCS) to do the graphic design, health literacy editing, and audience testing. Objective: Create an educational publication for parents about newborn screening that is engaging, modern, up-to-date, and easily adaptable to include additional conditions added to the screening panel in the future.
Content and Editing: To create the content for the new publication, NBS considered what information is required by regulation to be provided to parents, content of supplemental parent handouts NBS created in the last five years, and anecdotal feedback from healthcare partners on what information parents want to know about screening. Additionally, NBS performed an informal literature review of other state and national newborn screening educational materials to identify key information for inclusion. Content was then edited for readability and structure and put into Plain Talk, when possible, by the HPCS Health Communicator. Graphic Design: Products from various state and national newborn screening programs were given to HPCS as design inspiration. The HPCS Graphic Designer created an expanded booklet that

includes an updated style, composition, and layout. Using type, image, and visual continuity, NBS now has an engaging and modern publication.
Audience Testing: A HPCS Health Educator, with NBS input, developed an audience testing protocol to obtain feedback from parents on the newly designed publication. The questions focused on readability, comprehension, and overall design. The Health Educator determined appropriate audience testing locations for a representative sample of the state.
Next Steps: Audience testing is in progress and will continue until thematic saturation is reached. Audience responses will be reviewed by HPCS and NBS; feedback will be incorporated into the final version. Once finalized, the booklet will be translated into the top 12 languages spoken in Washington and will be available on the NBS website. English copies will be included with all NBS specimen cards and printed Spanish copies will be available upon request. As conditions are added to the NBS panel, the booklet has space to easily include the new disorder descriptions.

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Clinical Utility of an Expanded Next Generation Sequencing Panel and Next Generation Sequencing Data Reanalysis in Newborn Screening for Cystic Fibrosis

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Newborn screening for cystic fibrosis (CF) is widespread in the United States but molecular CFTR variant testing still varies widely among different states. In Wisconsin, immunoreactive trypsinogen (IRT) testing is the first-tier newborn screening (NBS) test for CF. Specimens in the daily highest 4% of IRT values then undergo second-tier a predefined panel of CF-causing variants analysis. Next generation sequencing (NGS) technology is utilized to fully sequence the CFTR gene and that data is filtered to display a defined panel of CF-causing variants and selected variants of varying clinical consequence when one CF-causing variant is identified. Any newborn with one or more CF-causing variants identified is referred to follow-up with a pilocarpine iontophoresis sweat chloride test as confirmatory testing. Here we report the outcome of our practice that next generation sequencing data is reanalyzed on the specimens with sweat test results greater than 30 mmol/L after one CF-causing variant was identified through a regular NBS protocol. The NGS data reanalysis was performed with removing preset panel limitation and viewing all variants. So far, five specimens went through such practice, and a second pathogenic or likely pathogenic variant was found in three cases. This observation indicates that additional next generation sequencing data reanalysis is a cost-effective practice in identifying the second pathogenic or likely pathogenic CFTR variant in the infant with a likely CF or CFTR-related metabolic syndrome diagnosis.

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Validation of a Multiplexed Real-time PCR Assay to Detect SCID and SMN1 Homozygous Exon 7 Deletion and Droplet Digital PCR Assay to Assess SMN2 Copy Numbers in Newborn Screening for Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is one of the most common lethal recessive genetic conditions, with an incidence of 1 in 11,000 births. The condition is associated with significant motor disability, respiratory and nutritional compromise, and death in infancy or childhood in more than 50% of affected children. Infants with homozygous deletion of SMN1 and 2 SMN2 copies typically manifest the most severe infantile form of SMA, SMA type I. However, symptom onset and disease severity in infants and children with 3 or more copies of SMN2 is more variable. In 2017, FDA approved a novel treatment that could be used in the neonatal period. In 2018, the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) recommended expanding the Recommended Uniform Screening Panel (RUSP) to include SMA caused by homozygous deletion of exon 7 in SMN1, and the Secretary of Health and Human Services accepted the recommendation.

We successfully developed and validated a real-time PCR assay to simultaneously screen for SMA and SCID. The PCR assay identifies the absence of exon 7 in the SMN1 gene while simultaneously evaluating the copy number of the T-cell receptor excision circles (TREC). Additionally, the amplification of a reference gene, RPP30, was included in the assay as a quality/quantity indicator of DNA isolated from the 3.2 mm dried blood NBS specimens. The additional cost for SMA screening test is minimal because it is multiplexed with an existing screening assay used to identify severe combined immunodeficiency (SCID) infants. Moreover, we have further developed and validated a droplet digital PCR (ddPCR) assay to assess the SMN2 copy numbers. We plan to use this test to provide “just in time” information to help clinicians manage the care of newborns who have positive for SMA results. We used blood spots from a well-characterized cohort of subjects with SMA, SMA carriers, and control subjects enrolled in the Newborn Screening Translational Research Network (NBSTRN) Longitudinal Pediatric Data Resource (LDPR) study. Subjects were diagnosed with type 0, I, II, III or IV SMA and had 2, 3, 4 or more than 4 SMN2 copies. Samples from these subjects were used to validate the accuracy, reproducibility and clinical validity of this assay.

Because SMN2 copy number is a major modifier of SMA disease where the higher SMN2 copy numbers are associated with later onset and milder phenotype, identifying infants with 2 or 3 SMN2 copies and treating at the earliest possible timepoint is critical to ensure the best outcomes. The comprehensive approach of NBS for SMA, that includes “just in time” knowledge of SMN2 copy numbers in newborns who have homozygous deletion of exon 7 in SMN1, will facilitate early clinical follow-up, family counseling, and treatment planning.

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P-132

Establishment of Risk Assessment Threshold in Newborn Screening for Pompe Disease: Wisconsin Pilot Project Experience

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Introduction and Objective: Pompe disease is an autosomal recessive lysosomal storage disorder with an estimated incidence of 1:50,000 characterized by myopathy, cardiomyopathy, and deficiency of alpha-1,4-glucosidase (GAA) enzyme activity due to pathogenic variants in the GAA gene. GAA enzyme replacement therapy can be an effective treatment for Pompe disease if initiated early in the course of the disease. However, newborn screening (NBS) for Pompe disease that can systematically and pre-symptomatically identify newborns with GAA deficiency is still nascent. Effectively identifying infantile Pompe disease remains a widespread challenge. Moreover, identifying late onset Pompe disease and distinguishing this from the infantile onset form of the disease remains an even more challenging problem. Here we report a comprehensive NBS approach in the Wisconsin Pompe disease NBS pilot program, which includes GAA enzyme measurement, CLIR analysis, GAA variant analysis, and biochemical second tier testing. This innovative approach has provided a unique opportunity to compare each method's performance in each established risk assessment threshold.
Methods: In the Wisconsin Pompe NBS pilot project, GAA enzyme activity in dried blood spots is used as a primary screening tool. The assay involves incubating a 3.2-mm dried blood spot punch with substrate and internal standard for α-glucosidase at 37°C for 18 hours. The products are purified, dried, suspended, and then analyzed using flow injection tandem mass spectrometry. The enzyme activities are calculated as the function of product per hour compared to the internal standard for the sample. The enzyme reaction mixture contains five additional enzymes. The results of the other five enzyme activities were used to judge specimen quality. Specimens with multiple low enzyme activities are deemed poor quality specimens. All GAA enzyme testing results were subject to the CLIR tool analysis. Specimens with GAA enzyme lower than 10% of the daily median or "likely Pompe" per CLIR undergo GAA gene variant analysis and biochemical second tier testing.
Results: In the one year period from July 14, 2017 to July 13, 2018, we screened 63,716 infants for Pompe disease, and reported twelve screening positive cases. We have identified two pathogenic or likely pathogenic GAA gene variants in every identified and reported screen positive case. Based on clinical confirmatory testing results, those twelve reported screen positive cases likely have late-onset Pompe disease. There were no discordant results in Pompe disease risk assessment among the enzyme cutoff method, CLIR analysis, GAA gene variant analysis, and biochemical second tier testing.
Conclusions: We have successfully established a process to conduct NBS for Pompe disease in a public health NBS laboratory, and there are multiple strategies to avoid carrier and pseudo deficiency identification.

