# APHL 2020 Newborn Screening Virtual Symposium
## Poster Abstract List

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Improving Newborn Bloodspot Screening Timeliness with Software: How Track-Kit Helped Increase Saturday Specimen Deliveries by 210%

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Weekend operations is a key way to improve the timeliness of newborn bloodspot screening (NBS). Samples collected on Friday are shipped for Saturday delivery and screening, which may identify screen positive infants on Sunday instead of the following Tuesday. This could make a critical difference in the baby’s health.

When Newborn Screening Ontario (NSO) launched weekend processing, the number of specimens received on a Saturday was far fewer than expected. One factor contributing to the low Saturday volumes was that hospital staff were reluctant to select the expensive next-day weekend courier delivery, even though NSO pays specimen transportation costs.

NSO implemented Track-Kit in mid-2018 to track the 150,000 specimens shipped to the NSO lab from 200 hospitals and midwives across Ontario. On Fridays, instead of selecting from the usual list of courier options, Track-Kit only presents hospital staff with the optimal next day option, best for NBS. Mandatory selection of the Saturday delivery option, combined with an educational blitz, resulted in a 210% increase in Saturday specimen deliveries when compared to before Track-Kit was used.

Track-Kit, a cloud-based specimen tracking solution by STACS DNA, delivers real-time status and location updates to the submitter and the lab for every specimen, plus alerts when a specimen is late so that recollections can be performed sooner.

This poster will graphically present data from NSO’s adoption of Track-Kit, showing:

1. The 210% increase in average Saturday specimen deliveries which supports a full day of Saturday operations, and, more importantly, screens infants earlier, leading to better outcomes in patients’ health
2. Improved one-day transit time by 2.6%
3. Reductions in inappropriate courier account use to save time and money

The streamlining of processes and increase in communication between submitting hospitals and the laboratory through Track-Kit has improved specimen transit times, allowed for earlier detection of delayed or lost specimens and faster recollect requests, and increased weekend screening – all contributing to improved NBS timeliness in Ontario.

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Age at Time of Collection Added to Iowa’s Newborn Screening for Congenital Hypothyroidism TSH cut-off Algorithm

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The Iowa Newborn Screening Program (INSP) uses elevated values of thyroid stimulating hormone (TSH) to assess risk for congenital hypothyroidism (CH). Iowa’s current algorithm to identify newborn’s at increased risk for CH is based on fixed cut-offs, a borderline range and a presumptive positive range. Due to the rapid decline in TSH values after the post-delivery surge, INSP developed an algorithm that uses age at time of collection bins and cut-offs within each bin appropriate for the age at time of collection. We anticipate a dramatic increase the specificity of Iowa’s CH screening algorithm, as well as improved sensitivity for late and repeat collections.

Iowa’s age at time of collection bins were determined by first binning 2015-2018 TSH data into 1-hour increments and calculating medians, means, and standard deviations. One-hour bins with similar statistics were combined to produce 8 bins (Bin 2: 24-28hrs, Bin 3: 28-33hrs, Bin 4: 33-38hrs, Bin 5: 38-45hrs, Bin 6: 45-53hrs, Bin 7: 53-59hrs, Bin 8: 59-66hrs, Bin 9: 66-216hrs). Bin 1, 0-24 hrs, consisted of early collection specimens. Repeat and late collection specimens were evaluated and were categorized into two additional bins (Bin 10: 216-408hrs, Bin 11: beyond 408hrs). The median and standard deviation (SD) within each bin (calculated using 2018 data) were used to select cut-offs. Targeting the expected incidence of primary CH of 1:1500 (average 25 cases in IA), the borderline cut-off was set at 3.5 bin SD above the bin medians, and the presumptive positive cut-off was set at 7 bin SD for bins 1-5 and 6 bin SD for bins 6-11 above the bin medians. Around 65-70% of Iowa’s collections fall into the second bin, between 24 and 28hrs of age at time of collection. Bins with smaller numbers of infants were given a more conservative cut-off (6 SD).

We assessed how the bin cutoffs would change retrospective call-out categories for confirmed CH cases – as tracked by INSP follow-up. Most of the lab categories assigned to true positive CH cases remained unchanged. The cases that would have theoretically changed category, specifically those who would have changed to within normal limits, were further examined with Iowa’s endocrinology consultant. The proposal was then presented to INSP stakeholders and Iowa’s state partners and was approved for implementation.

With the planned cutoff changes, there will be an estimated ~70% decrease in the number of borderline TSH specimens reported by the lab. Presumptive positive TSH specimens reported by the lab are projected to increase (~10%). This change will reduce the burden of false positive TSH screens in Iowa and states partnered with INSP. It also provides equality of risk assessment to those infants with specimen’s collected after 48 hours of age, avoiding previous risk of false negatives. Iowa’s new algorithm is scheduled to go-live at the end of March 2020, and performance will be assessed monthly after implementation.

Presenter: Anne Atkins, State Hygienic Laboratory at the University of Iowa, Coralville, IA, Email: anne-atkins@uiowa.edu
Optimizing the Severe Combined Immunodeficiency and Spinal Muscular Atrophy CDC Triplex Assay for New Real-time PCR Platforms
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Spinal muscular atrophy (SMA), an autosomal recessive condition, is the leading genetic cause of infant mortality, affecting an estimated 1:10,000 live births. Approximately 95% of SMA cases can be attributed to the absence of exon 7 in the survival motor neuron gene 1 (SMN1) gene on chromosome 5q, resulting in the functional loss of SMN protein that is critical for nerve function, leading to the degeneration of motor neurons and progressive muscle atrophy and weakness. The detection for the absence of SMN1 exon 7 can easily be multiplexed into the Severe Combined Immunodeficiency (SCID) real-time PCR assay. Currently, there are two FDA-approved treatments for SMA that can prevent or slow the progression of disease, with the greatest success being in those detected and treated pre-symptomatically. The U.S. Secretary of HHS added SMA to the recommended uniform screening panel (RUSP) for newborns in 2018.

All 50 U.S. states are screening for SCID and twenty states have since implemented newborn screening for SMA, many using a CDC-developed triplex real-time PCR assay that simultaneously screens for both SCID and SMA. The CDC assay was designed so that each of the three sequence-specific probes have a distinct fluorescent reporter dye to accurately differentiate between the targets in a single reaction, including: T cell receptor excision circle, TREC (FAM dye), SMN1 exon 7 (Cy5 dye), and reference gene, RPP30 (HEX dye). This assay was developed and optimized on the real-time qPCR instrument, Mx3005P. Laboratories using this instrument are transitioning to new systems due to the discontinuation of the Mx3005P. A number of laboratories, including CDC, have adopted the QuantStudio platforms. These instruments differ in some important features, such as sensitivity, multiplexing capabilities, optical systems, manufacturer-calibrated fluorescent channels, and software, making it necessary to re-optimize the CDC triplex assay to ensure the best sensitivity and performance on these new platforms.