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Improve Pompe Screening Assay Turnaround Time by Reducing Enzyme Incubation Time

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Pompe disease is an autosomal recessive lysosomal storage disorder with an estimated incidence of 1:50,000 characterized by myopathy, cardiomyopathy, and deficiency of alpha-1,4-glucosidase (GAA) enzyme activity due to pathogenic variants in the GAA gene. GAA enzyme replacement therapy can be an effective treatment for Pompe disease if initiated early in the course of the disease. Wisconsin Newborn Screening (NBS) has been conducting a Pompe NBS pilot program since July, 2017. We are currently using a 6-plex assay platform for Pompe screening. The assay involves incubating a 3.2-mm dried blood spot punch with substrate and internal standard for α-glucosidase at 37°C for 18 hours. The products are purified, dried, suspended, and then analyzed using flow injection tandem mass spectrometry. The enzyme activities are calculated as the function of product per hour compared to the internal standard for the sample. Besides GAA, the enzyme reaction mixture contains five additional enzymes. The results of the other five enzyme activities were used to judge specimen quality. Specimens with multiple low enzyme activities are deemed poor quality specimens. In order to improve the Pompe assay turnaround time, we have designed a study to compare Pompe screening performance between 18 hours enzyme incubation and 3.5 hours enzyme incubation. In this poster, we will present the study results.

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Withdrawn

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Wisconsin's Algorithm for Detecting Congenital Adrenal Hyperplasia—Incorporation of a Second Tier Test

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Background: In 1993, Wisconsin implemented newborn screening for congenital adrenal hyperplasia (CAH) and although affected children have been identified and effectively treated, the positive predictive value of the screening assay and algorithm was less than 3 percent. The poor specificity of the laboratory test was largely due to immune assay cross reactivity, as well as physiological changes in the concentration of 17-hydroxprogesterone, the key marker for the disease, during the first few days of life.

Methods: To improve screening for CAH, Wisconsin developed a second tier assay to quantify five different steroids (17-hydroxyprogesterone, 11-deoxycortisol, 21-deoxycortisol, androstenedione, and cortisol) using liquid chromatography tandem mass spectrometry (LC-MSMS) in dried blood spots. Wisconsin established its own unique reporting algorithm that incorporates analyte concentrations, as

well as two different ratios to identify babies at risk for CAH. From validation studies, patients with both classic salt wasting and simple virilizing CAH in addition to non-classical forms were identified. Additionally, the normal population was stratified by birth weight to allow for differences in steroid concentrations between premature and healthy, full-term newborns.
Results: Nine months after implementation of the second tier assay, the number of false positive specimens was dramatically reduced, improving the overall positive predictive value of the screening assay. However, a more detailed analysis of normal fluctuations of steroid concentrations, within the first 72 hours after birth, revealed that additional adjustments to cutoffs could be made to enhance detection. The Wisconsin algorithm for identifying cases of CAH now includes stratification for both birth weight and the timing of collection.
Conclusions: This study replicates and expands upon previous work to implement a second tier screening assay and reporting algorithm that incorporates the birth weight and collection time, thereby substantially improving the positive predictive value of the CAH screening assay.

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Wisconsin's Approach to Addressing Timely Specimen Re-collection after Unsatisfactory Specimen Submission

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Background: Newborn screening laboratories have differing practices regarding the testing of unsatisfactory specimens. Some laboratories avoid testing unsatisfactory specimens because poor sample quality may lead to erroneous test results ('garbage in equals garbage out'). Other laboratories test every specimen, regardless of quality issues, so as to not miss an opportunity to detect a disease state. Wisconsin's practice is to test all specimens, even if they are of unacceptable quality; however test results are reported as inconclusive, unless an abnormality is identified. Additionally, Wisconsin notifies the submitter of a need to re-collect and performs follow-up to increase the likelihood that an acceptable specimen will be received.
The Wisconsin newborn screening program distributes a monthly quality assurance report to submitters that highlights unsatisfactory specimens. Wisconsin sought to extend the focus of this pre-analytic metric to include the timing of the required re-collection.
Methods: The Wisconsin newborn screening laboratory developed a quality assurance report for follow-up of unsatisfactory specimens. Data is tabulated as a percentage of unsatisfactory specimens with re-collection received within the recommended time frame (7 days or less), or greater. The report highlights the percentage of unsatisfactory specimens for which re-collection remains outstanding, as well as the "case closed" specimens in which re-collection will no longer be pursued. A companion report is also available providing patient-level details for those specimens with re-collection outstanding.
Results: The follow-up report was released in January 2018 to submitters who sent in an unsatisfactory specimen that month. Every month thereafter a new report is released updating the number of unsatisfactory specimens and highlighting the re-collection timeframe, as compared to the state average. As of August 2018, 2% of the specimens received, state-wide, were unsatisfactory. Approximately 73% of these unsatisfactory specimens were re-collected within 7 days after notification;

20% re-collected 7 days after notification; 3% remain outstanding with no re-collection; and 4 % were deemed “closed cases”.
The target audience for this report was quality assurance officers and supervisors, who typically do not receive the daily notification of an unsatisfactory specimen; allowing for a more comprehensive review and awareness of the process. The newborn screening program is currently evaluating methods for monitoring improvements in a submitter’s performance.
Conclusion: Wisconsin developed a new monthly quality assurance report that tracks the percentage of unsatisfactory specimens re-collected within the designated time frames. This report serves as a tool for submitters to assess the re-collection process within their facility.

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P-137*

Combining Newborn Metabolic and DNA Analysis for Second-tier Testing of Methylmalonic Academia

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Purpose: Improved second-tier tools are needed to reduce false-positive outcomes in newborn screening (NBS) for inborn metabolic disorders on the Recommended Universal Screening Panel (RUSP).
Methods: We designed an assay for multiplex sequencing of 72 metabolic genes (RUSPseq) from newborn dried blood spots. Analytical and clinical performance was evaluated in 60 screen-positive newborns for methylmalonic acidemia (MMA) reported by the California Department of Public Health NBS program. Additionally, we trained a Random Forest machine learning classifier on NBS data to improve prediction of true- and false-positive MMA cases.
Results: Of 28 MMA patients sequenced, we found two pathogenic or likely pathogenic (P/LP) variants in a MMA-related gene in 24 patients, and one pathogenic variant and a variant of unknown significance (VUS) in one patient. No such variant combinations were detected in MMA false-positives and healthy controls. Random Forest-based analysis of the entire NBS metabolic profile correctly identified the MMA patients and reduced MMA false-positive cases by 51%. MMA screen-positive newborns were more likely of Hispanic ethnicity.
Conclusions: Our two-pronged approach reduced false-positives by half and provided a reportable molecular finding for 89% of MMA patients. Challenges remain in newborn metabolic screening and DNA variant interpretation in diverse multiethnic populations.

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P-138

Development of an Automated Newborn Screening Assay for IRT: Semi-plexed with TSH and 17-OHP Assays

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Immunoreactive trypsinogen (IRT) is an important pancreatic enzyme precursor measured in dried blood spot (DBS) specimens as part of in vitro diagnostic newborn screening for cystic fibrosis (CF). Elevated

IRT concentration is an indicator for CF disease in neonates, and presumptive positive results must be followed with second-tier and confirmatory diagnostics. CF is a core condition on the U.S. Recommended Uniform Screening Panel and is screened for internationally, signifying the importance of this first-tier IRT assay. The SPOTCHECK® Neonatal IRT Screening Kit (in development) consists of a sandwich enzyme immunoassay for the quantification of IRT in dried neonatal blood spots. Using the automated SPOTCHECK® Pro neonatal screening system and employing multi-analyte DBS calibrators, IRT is processed concurrently with TSH and 17-OHP assays from a common mother plate. Preliminary performance data for the high throughput assay indicates presumptive clinical screening effectiveness.

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Beyond Enzyme Assays: The Versatility of Digital Microfluidics for Screening and Clinical Testing in Newborns

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

Newborn screening has changed dramatically over the first 50+ years of its existence, from a single bacterial inhibition assay for phenylketonuria to dozens of distinct physiological, biochemical and molecular tests for at least 40 different conditions today. To aid in the ongoing expansion of newborn screening, our team has applied digital microfluidics technology to automate a broad range of clinical assays onto disposable cartridges using nanoliter droplets of sample (DBS, saliva, urine, plasma, and whole blood) and reagents. In addition to enzyme activity assays for lysosomal storage disorders, we have successfully developed basic chemistry assays, molecular assays, and immunoassays on the digital microfluidic platform. These assays currently number over 50 and address many of the most common conditions affecting newborns, including endocrine (TSH, T3, T4, insulin), metabolic (GALT, BTD, PHE, beta hydroxybutyrate), blood/coagulation (G6PD, bilirubin, ATIII, FVIII, vWF), renal (creatinine, NGAL), neuromuscular (creatine kinase, GAA, IDUA), and infectious disease (cCMV, MRSA, Candida). Our assays are developed using substrates and detection modalities that maximize sensitivity and precision within each assay. Multiple cartridge formats are available to accept anywhere from 1 to 40 samples in a single cartridge run, and up to four individual cartridges can be run simultaneously from the same instrument workstation to customize testing throughput. The rapid turnaround time and flexible format of our technology enable testing in a variety of settings ranging from public health laboratories to clinics, including point-of-care testing in the nursery or neonatal intensive care unit. Importantly, the small volume format of our platform minimizes the amount of sample needed and enables frequent testing of conditions that require recurrent testing, thereby reducing risks for vulnerable or low birthweight newborns already under clinical distress.

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Combining Creatine Kinase Activity and Isoenzyme Measurements for Newborn Duchenne Muscular Dystrophy Screening on the Digital Microfluidic Platform

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Duchenne muscular dystrophy (DMD) is the most common pediatric form of muscular dystrophy affecting 1 in 5,000 male births. Several promising therapies for DMD are on the horizon and nomination of the disorder to the Recommended Uniform Screening Panel (RUSP) is widely anticipated. Pilot DMD newborn screening studies based on the quantification of total creatine kinase (CK) enzyme activity or expression of a skeletal isoform of CK (CK-MM) have provided important data about the natural history of each analyte. We have developed a combinatorial newborn screening approach for DMD that uses digital microfluidic (DMF) technology to automate quantitative assays for both CK enzyme activity and CK-MM isoenzyme protein expression in newborn dried blood spot (DBS) extracts. In contrast to single analyte CK activity screening tests, our combined approach will safeguard against false negative findings while minimizing false positives. The DMF CK enzyme assay is based on the protocol used in a previous Pennsylvania DMD pilot (Orofanos & Naylor) and has excellent sensitivity across the expected clinical range for newborn DBS. The CK-MM isoenzyme immunoassay is completely automated on the digital microfluidic cartridge using paramagnetic beads and magnets located in the instrument deck. Assay performance data including linearity, specificity and precision, including discrimination of normal newborn DBS from affected DMD patient samples will be presented for each assay. Our combined DMD screening approach will be optimized for newborn DBS samples and will efficiently identify babies requiring follow-up. Additional advantages of the digital microfluidic platform for DMD newborn screening include: reduced reagent costs due to the sub-microliter reaction droplet size, small instrument footprint with easy installation, and minimal hands-on effort required.

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A Digital Microfluidic Nucleic Acid Test for Congenital Cytomegalovirus Infection in Newborns

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Congenital cytomegalovirus (cCMV) infection is the most common congenital viral infection, affecting one in every 200 newborns in the United States. The vast majority of babies born with cCMV appear healthy at birth, but nearly one in every five will later develop permanent health problems such as hearing loss. In the absence of clear guidelines for universal newborn screening for cCMV infection, hearing targeted early cytomegalovirus screening by nucleic acid testing is gaining momentum and has been underway in Utah for more than 5 years. To support efforts to expand the identification of cCMV infection in newborns, we have developed a digital microfluidic (DMF) method for nucleic acid cCMV testing from saliva specimens. The small footprint DMF platform is suitable for use in either a public health lab or hospital setting. All steps including sample lysis, extraction, purification, and real-time PCR amplification are performed on a disposable cartridge using 300 nanoliter droplets. Saliva swabs collected from infants are placed in a medium, which is transferred to a DMF cartridge along with reagents. All subsequent steps are performed in an automated fashion without user intervention.

Analytical validations including limit of detection, specificity, precision, and interference were performed and met established requirements. An initial method comparison to the gold-standard PCR assay with archived and prospectively collected newborn saliva samples revealed excellent correlation between the methods. These results demonstrate that CMV in newborn saliva can be detected using a fully automated platform to enable early identification of infected infants.

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Early Initiation of Nusinersen Treatment on Motor Milestone and Motor Function Response in Symptomatic Infantile-onset SMA and in Infants in a Presymptomatic Stage of SMA

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Background and Objective: Nusinersen is an antisense oligonucleotide that is approved for the treatment of spinal muscular atrophy (SMA). The objective was to assess the effect that timing of initiation of therapy has on magnitude of benefit by examining data from infants who initiated treatment prior to SMA symptom onset in NURTURE (NCT02386553) and in infants who initiated nusinersen after symptom onset in ENDEAR (NCT02193074) and continued treatment in the SHINE extension study (NCT02594124).
Methods: The proportion of participants who met protocol-defined Hammersmith Infant Neurological Examination Section 2 (HINE-2) motor milestone response criteria (≥2-point increase or maximal score in kicking ability or ≥1-point increase in head control, rolling, sitting, crawling, standing, or walking and more categories improving than worsening) was evaluated in NURTURE infants and in subgroups of SHINE/ENDEAR participants who initiated nusinersen treatment before or after the median age at the time of first dose (≤5.42 vs >5.42 months). The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) mean total score and proportion of SHINE/ENDEAR participants by subgroup who met CHOP INTEND response criteria (≥4-point score improvement from baseline) was also assessed. Data cut off dates for NURTURE and SHINE were May 15, 2018 and June 30, 2017, respectively.
Results: At data cut off, 25 infants were enrolled in NURTURE and received ≥7 doses of nusinersen. Median age at first dose was 22 (range: 3-42) days and at last visit was 26 (14–34) months. At the interim analysis, all NURTURE infants were alive and none required permanent ventilation (≥16 hours/day continuously for >21 days in the absence of an acute reversible event). At the Day 700 study visit, 100% of NURTURE infants with 2 SMN2 copies (10/10) and 3 SMN2 copies (5/5) met the HINE-2 response criteria, and CHOP INTEND mean total score was 59.1 (SD 5.87; n=12). In SHINE/ENDEAR, at Day 698, 67% (8/12) of participants who were age ≤5.42 months at first dose met HINE-2 response criteria versus 28% (5/18) of participants who were age >5.42 months at first dose. At Day 698, CHOP INTEND mean total score in SHINE/ENDEAR was 42.5 (SD 12.82; n=17) and there was a greater proportion of CHOP INTEND responders in participants who were age ≤5.42 months at first dose (77%; 10/13) versus those who were age >5.42 months at first dose (47%; 8/17).