To this end, CDC is evaluating the performance of each of the currently used reporter dyes and how they interact with each other in the CDC triplex assay on these new platforms. Preliminary results have shown that when the HEX dye is detected in the new platform’s VIC channel, as is commonly done, there is an inappropriate background correction in the FAM channel, causing a baseline distortion. The CDC assay re-optimization will test two approaches on the new platform: alternative dyes for each target to see if they increase the sensitivity and detection of each target; and custom calibrations for the HEX dye to see if this will eliminate the inappropriate baseline correction. We expect the results from these studies will offer alternative assay options to NBS labs beginning to implement SMA screening.

Presenter: Nicolle Baird, Centers for Disease Control and Prevention, Atlanta, GA, Email: mmy1@cdc.gov
Intensive research in early detection of specific illnesses and genetic disorders in newborns is essential for prevention of permanent disabilities or death of infants. In addition to Severe Combined Immunodeficiencies (SCID), Spinal Muscular Atrophy (SMA) has been recently added to the US-RUSP list. SMA is a motor neuron disorder caused by mutation in the SMN1 gene, whereas SCID constitutes a series of immune system functionality diseases exhibiting low levels of T-cell receptor excision circles (TREC). Since the implementation of a single test for both SMA and SCID is currently sought after globally, we developed a real-time SMA/SCID multiplex assay that permits concomitant measurement of SMN1, TREC, and RNaseP reference. We designed the SMA assay to target exon 7 of SMN1 gene and effectively eliminated non-specific detection of the highly similar SMN2 gene by competitive inhibition. This high SMN1 target specificity of SMA assay limits both ambiguous calls and requirement for retesting. We also confirmed TREC target specificity and the SCID assay’s high sensitivity with TREC copy number detection capability of as low as 9 copies per reaction. Additionally, we were able to substantially improve DBS sample preparation method and reduce the number of steps to a minimum. In conclusion, we have developed a highly specific, sensitive, and robust multiplex assay for SMA and SCID testing with a rapid and streamlined turnaround workflow to aid further research efforts.

**Presenter:** Sonu Baral, Thermo Fisher Scientific, Santa Clara, CA, Email: sonu.baral@thermofisher.com
Arkansas Newborn Screening Long-Term Follow-up Database Study – ANGELS Newborn Screening 2018 Annual Report

J. Bolick, University of Arkansas for Medical Sciences/Arkansas Children’s Hospital, Little Rock, AR

**Problem/Objectives:** Every year approximately 38,000 Arkansas newborns receive a newborn screen for 29 primary core panel metabolic conditions (including Severe Combined Immunodeficiency), hearing loss, and Critical Congenital Heart Disease; 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). The 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 Arkansas Medicaid Program to assure the best possible outcomes for high-risk pregnancies. 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 2018 marked the seventh full year of implementation of the Database. The 2018 Annual Report provides a summary of the data gathered between 01-01-2018 and 12-31-2018 along with some cumulative data. Calendar Year 2018 marked the first full year of collecting data on kept/missed clinic visits to the ACH primary specialist. With 126 new subjects/cases in 2018, the Database contained 1,088 subjects representing 1,095 NBS cases at the end of 2018.

**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.

**Presenter:** Jo Bolick, University of Arkansas for Medical Sciences/Arkansas Children’s Hospital, Little Rock, AR, Email: jabolick@uams.edu
Rapid UPLC-MS/MS Dried Blood Spot Analysis of Steroid Hormones for Clinical Research

H. Brown, D. Foley and L. Calton, Waters Corporation, Wilmslow, England, United Kingdom

Background: Dried Blood Spots (DBS) are an established microsampling technique providing a low-cost approach to collecting, shipping and analyzing samples in clinical research. Despite the convenience and speed of immunoassay, the relatively low analytical specificity of this technique creates a need for repeat analysis using methods with higher analytical specificity such as liquid chromatography – tandem mass spectrometry (LC-MS/MS). The ability to analyze more than one marker at a time (multiplexing) allows the generation of a richer dataset. The challenge was to create a fast, analytically sensitive and selective LC-MS/MS methodology for analysis of multiple steroid hormones.

Methods: DBS calibrators and quality control (QC) samples were prepared from synthetic whole blood, prepared by mixing equal volumes of saline-washed red blood cells with stripped serum enriched with androstenedione, 17-OHP, cortisol, 11-deoxycortisol and 21-deoxycortisol from certified reference material (Sigma Aldrich, Poole, UK). Synthetic whole blood was applied to Whatman 903 Protein Saver Blood Spot cards, dried, and stored at -20ºC. Two 3mm DBS punches were mixed for 5 minutes with a methanol-based extraction solvent containing stable-isotope labelled internal standards. Further matrix clean-up using solid phase extraction (SPE) removed interferences which might otherwise co-elute with steroid hormones in the rapid UPLC method. Extraction was carried out with a Waters Oasis™ MAX µElution 96-well SPE plate, allowing direct injection of the prepared sample. Sample preparation was automated using a Tecan® Freedom Evo 100 liquid handling robot. Samples were injected onto a 2.1mm x 50mm CORTECS C18 2.7µm UPLC column using an ACQUITY UPLC I Class system, and steroids were detected and quantified with a Xevo™ TQ-S micro mass spectrometer.

Results: Rapid separation of 17-OHP, androstenedione, cortisol, 11-deoxycortisol and 21-deoxycortisol with baseline resolution of steroid isobars was achieved within 1.4 min, and the injection cycle time was 2.3 min (allowing for column regeneration and equilibration). Calibration lines were linear from 0.5 – 500 ng/mL for androstenedione and 11-deoxycortisol; and 1.0 – 500 ng/mL for cortisol, 17-OHP and 21-deoxycortisol with correlation coefficients (r²) >0.99 over five occasions. Coefficients of variation (CV) for total precision and repeatability over five occasions at four concentrations: 2, 5, 50 and 400ng/mL, were ≤ 9.3% (n = 25) with accuracies ranging from 94 – 110%.

Conclusions: UPLC separations was achieved at high linear velocity, with minimal loss in column performance. Automation of sample preparation created opportunities for improved laboratory efficiency, and the method itself demonstrated excellent linearity, analytical sensitivity, precision and accuracy.<br>For Research Use Only, Not for use in diagnostic procedures.

Presenter: Dominic Foley, Waters Corporation, Wilmslow, England, United Kingdom, Email: dominic_foley@waters.com
Verification and Implementation of NeoBase™ 2 Non-derivatized MSMS Kit: The New Jersey Experience

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Objective: The New Jersey Newborn Screening laboratory recently verified the performance of the NeoBase™ 2 Non-derivatized MSMS kit to replace Neogram Derivatized MSMS reagent for the measurement of amino acids (AA) and acylcarnitines (AC) from newborn heel prick blood specimens. Quantitative analysis of these analytes aids in screening newborns for over 30 metabolic disorders. In addition to detecting AA and AC, the NeoBase™ 2 kit also measures argininosuccinic acid (ASA), succinylacetone (SUAC), nucleoside and lysophospholipid, providing the potential to screen for additional disorders in the future.