Conclusions: After nearly 2 years of treatment, all infants who initiated nusinersen in the presymptomatic stage of SMA met the protocol-defined criteria for HINE-2 motor milestone response (15/15) and mean CHOP INTEND total scores were near the maximum scale value of 64 points. Among symptomatic infants, earlier treatment with nusinersen was associated with greater benefit. These results highlight the benefits of early treatment and value of newborn screening initiatives. Study support: Biogen

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Newborn Screening for Six Lysosomal Storage Diseases in a Cohort of Mexican Patients: Six-year Findings from a Screening Program with a National Coverage

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Lysosomal storage diseases (LSD) are a group of more than 50 genetic disorders in which there is a decrease or an absence of activity, inadequate lysosomal enzyme or carrier proteins biogenesis producing progressive accumulation of precursor metabolites within the lysosomes that result in cellular dysfunction and multiple organ system failure. Currently, there are no comprehensive studies that show the incidence in Mexico for LSD.

We retrospectively analyzed 70,403 newborn screening (NBS) reports storage in our database between January 26, 2012 and September 27, 2018. The results were obtained from samples placed on filter paper and processed by tandem-mass spectrometry at PerkinElmer Genomics (Bridgeville, PA, USA). Six different LSD were studied, including Pompe disease, Fabry disease, Gaucher disease, mucopolysaccharidosis type I (MPS-I), Niemann-Pick type A/B disease, and Krabbe disease. The protocol followed to get an LSD diagnosis began with a NBS first sample showing a decreased enzymatic activity, a second sample with a consistent abnormal result, performing afterwards a confirmatory testing through serum enzymatic activity and/or gene sequencing.

For the 70,403 NBS reports analyzed, 34,416 were female and 35,987 were male. On average, the NBS was performed at 5.37 days of age. After the second sample analysis, we found 73 LSD abnormal results. The diagnosis was confirmed in 24 newborns (1 for Niemann-Pick, 6 for Pompe, 1 for Krabbe, 13 for Fabry, and 3 for MPS-I). Furthermore, 13 newborns were identified as heterozygous (4 for Niemann-Pick, 8 for Pompe, and 1 for Krabbe), and 19 newborns with pseudodeficiency (1 for Niemann-Pick, 17 for Pompe, and 1 for MPS-I). The last 17 newborns were ruled out after a normal confirmatory test. The false positive rate for the NBS was 0.07%. On the other hand, the positive predictive value was 32.88%. Noteworthy that the newborns confirmed with an LSD were diagnosed approximately at 52 days +/- 22 days after the collection of the first sample.The incidence of the six LSD studied in the Mexican newborn population studied was 3.41 cases per 10,000 newborns, being Fabry disease the most frequent pathology (1.85:10,000). Carrying out a newborn screening that includes these LSD has allowed us to establish an early diagnosis, initiate an appropriate and timely treatment in order to improve their quality of life. Moreover, genetic counseling was given.

In our experience, a prompt diagnosis is achieved thanks to a close follow up by the integration of all parties participating in the NBS program (laboratory, medical staff, parents, social workers, among others).

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Tier 1 Screening for Enzyme Deficiencies Associated with GAMT (guanidinoacetate methyltransferase) using Flow-injection MS/MS

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GAMT deficiency is a severe neurological disorder, caused by the toxic accumulation of guanidinoacetate (GAA), resulting in impaired cognitive and motor development. While no therapies for GAMT deficiency have been approved, research has shown that the physiological amino acid ornithine is a competitive inhibitor for AGAT, which produces the neurotoxic GAA. Thus, it is crucial to diagnosis GAMT deficiency during the early days of life, a critical neurological development window for neonates. Here we describe a flow-injection, non-derivatized method for detection of GAA in contrived positive dried blood spots (DBS). Briefly, blood was spiked with clinically relevant concentrations of GAA to mimic contrived positive samples for GAMT deficiency. Dried blood spots were then made by spotting blood onto filter paper, allowing it to dry, followed by subsequent extraction of GAA. A PerkinElmer QSight® 210 MD mass spectrometer was used, for research purposes only, to demonstrate the capability of the assay. Contrived positive DBS with elevated GAA were successfully flagged via the tier 1 methodology and separated from presumed negative DBS. We conclude that the assay and instrumentation used here can accurately screen for enzymatic defects associated with GAMT, as done in a newborn screening laboratory.

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Tier 1 and Tier 2 Mass Spectrometry Methodologies for Determining Enzymatic Defects Associated with Pompe Disease

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Pompe disease is a rare lysosomal storage disorder caused by a defect in the acid alpha-glucosidase (GAA) enzyme, rendering it unable to break down glycogen. In particular, glycogen accumulates in skeletal and cardiac tissue, resulting in neuromuscular degeneration and cardiomyopathy. Enzyme replacement therapy has proven to be an effective treatment, with the product Lumizyme®, especially if administered before pathogenesis of the disease. Therefore, it is crucial to detect Pompe disease during the early days of life, a critical development window for neonates. Here we describe two mass-spectrometry based assays which determine GAA activity through first tier rapid screening and subsequent confirmatory analysis of tier 2 markers ((creatine/creatinine)/GAA) using LC-MS/MS in contrived positive samples. Contrived positive dried blood spots (DBS) were made by using leukocyte depleted red blood cell concentrate, spiked with creatine and creatinine. Presumed negative DBS were

made by using whole blood, spiked with creatine and creatinine. These were first subjected to an enzymatic assay, followed by a rapid flow-injection based MS/MS to distinguish contrived positives from presumed negative DBS for Pompe. Flagged contrived positives were then subjected to a separate tier 2 LC-MS/MS assay capable of separating interfering isobars, to screen for elevated ((creatine/creatinine)/GAA) ratios . Briefly, for tier 2 testing, DBS were extracted for creatine, creatinine, and GAA, then analyzed against a standard curve, in order to identify contrived positives. A PerkinElmer QSight® 220 CR instrument was used for analysis. Here, we demonstrate that the activity-based flow-injection MS method is able to determine deficient GAA activity in contrived positives for Pompe disease. The second tier LC-MS/MS assay is capable of flagging contrived positives with high, clinically relevant ((creatine/creatinine)/GAA) ratios. With this approach, we demonstrate a single LC-MS instrument which is capable of both tier 1 and tier 2 testing for Pompe disease.

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Automation of Tier 2 Newborn Screening from Positive Tier 1 Results using the PerkinElmer QSight® 225 MD

C. Hill, R. Maharajh, S. Smith, T. Au Yeung and B. Williamson, PerkinElmer, Waltham, MA

Newborn screening labs are responsible for the rapid testing of several hundreds, if not thousands of samples a day for various inborn errors of metabolism. Typically, this is done by a flow-injection MS/MS approach (Tier 1) which allows for quick turnaround of clinical data. While the incidence of flagged potential positives for any disease is very low, those that are flagged must be retested via a separate Tier 2 approach. Often, this is done with a longer chromatography-based methodology which is capable of separating out interfering isobars before the biomarker of interest is introduced into the mass spectrometer. Retesting of these samples often becomes a bottleneck, as the samples must be re-prepared and then subjected to subsequent analytical tests, which adds time, complexity and room for human error to the process. Here, we demonstrate an automated workflow using the PerkinElmer QSight® 225 MD (in development) mass spectrometry system, where samples that are positive for tier 1 markers are automatically retested via tier 2 methodologies, without the need for human intervention. Contrived positive samples for both tier 1 and tier 2 markers were made and subjected to the automated methodologies described here. Examples of diseases associated with enzymatic effects tested include Krabbe disease and X-linked adrenoleukodystrophy. This approach allows for quicker retesting via tier 2, resulting in important clinical data being delivered to health care professionals in a timelier fashion and greatly reduces the risk of human error during sample transport and reinjection. This is of particular importance in the newborn screening community where time is critical and confirmation of disease status is required before significant onset of pathogenesis.

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A Six-plex Assay for Lysosomal Storage Disorders using the PerkinElmer NeoLSD Kit Coupled with a QSight® 210 MD Mass Spectrometer

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Lysosomal storage disorders (LSD) are inborn errors of metabolism caused by enzyme deficiencies, resulting in accumulation of toxic species in the lysosome. While more than fifty LSDs have been identified, only some of the most common disorders are being screened in neonates. Accurate diagnosis of these diseases during the early stages of life is critical, as therapies may be available for children during a critical development window. PerkinElmer has developed a multiplexed IVD newborn screening kit testing for six of the LSDs: Pompe, Fabry, Gaucher, MPS-1, Nieman-Pick and Krabbe which are due to deficiencies in the respective enzymes, GAA, GLA, ABG, IDUA, ASM, and GALC. The biochemical NeoLSD™ assay used here supplies surrogate substrates for the six enzymes and when coupled with a PerkinElmer QSight® 210MD MS/MS device, a complete solution for newborn screening labs is available. In this study, contrived dried blood spots (DBS) with varying levels of enzyme activity were tested for numerous analytical parameters including: 21 day precision, linearity, limit of detection, and interference. We demonstrate excellent performance of the assay in all analytical tests, for all six of the enzymes. With this in mind, the PerkinElmer NeoLSD™ kit and QSight® 210 MD mass spectrometer offer an excellent solution for newborn screening labs to screen for six of the lysosomal storage disorders.

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MigeleTM Gel Electrophoresis Unit for Hemoglobinopathies Detection

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MigeleTM Gel Electrophoresis Unit to be used with RESOLVETM Hemoglobin kits was launched in July 2018. This instrument has been developed to be used by the hemoglobinopathies screening customers to detect infants at risk of hemoglobinopathies within the neonatal period.

A method comparison study was performed with the new MigeleTM Gel Electrophoresis Unit and the existing electrophoresis unit. The study was made with RESOLVETM Hemoglobin kits FR-9120 and FR-9400 and using in total of 122 normal dried blood spot, 56 abnormal dried blood spots, 37 cord blood and 20 whole blood samples for the analysis. 56 abnormal samples were classified as abnormal and 179 normal samples were classified as negative with both instruments and kit versions FR-9120 and FR-9400. The overall agreement was 100% and the screening efficacy of confirmed positive specimens is identical between MigeleTM Gel Electrophoresis Unit and existing electrophoresis unit.

Based on the results of the performance comparison study it can be concluded that the screening efficacy of the MigeleTM Gel Electrophoresis Unit is substantially equivalent to the existing electrophoresis unit and is suitable to be used for aid in the diagnosis of neonatal and adult hemoglobinopathies.

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Sequential Elution: Demonstration of a Novel, Multi-Sampling Method for Dried Blood Spots
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The challenge for laboratories to decide which assays to implement is ever-present with the continual advances in science. With more options available than most laboratories have resources, there is a demand to make testing even more efficient than previously required. In newborn screening, testing can be readily performed using a punch from a dried blood spot (DBS) made from a newborn heel stick. Traditionally, each assay performed in the lab needs an individual punch from the DBS card. There is a limited number of times that a DBS card can be punched; thus, capping the number of tests than can be done on that specimen. A common way to maximize the number of analytes than can be tested in a single DBS punch is to favor a multiplex assay over a single-plex test, which also decreases the cost per analyte. A new approach, presented here, is the ability to perform two multiplex assays on the same DBS punch, which will allow obtaining adequate results while reducing the required sample material by half. Sequential Elution is a method that can expose the DBS punch to conditions that will measure enzyme activities via mass spectrometry followed by immediate processing to extract DNA. The DNA is then used to quantify four genomic and extra-genomic loci via real-time PCR. This method was demonstrated on over one thousand, putative normal newborn DBS and run with PerkinElmer's NeoLSD, NeoBase 2 mass spectrometry kits as well as a four-plex real-time PCR assay. The PCR assay identifies the absence of exon 7 in the SMN1 gene without the amplification detection of the homologous SMN2 gene as well as T-cell receptor excision circles (TREC) and for K-deleting recombination excision circles (KREC). The performance was further characterized using DBS reference samples for both the mass spectrometry and real-time PCR assays. The results from this study demonstrate a new workflow for newborn screening labs that will reduce the required samples from each patient as well as the hands-on time for each assay.

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A High-throughput Multiplex qPCR Screening Assay For SCID, SMA, and XLA in Newborns
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A four-plex, real-time PCR assay was developed to effectively identify the homozygous deletion of exon 7 in the SMN1 gene and simultaneously evaluate the copy number of T-cell receptor excision circles (TREC) and Kappa-deleting recombination excision circles (KREC) in the DNA extracted from a 3.2 mm newborn DBS punch. The lack of TREC and KREC molecules in the blood and the homozygous deletion of SMN1 exon 7 are widely-accepted biomarkers of SCID, XLA and SMA for newborn screening, respectively. The assay is integrated into a semi-automated, high-throughput system, which consists of PerkinElmer's JANUS® G3 Automated Workstations for automated DNA extraction and PCR setup, a thermocycler supporting 384-well plates, and stand-alone analysis software. The system has the capacity of processing more than 1,500 DBS samples from sample to result in less than 8 hours with

minimal hands-on time and sample tracking capability. The system was tested with over 1,000 newborn DBS samples as well as contrived samples and its analytical performance was validated intensively following CLSI guidelines. The results confirm the viability of this testing system and demonstrate its upcoming benefits to newborn screening programs.

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Screening of Inborn Genetic Disorders X-ALD, ADA-SCID, ASA-LD and OTCD with Specific New Analytes Included in the NeoBase™ 2 Non-derivatized MSMS Kit

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In total 51 analytes can be screened with the FDA-approved next generation NeoBase™ 2 Non-derivatized MSMS kit, providing more conclusive screening results and possibility to screen more inborn errors of metabolism when compared to the previous non-derivatized MSMS kit, NeoBase™. New analytes include very long-chain lysophosphatidylcholines C24:0-LPC and C26:0-LPC, which levels reflect the abnormal very long-chain fatty acid (VLCFA) profile in the X-linked adrenoleukodystrophy (X-ALD) patients. X-ALD occurs, when mutated ABCD1 gene encodes defected adrenoleukodystrophy protein (ALDP) involved in VLCFA transport and thus leading to VLCFA accumulation in body. Specific markers for Adenosine deaminase severe combined immunodeficiency (ADA-SCID), Adenosine (ADO) and 2-Deoxyadenosine (D-ADO), have been included to effectively screen ADA-SCID patients. Deficient ADA enzyme has reduced ability to convert deoxyadenosine to non-toxic metabolites causing eventually severe combined immunodeficiency (SCID). 10–15% of all SCID cases have ADA deficiency, which onset may be delayed. Additional specific markers, Argininosuccinic acid (ASA), Glutamine (Gln) and Glutamic acid (Glu) were included to improve screening performance of two of the most common Urea cycle disorders, Argininosuccinic acid lyase deficiency (ASA-LD) and Ornithine transcarbamylase deficiency (OTCD). NeoBase™ 2 Non-derivatized MSMS kit controls include now 29 analytes containing C26:0-LPC, Adenosine, Argininosuccinic acid and Glutamine in low and high levels. Two external studies were conducted in US*. In both studies approximately 2000 newborn specimens were tested for population distribution and 2500 specimens to demonstrate screening performance including 4 X-ALD, 4 ADA-SCID, 3 ASA-LD and 3 OTCD confirmed positive specimens. All listed specimens were identified, or screening performance was shown to be improved when NeoBase™ 2 assay with new analytes was used.