Methodology: To verify the performance of this FDA approved assay, linearity, precision, and accuracy studies were performed following CLSI guidelines. The performance of Neobase 2 was compared to Neogram by analyzing 148 patient specimens using both methods and calculating linear regression statistics to determine the relationship between the assays. To define screening cutoffs, the normal distribution of all analytes measured using Neobase 2 was determined. Specifically, 2053 blinded specimens were analyzed and the data collected in SpecimenGate Cutoff Analyzer software. To maintain continuity, Neobase 2 screening cutoffs were defined using the same population percentiles used when screening with Neogram. For the new analytes included in Neobase 2, screening cutoffs were established based on the normal distribution in the New Jersey population and using the information provided in the package insert. Finally, to confirm accuracy, proficiency testing (PT) specimens provided by the Centers for Disease Control were analyzed and evaluated for acceptable performance.

Results: Linearity and precision studies verified the assay performance characteristics as provided in the package insert. Results of PT specimens were evaluated against the Neobase2 peer group summary data provided by the CDC and all results were acceptable. Regression analysis for the correlation studies revealed a range of correlation coefficients (R² = 0.64 - 0.96). This was not surprising as Neobase 2 uses a non-derivatized methodology as compared to Neogram which uses a derivatized method. The screening cutoffs for 20 primary analytes were evaluated and adjusted based on correlation data and the population distribution. The NJ NBS lab went live with Neobase 2 on January 21, 2020, screening statistics are being monitored and results will be presented.

Conclusions: NeoBase™ 2 Non-derivatized MSMS reagent provides enhancements both in the number of analytes detected as well as simplification of specimen processing. The transition from Neogram to Neobase 2 was complex, requiring a coordinated effort involving LIMS configuration, new instrumentation optimization, and assay verification. The impact on screening statistics will be evaluated over the next 6 months and the results presented.

Presenter: Mary Carayannopoulos, New Jersey Department of Health, Newborn Screening Laboratory, Trenton, NJ, Email: mary.carayannopoulos@doh.nj.gov
Das KIND: A Novel Approach to Newborn Screening Follow-up in Rural Indiana
S. Chupp, J. Werker, R. Evans and Z. Ammous, The Community Health Clinic, Topeka, IN

Newborn screening (NBS) is widely recognized as a vital component of public health. While state NBS programs have proven successful, improvements in service delivery may benefit special populations. The Community Health Clinic (CHC), a rural Indiana genetics clinic, presents a novel approach to NBS follow-up for Plain (Amish and Mennonite) and other local communities.

The CHC aims to improve access to healthcare by overcoming barriers including communication, distance to specialty care, and cost. The CHC identified NBS follow-up for inborn errors of metabolism (IEMs) as an area of need in the northeastern Indiana Plain community. In 2013, the CHC began to provide follow-up for infants with abnormal NBS results for IEMs in northeast Indiana through the Das KIND program.

The Das KIND program provides short-term and long-term follow-up for IEMs. Short-term follow-up aims to improve outcomes by reducing time to confirmatory testing and treatment, while improving communication with families and primary care providers (PCPs). This is accomplished by:

1. Rapid and direct contact between the CHC and the family upon a positive screen referral,
2. Education for the family and 24-hour access to an RN throughout the confirmatory testing process and after diagnosis, and
3. Collaborative relationships with local PCPs, providing relevant education and serving as a resource to promote effective care of the mutual patient.

The CHC offers all confirmed affected infants long-term follow-up (LTFU) with the CHC’s clinical geneticist, physician assistant, and dietitian. Since its inception, the CHC’s Das KIND program has received 221 referrals for positive screens. Of these, 108 were confirmed positive, and 68 patients continue LTFU with the CHC. 77% of the Plain patients who were confirmed positive continue LTFU with the CHC.

The Das KIND program has realized both challenges and successes. Challenges include gaining recognition with PCPs and other follow-up providers as a new NBS provider, communication with Plain families who often do not have telephones or a PCP, and limited resources of a small clinic to execute a high-quality NBS program. Successes include rapid times to diagnosis and treatment, empowering the local Plain community to better utilize NBS and care for their affected children, and building rapport with local providers and families. Housing short-term and long-term follow-up within the same program allows improved coordination of care for the immediate newborn, as well as faster diagnosis for future siblings of confirmed positive patients through utilization of cord blood testing. These results are typically available prior to NBS results, allowing earlier intervention for affected infants. While it may not be possible in every NBS program to implement this type of follow-up service, the CHC demonstrates a highly effective way to improve care for vulnerable populations.

Presenter: Sharon Chupp, The Community Health Clinic, Topeka, Indiana, Email: chupp@indianachc.org
Poster #9

Results of a Newborn Screening Program in Mexico: 2020 IEM Global Prevalence Comparison

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Introduction: The inborn errors of metabolism (IEM) are a group of more than 1,000 rare inherited disorders with an estimated global prevalence of 1:800 newborns (NB). Only a few can be early diagnosed by newborn screening (NBS). Currently there is a great variability in the IEM’s statistics detected by NBS, mainly explained by different laboratory methodologies, suboptimal coverage programs, population distribution, ethnicity, and consanguinity. The NBS coverage in Mexico is over 80% and it mostly targets congenital hypothyroidism. The healthcare system segmentation is one of the challenges the country is facing, in which private NBS programs -with broader disease panels- covered a small percentage of the population and databases cannot be comparable to each other.

Objective: To address the limitations regarding coverage, size, reliability, comparability, and scope for international NBS programs. We present the largest private NBS database recorded to date in Mexico and a worldwide systematic review for IEM included in the RUSP.

Methodology: We analyzed Genomi-k’s 279,387 NBS reports performed since June 2007 to January 2020. All DBS were processed by PerkinElmer Genomics for amino acid metabolism disorders (AA), fatty acid β-oxidation defects (FAOD), organic acidemias (OA) and lysosomal storage diseases (LSD) –only for 40% of the population- by MS/MS. While other biochemical assays were used for galactose metabolism disorders (GALD) and biotinidase deficiency (BIOT) screenings. Abnormal results were followed by diagnostic tests. Furthermore, we conducted a systematic review for worldwide IEM prevalence extracting RUSP conditions for equivalent comparison (40 scientific reports: 8 Latin America, 11 North America, 10 Europe, 2 Middle East, 8 Asia and 1 Oceania).

Results: For the last 12.6 years, our program presented an IEM prevalence of 4.92:10,000 NB, with a confirmed cases distribution of: 54.3% LSD, 21.1% AA, 10.2% OA, 6.5% BIOT, 4.3% FAOD, 3.6% GALD. Furthermore, 4.44:10,000 NB had a pseudodeficiency or were heterozygotes -72.3% were Pompe disease and BIOT-. In relation to our NBS program for IEM, we had a high specificity (99.94%) and sensitivity (~100%). Moreover, 0.11% NB were lost to follow up. The systematic review for the IEM (included in the RUSP) ended with an estimated worldwide prevalence of 3.5:10,000 NB.