* Outcomes of the two external studies have been published in the 11th ISNS European Regional Meeting (Bratislava, Slovakia)

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Clinical Performance Evaluation of the NeoLSD™ MSMS Kit for Lysosomal Storage Disorder (LSD) Newborn Screening

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Lysosomal storage disorders (LSD) are a collection of nearly 50 genetically acquired diseases with an estimated combined worldwide incidence of approximately 1 in 7000 - 8000 live births. These disorders result from the dysfunction, deficiency or absence of a lysosomal enzyme. Affected individuals are unable to metabolize the disease specific substrate of the deficient enzyme, which leads to its progressive accumulation in the lysosomes of tissues. The NeoLSD™ MSMS Kit (3093-0010, 3093-001U) is a new multiplex assay for the quantitative measurement of the activity of the enzymes acid-β-glucocerebrosidase (ABG), acid-sphingomyelinase (ASM), acid-α-glucosidase (GAA), β-galactocerebrosidase (GALC), α-galactosidase A (GLA) and α-L-iduronidase (IDUA) in dried blood spots (DBS) from newborn babies. The analysis of the enzymatic activity is intended as an aid in screening newborns for the following lysosomal storage disorders (LSD) respectively; Gaucher Disease, Niemann-Pick A/B Disease, Pompe Disease, Krabbe Disease, Fabry Disease, and MPS I Disease. This assay is the first IVD kit bearing CE mark and cleared by FDA to screen newborns for the 6 LSDs.The objectives of the clinical performance study were to produce newborn population distribution data for each of the 6 enzymes using the NeoLSD™ MSMS assay and demonstrate that the subjects affected with any of the screened disorders can be distinguished from the normal population by using the site-specific cut-offs derived from the population distribution data. The specimens were archived newborn screening dried blood spot samples from the Danish Newborn Screening Biobank collected from 30 affected - 5 Gaucher, 1 Niemann-Pick A, 10 Krabbe, 5 MPS I, 5 Fabry, and 4 Pompe - and approximately 4000 unaffected newborns. Clinical outcome was obtained from all samples and used as a comparator for the NeoLSD™ MSMS assay. NeoLSD™ MSMS kit was found to correctly identify affected subjects with low lysosomal enzyme activity from the normal population. The sensitivity of the NeoLSD™ MSMS assay was 92.9% and 100 %, when two screen negative Fabry samples were included and excluded, respectively. The specificity of the assay was 99.4%, leading to 0.6% false positive rate. The NeoLSD™ MSMS kit showed excellent screening performance. Since the enzyme activities of the lysosomal enzymes change with the age of the newborn laboratories should take this into account when establishing cut-off values.

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Seven-plex MS/MS Method to Measure I2S, NAGLU, GALNS, GLB1, ARSB, GUSB, and TPP1 Enzyme Activities in DBS on a PE QSight® 220 CR

J. Trometer, C. Hill, M. Timmons, T. Au Yeung and B. Williamson, PerkinElmer, Waltham, MA

The mucopolysaccharisodes (MPS) family of lysosomal storage disorders (LSDs) is caused by defects in the metabolic breakdown of glycosaminoglycans (GAGs). This work demonstrates a novel MS/MS assay that simultaneously monitors the activity of seven different MPS enzymes. Using a single 3.2 mm dried blood spot (DBS) punch and incubation cocktail, our assay has the ability to identify samples with low enzyme activities for I2S (MPS II), NAGLU (MPS IIIB), GALNS (MPS IVA), GLB1 (MPS IVB), ARSB (MPS VI),

GUSB (MPS VII), and TPP1 (CLN2). The seven-plex is incubated overnight in the presence of incubation cocktail at 37 °C followed by a post-incubation, fully automated workup that is less than 30 minutes per plate. Enzyme activities are measured by LC-MS on a PE QSight® 220 CR triple-quad Mass Spectrometer. Sample-to-sample time using MS/MS analysis can be as low as 2 minutes, which allows the possibility to obtain more than 5000 results per day if desired. Method performance studies show good linearity for each enzyme in their respective activity range. Furthermore, a study consisting of several hundred presumed healthy neonates, confirmed low I2S, NAGLU, GALNS, GLB1, ARSB, GUSB, and/or TPP1 activity and CDC control DBS showed excellent resolution and clear distinctions between the different enzyme activity levels.

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Hemoglobinopathies Detection with the MigeleTM Gel Electrophoresis Unit in Nevada

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Newborn screening laboratories are utilizing different methods for hemoglobinopathies detection [1]. Isoelectric focusing based method (RESOLVETM Hemoglobin Kits, PerkinElmer) has already been in the market for multiple decades and it can be used as a primary or secondary screening method for children and adults. This solution ensures robust, accurate and cost-efficient choice for detection of hemoglobinopathies. [2] MigeleTM Gel Electrophoresis Unit was launched in July 2018 to be used with RESOLVETM Hemoglobin kits. During the development of the instrument, it has been shown to have equal performance with the predecessor. Easy to use design, increased stability and four units that can be connected into series have proven that this instrument is clearly a preferred solution to be used with RESOLVETM Hemoglobin kits. During the unit development, Nevada State Newborn Screening Laboratory kindly tested the unit using the control material and provided feedback that the unit can be used in the hemoglobinopathies detection workflow. Currently, the method is being taken into use in a routine hemoglobinopathy screening laboratories and we see that this method is a choice for the hemoglobinopathies detection now and in the future.

[1] Benson J.M., Bradford, L. & Therrell Jr. MS. (2010) History and Current Status of Newborn Screening for Hemoglobinopathies. Seminars in Perinatology, 34: 134-144.
[2]https://www.cdc.gov/ncbddd/sicklecell/documents/nbs_hemoglobinopathy-testing_122015.pdf

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A Two-Tiered Approach for Newborn Screening of X-Linked Adrenoleukodystrophy

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X-linked adrenoleukodystrophy (X-ALD) is an inherited peroxisomal disorder affecting approximately 1 in 17-20,000 births. Due to the severity of the disease, X-ALD is currently part of numerous state newborn

screening (NBS) panels, as early detection can allow for life saving intervention. Screening is accomplished through the measurement of a biomarker, C26:0 lysophosphatidylcholine (C26:0 LPC) from dried blood spot samples using LC-MS/MS. Primary screening is accomplished using flow injection analysis (FIA). Samples with a measured C26:0 LPC concentration above an established threshold, will undergo second tier testing. This involves utilizing liquid chromatography to separate C26:0 LPC from an isobaric endogenous interferent that is often present within DBS samples. This two-tiered approach allows for increased sample throughput and reduced cost. Recently, the PerkinElmer QSight® MD 210 screening system has become available. The novel source design provides a self-cleaning ability, which was found to be advantageous in X-ALD screening. After performing approximately 1,000 injections, the precision of internal standard response was within 20 %, without performing any routine cleaning of the ion source. This instrumentation was also found to provide a 10-fold increase in sensitivity and a 30 % reduction in sample-to-sample analysis time when compared with current methodology. Overall, the QSight® MD 210 screening system was found to provide a comprehensive solution for newborn screening of X-ALD.

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Clinical Performance Evaluation of the GSP® Neonatal Creatine Kinase – MM Kit for Duchenne Muscular Dystrophy (DMD) Newborn Screening in the US

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Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy among children affecting mostly males (1:5000) and caused by mutations in the dystrophin gene. With DMD, muscle specific creatine kinase isozyme CK-MM is released into bloodstream enabling screening for DMD with this biomarker. GSP® Neonatal Creatine Kinase –MM kit is a new fully automated assay based on DELFIA® technology for the GSP platform. The assay is currently in development.

The objectives of the clinical performance study were to produce newborn CK-MM distribution data in the US population using the GSP CK-MM assay and evaluate the assay ability to distinguish newborns at an increased risk for DMD from the normal population.

The study specimens included 3041 de-identified residual routine newborn screening dried blood spot samples provided by a Newborn Screening Laboratory in the US. The routine newborn specimens were collected between >24 to 730 hours after birth. To enrich the study population, 30 confirmed positive samples that had been collected from DMD affected newborns and archived in California Department of Public Health- Biobank Program were also analysed. The archived positive specimens were collected between16 to 65 hours after birth.

With 99.5th percentile cut-off, which corresponded to CK-MM concentration of 2040 ng/mL, all known 30 DMD positive specimens were classified as screening positive with CK-MM ranging from 2390 to

22400 ng/ mL. Second tier testing by dystrophin gene mutation analysis using the Next Generation Sequencing method was performed. Among 21 samples having a CK-MM concentration value above the 99.5th percentile, four male samples were found to have a DMD pathogenic gene mutation. The median values for presumed unaffected and DMD positive specimens were 348 ng/ml and 14 300 ng/ml, respectively. Based on this sample set, CK-MM concentration was shown to correlate inversely with the age of the newborn in non-DMD specimens.

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Improved GSP® Neonatal GALT Kit Performance

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Classical galactosemia caused by galactose-1-phosphate uridyltransferase (GALT) deficiency is one of the most urgent disorders in the recommended uniform screening panel (RUSP). PerkinElmer GSP® Neonatal GALT kit (3303-001U) was the first enzymatic assay to be developed on the automated GSP® platform in 2010. Based on the customer feedback collected in the US in 2017 the GSP Neonatal GALT assay was updated to better meet the current user requirements for galactosemia newborn screening. The most important requirements identified for the assay were to reduce the number of false positive results and to minimize unnecessary recalls.

The required improvements to the product translated from the customer needs were identified as: • to enable GALT activity measurement from floating disks which were classified as presumptive positive • to improve the assay resolution in the clinically relevant range • to reduce false positive results due to G6PD deficiency • to extend reagent on-board stability

The GALT activity measurement from floating disks was enabled by changing the measurement method of the assay well. This improvement required no reagent modification, simply an update to the GSP software. Internal verification studies showed that the updated assay was able to measure GALT activity from the floating disks accurately and consistently.

To reduce the number of false positive results, the assay protocol was modified, and the buffer composition was re-optimized. The on-board stability of the kit reagents was also improved by using the optimized assay buffer. In internal feasibility testing, protocol and chemistry modification in GSP Neonatal GALT assay gave less positive results for G6PDS deficient samples and the on-board stability increased to a minimum of 7 days from the current 48 hrs. These kit design changes require a clinical validation study in the US before implementation.

After the update floating disks are not classified as presumptive positives but the GALT activity can be measured. By omitting the unnecessary recall of the newborn the updated GSP GALT kit reduces the burden on the healthcare system. In addition, the parent anxiety will be avoided as it is not necessary to halt breastfeeding to a false positive result caused by floating disks.

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Screening Performance of the NeoLSD(TM) MSMS Kit on the PerkinElmer QSight(TM) 210 MD Screening System

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Introduction: The NeoLSD MSMS kit is intended for the quantitative measurement of the activity of the enzymes acid-β-glucocerebrosidase (ABG), acid-sphingomyelinase (ASM), acid-α-glucosidase (GAA), β-galactocerebrosidase (GALC), α-galactosidase A (GLA), and α-Liduronidase (IDUA) in dried blood spots (DBS) from newborn babies. The analysis of the enzymatic activity is intended as an aid in screening newborns for the following lysosomal storage disorders (LSD) respectively; Gaucher Disease, Niemann-Pick A/B Disease, Pompe Disease, Krabbe Disease, Fabry Disease, and mucopolysaccharidosis Type I (MPS I) Disease. The NeoLSD MSMS kit was cleared by FDA for use with the Waters TQD MSMS instrument (TQD). PerkinElmer is extending use of the NeoLSD MSMS kit to the PerkinElmer QSight 210 MD MSMS Screening System (QSight platform). The intended use of the NeoLSD MSMS kit will not be changed.

Objective: This study is designed to demonstrate the NeoLSD MSMS kit screening performance on the QSight platform is equivalent to its screening performance on TQD platform.

Methods: One DBS punch is prepared according to the NeoLSD MSMS kit insert and used for testing on both MSMS platforms. Two aliquots of the organic layer of the extracted sample will be transferred to two different sampling plates, one plate analyzed on the QSight platform and one plate on the TQD platform. Transferring the samples to two different sampling plates will reduce variation in the assay due to sample preparation.

The Florida Department of Health Newborn Screening Laboratory will conduct this study with the TQD platform used during the predicate validation study. The subject population in this study will be the same population from which cut-offs were calculated for use in the TQD validation study. Prior to the screening performance study, a 300-sample method comparison will be performed to confirm the cut-off values determined with the NeoLSD MSMS kit on the TQD platform are applicable to the NeoLSD MSMS kit on the QSight platform. TQD cut-off values will be acceptable for transfer to the QSight platform if bias at medical decision point is smaller than ±10% for all enzymes. If correlation of the assay results is not confirmed by method comparison, a 2000 sample population distribution study will be conducted to establish cut-offs for use with the QSight platform in the screening performance study.

For screening performance, a minimum of 2000 leftover deidentified newborn DBS specimens, submitted to the laboratory for routine 1st tier screening, will be assayed with NeoLSD MSMS kit in parallel on both platforms. This routine sample cohort will be enriched with leftover, LSD-screen positive samples, if available, and sponsor-provided contrived samples, simulating LSD-positive samples. Established cut-offs will be applied to classify the study samples into screen positives and screen negatives.

Results: Pending.

Conclusions: Pending.

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

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Introduction: Hereditary Tyrosinemia Type I (HT-1) is a rare autosomal recessive disease that affects 1 in 100,000 people worldwide, and presents as a deficiency in fumarylacetoacetate hydrolase (FAH). If not detected early and treated, patients can develop liver failure with death likely in the early years of life. Asymptomatic detection of HT-1 can be determined by measuring tyrosine or succinylacetone (SUAC) levels. Traditionally, elevated tyrosine was used as a diagnostic marker but it has been determined that it is not sufficiently specific for the disease and can yield false positive and false negative results. SUAC is now the method of choice as it is more sensitive and specific than tyrosine as a diagnostic marker for HT-1. In 2015, 39 of 50 states tested HT-1 with SUAC as a primary marker, while 5 used SUAC as a secondary marker and 6 states used tyrosine alone as marker for HT-1 in newborn screening. The objective of this study was to determine whether the 11 states using tyrosine as a primary HT-1 marker in 2015 have moved to SUAC as their primary detection method in 2018.
Methods: The 11 states that used tyrosine as a primary HT-1 marker (Alabama, Connecticut, Delaware, Georgia, Illinois, Kansas, Maryland, Missouri, New Jersey, Oklahoma, West Virginia) in 2015 were contacted by email or telephone August to September 2018. Newborn screening websites for each state were also reviewed for information.
Results: Six (Alabama, Connecticut, Delaware, Georgia, Illinois, Kansas) of the 11 states have confirmed the use of SUAC as a primary marker for HT-1 detection, and 4 states (New Jersey, Maryland, Oklahoma, West Virginia) still use tyrosine as a primary marker. The state of Missouri declined to participate, but based on internet research, it appears likely that it still measures tyrosine levels for HT-1 detection. All states who confirmed the use of tyrosine for detection, have stated that they are planning to move to SUAC testing in the next year. New Jersey, Oklahoma, and West Virginia are waiting for Perkin Elmer's new detection SUAC test, the NeoBase™ 2 Non-derivatized MSMS kit, to be validated for use. Maryland is in the process of hiring staff to validate their own SUAC assay. Developing the assay, assay validation, lack of reagents, and cost of implementing a SUAC diagnostic test were cited as barriers to implementation.
Conclusion: Progress has been made toward uniform newborn screening of HT-1 in the United States with SUAC; 90% (45 of 50) of the states have already implemented the use of SUAC for HT-1 detection. Of the remaining 5 states, at least 4 are attempting to implement SUAC detection tests in the next year.