Conclusions: The specific IEM estimated prevalence ranges from 1.71 (Oceania), 2.59 (Latin America) to 6.75 (Middle East) per 10,000 NB. Our IEM rate is higher than those reported for Latin America, mainly due to a broader NBS panel. The current challenge is to improve the long term follow-up. More extensive epidemiological studies with RUSP-based programs and timely therapeutic approaches will show a clearer picture of IEM distribution and benefit; thus, decreasing the global burden of disease, as well as morbidity due to IEM.

Presenter: Hector Cruz-Camino, Genomi-k, Monterrey, Nuevo Leon, Mexico, Email: hcruz@genomi-k.com
A Novel 2-plex qPCR Assay to detect Congenital Cytomegalovirus
S. Dallaire, H. Polari, M. Velling and Y. Tong, PerkinElmer, Waltham, MA

Congenital cytomegalovirus (cCMV) infections in infants causes a greater number of permanent disabilities than Down Syndrome, Fetal Alcohol Syndrome, and pediatric HIV/AIDS combined. While 99.5% of the population test negative for cCMV, 10-15% of the cases that test positive are symptomatic with long-term sequelae including hearing loss. Early intervention and promising antiviral treatments are available for these children. The RUSP recommends hearing loss screening, but 50% of cCMV cases are missed during the traditional screen, and not detected as most cCMV disability is not evident at birth.

A two-plex real-time PCR assay was developed to detect cCMV loci using DNA isolated from a single 3.2mm punch of a dried blood spot (DBS) using the NeoMDx RUO Extraction kit. The amplification of the house-keeping gene, RPP30, is included in the assay as a positive control of DNA purification and can be used to as an internal control to determine relative copy number. The qPCR assay is compatible with the existing RUO NeoMDx molecular screening system, utilizing a simple, alkaline based DNA extraction, and 2-part qPCR set up. The assay can be fully automated in the same fashion as the NeoMDx assay.

The cCMV real-time PCR assay performance was demonstrated on +1000 DNA samples extracted from 3.2mm punches of putative normal newborn DBS, as well as DNA from several characterized reference cCMV positive samples and controls. The results from this study with a two-plex real-time PCR assay further demonstrates the potential of future molecular DBS based assays.

Presenter: Stephanie Dallaire, PerkinElmer, Waltham, MA, Email: stephanie.dallaire@perkinelmer.com
Poster #11

Development of Dried Blood Spot Reference Materials for Creatine Kinase MM Isoform Assays Used to Detect Duchenne Muscular Dystrophy in Newborns
P. Dantonio, E. McCown, C. Haynes, R. Vogt and K. Petritis, Centers for Disease Control and Prevention, Atlanta, GA

Duchenne Muscular Dystrophy (DMD) is an X-linked genetic disease that affects dystrophin protein in muscle cells. DMD is the most common of all the muscular dystrophies, with an estimated birth prevalence of one per 3500 males. The loss of functional dystrophin causes damage to muscle cells, which then release the MM isoform of creatine kinase (CK-MM) into the newborn’s bloodstream. CK-MM assays on newborn dried blood spots can therefore be used to detect DMD. Newborn screening (NBS) for DMD is not routinely performed by public health NBS laboratories in the United States, but a large-scale pilot study is currently in progress. This pilot study is using an FDA-authorized DMD screening test that measures the level of CK-MM in newborn dried blood spot (DBS) specimens using a time-resolved fluorescence immunoassay. Because the amount of CK-MM in newborns is transiently elevated after the birthing process, a normal result varies depending on how many hours after birth the specimen is collected.

The Newborn Screening Quality Assurance Program (NSQAP) at the US Centers for Disease Control and Prevention (CDC) produced DBS reference materials with different levels of CK MM to correspond to different cutoff values based on the variability of collection times. Starting with a base pool of leuko-depleted blood, different amounts of CK-MM enzyme were spiked into separate blood pools to approximate normal and abnormal NBS specimens with different post-birth collection times. DBS materials made from these pools were evaluated at CDC using the FDA-authorized immunoassay. Initial results show that these prototype quality assurance materials can be produced with measured CK-MM levels ranging from those typically found in unaffected newborns to those found in newborns with DMD.

Disclaimer: The findings and conclusions in this study are those of the authors and do not necessarily represent the views of the US Department of Health and Human Services, or the US Centers for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the US Department of Health and Human Services, or the US Centers for Disease Control and Prevention.

Presenter: Paul Dantonio, Centers for Disease Control and Prevention, Atlanta, GA, Email: pbd1@cdc.gov
**Poster #12**

**Forecasting Bioinformatics Methodologies for Heterogeneous Disorders in Newborn Screening Systems**

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Next-generation and Sanger sequencing methodologies are increasingly implemented in newborn screening systems as more complex disorders are added to the recommended uniform screening panel (RUSP). With the emergence of new disorders comes a greater need for tiered workflows to augment traditional screening methods in newborn screening systems. Incorporating bioinformatics-based workflows for targeted conditions has been shown to improve clinical outcomes where early detection and treatment may result in life changing outcomes. The addition of bioinformatics workflows can foster follow up initiatives and support the continuum of care to reduce stressful circumstances for parents.

The importance of genomic analysis is evolving in disorders such as severe combined immunodeficiency (SCID), cystic fibrosis (CF), and other disorders that were recently added to the Recommended Uniform Screening Panel (RUSP) such as mucopolysaccharidosis (MPS-1) and Pompe. Bioinformatics approaches may especially be helpful for some of these disorders in which there may be similar, asymptomatic, or late onset symptoms and it is otherwise difficult to pinpoint the root causation. In these circumstances, variant analysis can complement biochemical methodologies and increase positive predictive value.

The poster aims to forecast the various approaches to utilizing sequencing and DNA analysis to augment current practices in Virginia, Utah, New York, and Texas newborn screening laboratories. We highlight laboratory algorithms, cost analysis, turnaround time, and positive predictive value for the aforementioned disorders. The implication of this poster is to share bioinformatics approaches as programs adopt next-generation sequencing and Sanger sequencing technology, highlight challenges, and summarize data to anticipate future goals and directions for the newborn screening programs.

**Presenter:** Bryce Asay, Utah Public Health Laboratory, Taylorsville, UT, Email: aphlnbsba1@utah.gov
CDC’s Newborn Screening Quality Assurance Program provides quality assurance materials to newborn screening laboratories worldwide to ensure and maintain the quality and accuracy of newborn screening results. CDC maintains a molecular repository of specimens representing various newborn diseases for which there are molecular tests used in routine screening. This repository contains different sample types including dried blood spots, cryopreserved white blood cells, and Epstein Barr Virus (EBV) transduced cell lines, all made from patients or family members impacted by newborn diseases. The transduced cell lines, originally isolated from B-lymphocytes, are used as a sustainable source for quality assurance materials. Cells are grown in culture and used to make lab created Dried Blood Spots (LcDBS) quality assurance materials. Occasionally, a transduced cell line will display inhibited or very slow growth even after successful transduction, making it hard and, in some cases, impossible to grow enough cells needed to create LcDBS materials. Studies in literature suggest that various media supplements including vitamin solution, non-essential amino acids, or additional FBS will enhance cell growth. This study seeks to determine the effect of various media supplements for slow growing cell lines by examining the effects of selected supplements on two slow growing cell lines – one from a CF patient and a second from a galactosemia patient. Slow growing cells from the two cell lines were split into flasks, each containing one of the following: media containing non-essential amino acids, vitamin solution, 30% FBS, filtered media from a well growing cell line, or normal complete media. The cells were grown in the supplemental media for 1 month. The total live cell growth was compared throughout the experiment to determine which supplement(s), if any, worked the best. Lab created dried blood spots will be created from these samples and tested to determine if adding media supplements affects any downstream molecular analysis.