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Development of a Simple and Low Cost Workflow for Simultaneous Screening for SCID and SMA From a Single Dried Blood Spot Punch

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Recently, the Recommended Uniform Screening Panel includes evaluation of two conditions - Severe Combined Immunodeficiencies (SCID) and Spinal Muscular Atrophy (SMA). Health economic models have been favorable for the screening of SCID while assessments to compare and model treatments of SMA have been initiated.

SCID constitutes a series of immune system functionality diseases. Real-time PCR screening assays to measure levels of T-cell receptor excision circle (TREC) and kappa-deleting recombination excision circles (KREC) helps delineate perturbations of T and/or B-cells development.

SMA is a motor neuron disorder caused by the absence of the SMN1 gene. Severity and age of onset for this disease are mediated by the copy number of a highly related pseudogene, SMN2. To accurately delineate the homozygous loss of SMN1, a real-time PCR designs employing a specific TaqMan probe permit both genes to be distinguished upon the basis of a one-base polymorphism.

To date, the implementation of a single test for both SCID (both TREC and KREC) and SMA is not routine globally. To meet this objective we have sought to develop and trial a real-time assay possessing a number of promising attributes.

The scope of the project was to: (i) Design an assay permitting concomitant measurement of SMN1, TREC, KREC and RNase P reference. (ii) Reduce the number of steps to the minimal number possible. (iii) Enable a rapid and sensitive assay. (iv) Obtain highly concordant data thereby limiting both ambiguous calls and the requirement for retesting. (v) create double-stranded artificial templates for positive control and quality assessments.

Single 1.5 mm dried blood spots were punched into a 96-well PCR plate. A streamlined workflow involves dispensing three sequential additions of reagents into the well in preparation for the multiplex real-time PCR reaction. The in situ process (<20 minutes per 96-well plate from receipt of plated blood spots to real-time PCR reaction) minimizes costly and time-consuming DNA extraction and the chance of contamination. Further, the simplicity of the workflow is amenable to low-cost reagent dispensing automation.

Four different fluorescent dyes, as well as fast thermal cycling in <40 minutes, was undertaken to allow faster turnaround times per instrument without sacrificing sensitivity and robustness. Compared to traditional methods, fast cycling is 2 to 3 times faster. We tested 144 randomized anonymous samples that included several affected individuals. The result showed that the normal samples exhibit robust amplification curves whereas affected individuals, with no or very attenuated amplification, can be readily identified.

In conclusion, we have designed and tested a robust multiplex assay with a low hands-on procedure and rapid turnaround workflow. This method aims to complement the goal of a high throughput and cost-effective screening of SMA and SCID from DBS samples.

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A Simplified, Rapid Approach to Targeted Resequencing of Dried Blood Spot Samples for Newborn Screening Research

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Newborn genetic screening (NBGS) is a developing ancillary approach to newborn screening (NBS) that paves the way for improved diagnosis and disease prevention research. Diseases such as cystic fibrosis (CF) and severe combined immune deficiency (SCID) have few, if any, reliable biomarkers to properly diagnose disease in a newborn. Basic research into the genotype-phenotype relationship will improve our ability to tie molecular diagnosis to the disease state. NBGS research requires the use of newborn blood samples in the form of dried blood spots and punches (BP). Current BP protocols require laborious, time consuming nucleic acid purification followed by quantification. This problem is only exacerbated as sample numbers increase. We present a simplified protocol for preparing DNA libraries using Ion AmpliSeq™ chemistry from BPs for analysis on next-generation sequencing (NGS) platforms. AmpliSeq™ is a PCR-based NGS library prep method that uses targeted primer panels to amplify DNA or RNA regions of interest for most any research application, including inherited and infectious disease research. In addition, AmpliSeq on Demand™ offers >5000 pretested genes enabling researchers to quickly and cheaply interrogate genetic loci of interest and advance the identification of diagnostic disease markers. To verify the efficacy of our method, a variety of AmpliSeq™ panels, including CF and SCID, were tested on BPs using the new protocol compared to traditionally purified, quantified blood samples; no substantial differences in sequencing read quality or variant calling were observed between the BPs and purified genomic DNA. Simplified sample prep methods combined with flexible, easy to configure test panels will facilitate the uptake of NGS in settings where BPs are a common sample type. For Research use only. Not for use in diagnostic procedures.

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A Rapid Multi-plex MS/MS Assay for LSD

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A new multiplex flow injection analysis - tandem mass spectrometry (FIA-MS/MS) method is described that simultaneously measures the activities of the enzymes ABG, ASM, GAA, GALC, GLA and IDUA, using a single 3.2 mm punch from a dried blood spot (DBS). The currently accepted methodology for multi-plex assays involving the GALC enzyme calls for an overnight incubation step. This study examines the effects on all enzymes of a shortened incubation time on the ability to discriminate between positive and negative samples. The multi-plex method was tested with DBS from 2 groups of presumed healthy subjects (each N=~700), contrived positive samples having low activities and DBS controls. The analytical analysis was done on a PE QSight® 210 MD triple-quad Mass Spectrometer.

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Hemoglobinopathies Detection with the MigeleTM Gel Electrophoresis Unit in New Jersey

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Universal sickle cell disease and hemoglobinopathies detection programs started in all states of the U.S. in May 2006 [1]. Many states started the screening even earlier (e.g. New Jersey, where the hemoglobinopathies screening started already in April 1990) [1]. Newborn screening laboratories can utilize different methods in combination for hemoglobinopathies detection. An isoelectric focusing based method (RESOLVETM Hemoglobin Kits, PerkinElmer) has been in the market for decades and it can be used as a accurate primary or secondary screening method for children and adults. This solution ensures robust instrumentation and cost-efficient choice for detection of hemoglobinopathies. [2]

MigeleTM Gel Electrophoresis Unit was launched in July 2018 to use with RESOLVETM Hemoglobin kits. During the development of the instrument, it was shown to have equal performance with the previous version. Easy to use design and increased scalability, four units can be connected into series, ensuring that this instrument is a a preferred solution to use with RESOLVETM Hemoglobin kits. During the development, New Jersey Department of Health, Division of Public Health and Environmental Laboratories kindly tested the unit using the control material and provided good feedback for the usability of the unit. Currently, the method is being utilized in a routine hemoglobinopathy detection workflow and we see that this method is an option for the hemoglobinopathies detection now and in the future.

References:

[1] Benson J.M., Bradford, L. & Therrell Jr. MS. (2010) History and Current Status of Newborn Screening for Hemoglobinopathies. Seminars in Perinatology, 34: 134-144.
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