**Presenter:** Katherine Duneman, Centers for Disease Control and Prevention, Atlanta, GA, Email: ywv3@cdc.gov
Enhancing Disease Detection and Building Data Analytic Capacity in Newborn Screening Programs

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NBS programs are experiencing increased data analytic needs as a result of the continued expansion of the number of newborn screening diseases, increased complexity of disease detection through biomarker profiles and molecular findings, and difficulties in correlating disease markers with disease risk. Further, the addition of late-onset diseases to NBS panels necessitates a better way to routinely capture clinical information and outcomes so that NBS programs can fully appreciate the spectrum of disease they are detecting.

The CDC’s Newborn Screening and Molecular Biology Branch, through the NBS Vision 2030, is working towards helping state newborn screening programs meet these challenges by supporting data modernization in newborn screening programs in three ways:

1) Facilitating enhanced participant engagement by implementing a new data portal. Over time, data volume increased from a growing participant pool and available testing materials which created a need for a modern participant interface focused on improving data management with robust reporting to enhance user engagement. The new portal is expandable, allows for streamlined submission of data, allows users real-time access to data with the ability their program performance to an aggregate result, user-defined reporting tools, better data visualization and dashboards.

2) Supporting workforce capacity by initiating a fellowship to train and prepare bioinformaticians to apply their expertise within public health and design tools to aid existing public health personnel in the use of bioinformatics. This will provide a high-quality training experience for fellows while providing workforce capacity to the public health laboratory community.

3) Developing tools and resources to help support the analysis and interpretation of screening data, provide tools and resources to streamline manual processes and create efficiencies in time-intensive program activities, and allow for states to conduct data harmonization across multiple screening platforms to promote better biomarker interpretation.

The impact of these initiatives is enhanced disease detection, increased workforce capacity and knowledge, and improved screening workflow. Disease detection of high-risk newborns is enhanced through improved data-driven decision-making tools and resources. CDC continues to support newborn screening laboratory best practices through standardized workflows, validated analytical tools, and educational resources.

Presenter: Amy Gaviglio, G2S/CDC/APHL, Minneapolis, MN, Email: agaviglio@cdc.gov
Poster #15

Maryland’s Experience Screening for LSD
F. Gulamali-Majid and P. Paudyal, Maryland Department of Health Laboratories, Baltimore, MD

Lysosomal Storage Disorders are a group of more than 50 disorders, most of which are inherited as autosomal recessive trait. Although, these disorders are rare, as a group they affect 1 in 5,000 to 1:10,000 live births. LSDs are caused by lysosomal dysfunction usually as a consequence of deficiency of an enzyme required for metabolism of lipids and mucopolysaccharides. Lack of such an enzyme results in accumulation of the unwanted material in the lysosomes hence, the term LSD.

Newborn screening for lysosomal storage disorders (LSDs) is becoming increasingly widespread. Population screening results indicate that demographic factors such as birthweight, gestational age, and age of specimen collection may affect the measured lysosomal enzyme activities. Such variation in enzyme activity should be considered while establishing cutoff values. Limited data are available on enzyme activity for specimens collected after the first week of life and even the available data are biased as it is mainly derived from specimens that are repeat specimens, which have much higher rates of transfusion, premature birth, or other health issues compared to initial screen samples. Maryland is one of the states that routinely performs screening on at least two specimens from every infant, regardless of health status. The first dried blood spot specimen is collected at 24-48 hours of age and a second specimen is collected between 7-14 days after birth. We will analyze lysosomal enzyme activity in both cohorts of specimens (1st vs 2nd screen) in order to objectively characterize the effect of age at specimen collection, Gestational age and weight at the time of specimen collection. Such information is not currently available.

The Maryland Department of Health began screening for Pompe (GAA) and Fabry (GLA) and MPS1 (IDUA) on June 15, 2019. We will present results of 50,000 specimens from each age cohort (24-48 hours and 7-14 days). Since all infants will receive routine testing at these two ages, the sample set will be closely controlled to prevent bias due to other demographic effects – for example, premature newborns will be excluded from the analysis since birth weight and gestational age may also affect lysosomal enzyme activity. These results may be informative for other laboratories that are currently screening – or planning to screen – for LSDs, especially programs who routinely perform two screens on every newborn.

Presenter: Fizza Gulamali-Majid, Maryland Department of Health Laboratories, Baltimore, MD, Email: fizza.majid@maryland.gov
Method Comparison for a 4-plex Real-Time PCR Assay that Detects SMN1, TREC, KREC and RPP30 in Dry Blood Spots

C. Gutierrez-Mateo, S. Dallaire and G. Filippov, PerkinElmer, Waltham, MD

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) as well as Kappa-deleting recombination excision circles (KREC) in the DNA extracted from a 3.2 mm newborn dried blood spot (DBS) punch. 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.

The NeoMDx™ assay consists of an alkaline DNA extraction followed by DNA amplification of the four targets using an extensively optimized PCR mix. To evaluate the effectiveness of the PCR assay, we compared alternative extraction methods currently used by newborn screeners to test for SCID, XLA and SMA. We compared our extraction method (Method A) with a different alkaline DNA extraction (Method B) and an in situ protocol (Method C), which is a different kind of DBS sample preparation, developed and promoted by the CDC. For both Methods B and C, two different elution solutions, the NeoMDx Elution Solution and the Extracta™ DBS solution, were compared on more than 1000 newborn samples.

The results of these tests suggest that the simple alkaline method (B) as well as the in-situ method (C) are compatible with our PCR chemistry to identify the homozygous deletion of exon 7 in the SMN1 gene and simultaneously evaluate the copy number of TREC and KREC.

Presenter: Cristina Gutierrez-Mateo, PerkinElmer, Waltham, MA Email: cristina.gutierrez@perkinelmer.com
A Prospective Pilot Study for Proximal Urea Cycle Defects Using Post-Analytical Tools

P. Hall¹, A. Hagar², A. Wittenauer¹, W. Wilcox¹; ¹Emory University, Decatur, GA, ²Georgia Department of Public Health, Decatur, GA

Proximal urea cycle defects (PUCD) are a group of three disorders (carbamoyl phosphate synthase deficiency, N-acetylglutamate synthase deficiency and ornithine transcarbamylase deficiency) in the urea cycle with enzyme deficiencies located before citrulline is transported into the cytosol. The hallmark clinical finding is hyperammonemia, often severe and very early in life. Biochemically, all PUCD have decreased citrulline concentrations. Early intervention can improve outcome in PUCD, however low citrulline is a non-specific finding in newborns, and screening with a simple cutoff resulted in many false positive (FP) screens.

Pursuant to a task order from NIH, Georgia started a pilot study to screen for PUCD on October 31, 2019. In order to accomplish this screening without an overwhelming number of FP results, we developed post-analytical tools utilizing the CLIR platform (https://clir.mayo.edu). Initially, we used a single condition tool with adjustments for age at collection and birthweight. For infants with missing or extreme (very low birth weight or late collections) covariates, we also created a tool without these adjustments.

As of January 31, 2020, 24,179 samples have been screened for PUCD in Georgia. 56 were reported as abnormals (screen positive rate of 0.2 %), and 20 of these have been confirmed as FP. Three families / physicians have refused follow-up. One child has been lost to follow-up and one child is being followed by the genetics service for further workup. The remaining 31 cases are in process, however there are no high suspicion cases in this group. We are unaware of any patients presenting clinically with a proximal urea cycle defect who were born during the study period.

Early in our study, we had a higher rate of FP results than expected. A review of citrulline data collected after the laboratory’s recent switch to the NeoBase2 assay showed a downwards shift in citrulline concentrations. To account for this, updated reference data was uploaded, and our protocol shifted to using location specific reference data. After several weeks of screening, we had confirmed outcomes of early screens, which allowed for the creation of a dual scatter plot to reduce FP screens further. The next step in improvement will be the inclusion of glutamine and glutamic acid as analytes after the completion of IT improvements.

Presenter: Patricia Hall, Emory University, Decatur, GA, Email: tricia.hall@gmail.com
NBS Molecular Automation Workgroup
L. Hancock¹, O. Akinsola², S. Cordovado¹, G. Zarbalian²; ¹Centers for Disease Control and Prevention, Atlanta, GA, ²Association of Public Health Laboratories, Silver Spring, MD

The Association of Public Health Laboratories (APHL) Newborn Screening Molecular Resource website, developed in 2013, was designed to share newborn screening (NBS) specific expertise and good practices as it relates to molecular testing in high throughput population-based screening laboratories. When screening for severe combined immunodeficiency (SCID) was being implemented by U.S. public health laboratories, it was necessary for laboratories to incorporate automation that could accommodate the unique needs of molecular testing. In 2015, APHL formed an automation workgroup comprised of seven state programs from the Molecular Subcommittee to leverage expertise from across newborn screening programs that represented different population sizes. This workgroup was tasked with developing resources that are now present on the Molecular Resources Website, including the description of different liquid handling instruments currently used in NBS laboratories that cover needs using both highly automated and semi-automated methods; detailed information on how NBS programs use their automation instruments and methods; considerations when selecting or purchasing a new liquid handler including questions to consider about instrument hardware, software, maintenance and support; and information on pipetting verification methodologies.

Since the automation section of the NBS Molecular Resources Website was developed, the number of U.S. laboratories using automation has increased, however most laboratories do not have the resources to have staff dedicated to automation method development and instrument troubleshooting. In addition, expertise is often lost with staff turnover. To address this gap, APHL’s NBS Molecular Subcommittee and CDC’s Newborn Screening and Molecular Biology Branch have co-sponsored the NBS Molecular Automation Workgroup with the goals of providing expertise and support to programs (whether experienced or just starting out with automation), leveraging existing resources, as well as developing any new resources to fill identified gaps. This workgroup will operate as a moderated forum where participants with diverse automation and molecular biology backgrounds can share experiences, best practices, identify challenges and work together to develop solutions, quality improvements, and educational resources to enhance the use of automation and molecular testing. The NBS Molecular Automation Workgroup is open to all participants. More information is available under Automation Methods at https://www.aphl.org/programs/newborn_screening/Pages/molecular-resources.aspx

Presenter: Laura Hancock, Centers for Disease Control and Prevention, Atlanta, GA, Email: lfn2@cdc.gov
Poster #19

Leveraging Strategic Partnerships to Educate Expectant and New Moms on a Newborn Screening Study in North Carolina


**Background:** Early Check is a large-scale, population-based research study based in North Carolina (NC) that offers free newborn testing for a panel of conditions to all babies born in NC. Mothers sign up for this additional testing between the start of their second trimester and when their baby is one month old. The study faces a substantial challenge in educating all women giving birth in NC (approximately 120,000/year) about the study. Extensive education efforts are necessary within multiple health care settings across NC, a challenge that is similar to state NBS programs’ efforts to educate parents about newborn screening.

**Methods:** We leveraged multiple state Department of Health and Human Services (DHHS) networks as well as connecting with hospital systems, OB/GYN and pediatric clinics, and other key public health stakeholders to increase awareness of our study. Since October 2019, we have successfully implemented an Early Check education program within multiple health care settings. Partner sites are implementing diverse strategies to educate parents about Early Check. In OB clinics, flyers are being included in prenatal resource packets and/or are handed out during the 28th week appointment. In hospitals, strategies include flyers incorporated into resource packets for new parents, in-person delivery of flyers along with verbal script of key points about the study, and iPads being utilized to educate and inform new parents about the study. In prenatal classes, iPads are being used along with in-person delivery of flyers with a script. Some hospitals and clinics have included information about the study on their website or smartphone apps. In all types of settings, posters are being displayed in waiting areas.

**Results:** To date, we have recruited over 6,700 families to consent for Early Check. Our outreach and education strategy has involved partnering with key stakeholders across NC, including: UNC Medical Center, Duke University Hospital, Wake Forest University hospital and OB/GYN clinics, Vidant Health, Novant Health, Cone Health, Wilson Medical Center, Cape Fear Valley Medical Center, Wake Med, Camp Lejeune Pediatric Clinic, HCA Healthcare, Asheville Women’s OB/GYN, and Mountain Area Health Education Center (MAHEC). We expect to see a marked increase in consents in mid-2020 due to the increase in prenatal outreach efforts over the past few months. At that point, using information about which outreach strategies women would have been exposed to (based on their birth hospital), we will be able to examine which outreach and education strategies or combination of strategies are more effective.

**Conclusion:** This presentation discusses the unique strategic partnerships required to conduct large-scale recruitment efforts targeting hard-to-reach populations. It also offers insight into how state NBS programs can educate expectant and new parents about their programs.

**Presenter:** Blake Harper, RTI International, Research Triangle Park, NC, Email: bharper787@gmail.com
Second Tier Screening for Mucopolysaccharidosis Type I and Pompe Disease by Sanger Sequencing Using the BigDye Direct Cycle Sequencing Kit, XTerminator Cleanup and ABI 3730 DNA Analyzer Platform

R. Haughton, P. Stuller, L-E. Lion, G. Cote, C. Nixon and P. Hetterich, Virginia Division of Consolidated Laboratory Services, Richmond, VA

Objective: Validation of a second tier Sanger sequencing method for the detection of IDUA and GAA sequence variants in Virginia.

Background: During 2018 Virginia worked on assay development for MPS I and Pompe screening with a proposed algorithm of first tier Digital Microfluidics (DMF) and second tier Sanger sequencing for all alpha-L-Iduronidase (IDUA) and acid alpha-glucosidase (GAA) coding regions and intron-exon boundaries, as well as detection of the common exon 18 deletion in GAA by polymerase chain reaction (PCR) and gel electrophoresis. Population screening was implemented in 2019.

Method: The Sanger method employs extraction of DNA from 3.2-mm dried blood spot (DBS) samples punched in duplicate, followed by PCR analysis for the respective gene (11 reactions for IDUA; 15 reactions for GAA) using the BigDye Direct Cycle Sequencing Kit. PCR products are verified by gel electrophoresis, followed by cycle sequencing and cleanup using BigDye XTerminator reagents. Plates are then loaded on the ABI 3730 DNA Analyzer platform. Post sequencing analysis involves variant detection using Variant Reporter (ThermoFisher) software and variant classification using American College of Medical Genetics (ACMG) guidelines, facilitated by an automated bioinformatics program: Newborn Screening Variant Interpreter (NBSVI).

Results: Secondary screening by Sanger sequencing has reduced the referral rate for MPS I and Pompe by 26.3% and 52.2%, respectively. More current data will be shared, including sequence variants detected in IDUA and GAA by their classification (Pathogenic, Likely Pathogenic, Variant of Uncertain Significance, Likely Benign and Benign), as well as diagnosed cases.

Conclusion: The Virginia NBS program has met the goal of implementing population screening for MPS I and Pompe utilizing first tier DMF followed by second tier Sanger sequencing. This approach has significantly reduced the number of patients referred to diagnostic testing and provides useful and pertinent variant classification for the genes studied.

Presenter: Richard Haughton, Virginia Division of Consolidated Laboratory Services, Richmond, VA, Email: richard.haughton@dgs.virginia.gov
**Poster #21**

**Eonis™ Platform for Newborn Screening of SMA, SCID, and XLA**

T. Helenius¹, M. Hjort¹, O. Sareila¹, K. Vahtera¹, M. Jaakkola¹, S. Dallaire², D. Hougaard³, J. Bybjerg-Grauholm³, M. Maekvad-Hansen³, D. Adamsen³, C. Gutierrez-Mateo²; ¹PerkinElmer Wallac, Turku, Finland, PerkinElmer, Waltham, MA, ³Statens Serum Institut, Copenhagen, Denmark

Newborn screening for Spinal Muscular Atrophy (SMA) enables early treatment of asymptomatic newborns. Multiplexing existing primary immunodeficiencies screening is beneficial most importantly for the outcome of the patient but also for reducing costs related to health care consumption. The recently developed high-throughput qPCR assay for early detection of SMA, Severe Combined Immune Deficiencies (SCID) and X-linked agammaglobulinemia (XLA) from a DBS empowers screening of multiple samples semi-automatically with complete sample tracking included.

The available Eonis™ SCID-SMA assay detects the absence of exon 7 in the SMN1 gene, which is a recognized marker of SMA. Simultaneously with the detection of SMN1, copy numbers of T-cell receptor excision circles (TREC) and Kappa-deleting recombination excision circles (KREC) are assessed for detection of SCID and XLA. The multiplex assay utilizes RPP30 as an internal amplification control monitoring the quality of the extracted DNA as well as for the basis of quantification for TREC and KREC. The assay is monitored using disease-positive and disease-negative DBS controls, which are extracted and processed simultaneously with the newborn samples throughout the whole workflow.

The reliability and accuracy of the Eonis assay was assessed by the clinical validation study. The study screened over 3000 confirmed normal samples and 52 SMA, 17 SCID, and 4 XLA disease-positive newborn samples. All disease-positive samples were detected. The results from this study validate the effectiveness of the assay and demonstrate its potential benefit for newborn screening programs.

Eonis™ platform can be set up for low, middle and high throughput labs. Assay workflow including all needed reagents and consumables from punching to result interpretation is available in 96 and 384-well formats including options for automation. As the result of the development of a semi-automated workflow, both assay-formats minimize hands-on time and the total workload for the screening lab. Current treatments for SMA and primary immunodeficiencies are effective and available, hence a fast, reliable and validated screening method is crucial for a successful treatment early in the life of a newborn.

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**Presenter:** Terhi Helenius, PerkinElmer, Turku, Finland, Email: terhi.helenius@perkinelmer.com
The Role of Second Specimens in Second Tier Testing for Newborn Screening: Accurately Identifying True Positives in Congenital Hypothyroidism and Cystic Fibrosis

V. Yakuta, K. Inkhamfong, S. Rao and G. Bonn, Colorado Newborn Screening, Denver, CO

Newborn screening (NBS) is the leading public health initiative that is positively impacting early detection and the rate of survival of newborns. Colorado is one of a number of states that performs a 1st screen, at 24 to 48 hours of life and 2nd screen, at 8 to 14 days of life. A 2nd screen allows the Colorado Newborn Screening program to tailor second tier testing specifically, for congenital hypothyroidism (CH) and cystic fibrosis (CF). The evaluation of CH involves two independent analyses, thyroxine (T4) and thyroid stimulating hormone (TSH). Congenital hypothyroidism (CH) has been shown to be much more accurate in testing after 48 hours of life. Each year additional cases of CH are found on the 2nd screen that otherwise tested normal on the 1st screen. In Colorado the primary screen for CH is T4 and the second tier screening is TSH. A T4 value of 6 μg T4/dL serum or less is the first indicator of CH. The State of Colorado then performs TSH testing, which is the second tier testing, on the lower 11% of the results of T4. A TSH value greater than 20 μU/mL blood and less than 40 μU/mL blood on the 1st screen is considered borderline and called to the primary care physician (PCP) in order to insure collection of a 2nd screen. A TSH value greater than 40 μU/mL blood on the initial screen is considered positive and called out to endocrine specialist for further diagnostic testing and treatment. Colorado tests all 2nd screens for T4 following the same referral algorithm to TSH as 1st screens, any TSH value greater than 20 μU/mL blood on the 2nd screen is considered positive and called out to endocrine specialist for further diagnostic testing and treatment.

Cystic fibrosis (CF) testing also includes a second tier test and 2nd specimen in its algorithm. The evaluation of CF involves two independent analyses, immunoreactive trypsinogen (IRT) and DNA testing. Colorado’s algorithm is IRT/IRT/DNA. Colorado also has a high initial IRT level, greater than 140 ng/mL blood, that triggers immediate CF DNA testing. For 1st samples with IRT greater than 60 ng/mL and less than 140 ng/mL, CF DNA testing will be performed on the 1st blood spot specimen within fourteen days of screening, if a 2nd specimen has not been received. An IRT value of greater than 60 ng/mL blood on the 2nd specimen will initiate CF DNA testing. An IRT value of less than 60 ng/mL blood on the 2nd specimen indicates a normal result. With two screens and the ability of second tier testing, false negatives and false positives of disorders such as CH and CF are decreased while controlling costs of DNA testing. Two screens with second tier testing provides conclusive, timely, and accurate results allowing treatment to be initiated as early as possible.

Presenter: Varvara Yakuta and Kathy Inkhamfong, Colorado Newborn Screening, Denver, CO, Email: varvara.yakuta@state.co.us & kathy.inkhamfong@state.co.us
Incorporating SMA into the Colorado SCID Assay: Lessons in Assay Development and Optimization
K. Jones, D. Tran, T. Holt and G. Bonn, Colorado Newborn Screening, Denver, CO

As Colorado Newborn Screening began looking at incorporating SMA in early 2019, there were several challenges that needed to be addressed. At the time, Colorado was running a single-plex SCID assay utilizing a standard curve. This single-plex method required additional wells and an additional curve to evaluate beta-actin, which increased time at the bench and potential for error. Since the inception of the Colorado SCID assay in 2012, new master mix and DNA extraction chemistries have emerged that potentially offered cheaper, better assay performance. Thus the effort to incorporate SMA into the SCID assay became a multi-tiered project to optimize the Colorado SCID assay which included four components: update the mastermix, optimize DNA extraction, eliminate use of the standard curve for reporting, and create a multiplex assay that included SMN1 and Rnase P. During the development and validation of these components, Colorado encountered unexpected problems including assay components that, once validated, did not perform as expected in production and a need to change reagent sources after initiating a validation. These problems highlight the importance of pre-validation testing in assay development and the need to consider the assay as it would function in production as well as in validation. Despite (and, indeed, because of) these problems, Colorado Newborn Screening has since completed each component of its goal and has gone live with the final assay as of January 20, 2020. As the assay performs in the production environment, continuous monitoring of production results will demonstrate how well each component was addressed and inform further optimization as needed.

Presenter: Kendra Jones, Colorado Newborn Screening, Denver, CO, Email: kendra.Jones@state.co.us
Investigation of Chip-Based dPCR Platform in the Newborn Screening Laboratory for Enumeration of SMN2
K. Jones, T. Holt, and G. Bonn, Colorado Newborn Screening, Denver, CO

Currently, the established platform for dPCR used in Newborn Screening Laboratories is the Biorad QX200 ddPCR system. It is capable of running multiple samples simultaneously and has kits available for SMN1/SMN2 copy number determination. However, the initial cost can be prohibitive and smaller labs using dPCR as a second tier assay may not have enough samples to justify the purchase of this platform. The use of a cheaper, chip-based platform like the QuantStudio 3D may be more suitable for these laboratories. To determine the feasibility of using chip-based dPCR in the newborn screening laboratory, the Colorado Newborn Screening Laboratory engaged in testing for SMN2 copy number using Thermo Fisher’s QuantStudio 3D chip-based dPCR. SMN2 copy number is a useful second tier assay for SMA as it can indicate SMA disease severity and impact treatment plans. As there are currently no pre-made dPCR kits available for determining SMN2 copy number on a chip-based the system, Colorado Newborn Screening is developing its own SMN2 dPCR assay. Preliminary results demonstrate that this assay is both accurate and sensitive, indicating that the chip-based platform may be a suitable dPCR option for newborn screening laboratories. Colorado Newborn Screening aims to complete optimization of chip-based dPCR assay for SMN2 copy number determination and develop a dPCR assay for TREC to expand the use of this platform in the newborn screening laboratory.

Presenter: Kendra Jones, Colorado Newborn Screening, Denver, CO, Email: kendra.Jones@state.co.us
Updating & Revising Newborn Screening Card Artwork: Michigan’s Experience Bringing Key Stakeholders to the Table

M. Kleyn¹, S. Moloney¹, L. Turbett¹, K. Andruszewski¹, R. Robinson², M. Seeterlin¹; ¹Michigan Department of Health and Human Services, Lansing, MI, ²Michigan Medicine, Lansing, MI

Background: The newborn screening (NBS) card demographic information is time-consuming for hospital staff to complete and for NBS staff to follow up on missing information. To ensure the best use of hospital and program staff time, Michigan’s NBS Program created a workgroup to review the data elements on the first and repeat sample cards. The workgroup was tasked to confirm every element has a purpose and the need is clearly communicated to hospital staff.

Methods: A workgroup was formed with stakeholders representing different aspects of the NBS process including nurses from regular and NICU nurseries, NBS laboratory, and NBS follow-up staff. The group met monthly via conference call to review each data element, focusing on five main questions: Why is it needed? How is it used by the NBS lab or follow-up area? Is it clear for hospital staff what is being requested? Does the NBS Guide for Hospitals need to be revised to more clearly explain the request? Are hospital staff experiencing any difficulty gathering that information?

Results: The workgroup completed its review after seven meetings. The final list of suggested changes was reviewed with personnel involved with setting up HL7 messaging and the vendor that prints Michigan’s NBS cards and manages the laboratory information management system (LIMS). The list of changes was narrowed down based on feasibility. The final changes involved updating the format to allow for more space, adding a reminder checklist about the components of the NBS on the flap that covers the filter paper, adding a “Notes” box, updating labels for clarity or consistency, and removing some fields on the repeat cards that are already collected on the first sample card. Proofs with the revised card artwork were printed and tested by NBS program staff. Prior to sending out revised cards to hospitals, the vendor worked on changes to the LIMS to accommodate the revisions and the changes were communicated to hospital staff. To inform hospital staff, updates were made to Michigan’s NBS Guide for Hospitals, the NBS quarterly newsletter for hospitals contained an article detailing the changes, and a presentation about the changes was given at an annual NBS conference for hospital staff.

Conclusions: Performing a detailed review of NBS cards to minimize and clarify requested data elements is beneficial. We created an updated NBS card that added space, updated labels, and removed unnecessary fields. However, the process of making significant changes to the card artwork is lengthy and requires input from and communication with multiple stakeholders to ensure a smooth transition.

Presenter: Mary Kleyn, Michigan Department of Health and Human Services, Lansing, MI, Email: kleynm@michigan.gov
Integration of Classic Galactosemia Screening and Galactose-1-phosphate Measurement into the New NeoBase 2 Non-derivatized MSMS Kit Assay

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Classic Galactosemia (type I) is an inborn error of galactose sugar metabolism, which is caused by a deficiency in the galactose-1-phosphate uridyltransferase (GALT) enzyme. Because the defected GALT enzyme cannot convert the galactose to glucose, the galactose, galactose-1-phosphate (Gal-1-P) and other galactose metabolites accumulate in blood and tissues. Since classic Galactosemia can be effectively treated by a galactose-restricted diet, it has been included in many newborn screening programs for several decades. Currently, the predominant methods are based on separate GALT enzyme activity or total galactose concentration measurements, which however cannot readily be multiplexed with routinely used tandem mass spectrometry (MSMS) assays to screen an expanded panel of other metabolic disorders.

Based on the recent findings that selective Gal-1-P measurement can be integrated into the currently used non-derivatized MSMS assays [1], we further demonstrated in this work that this novel approach can be also used with the new PerkinElmer NeoBase™ 2 Non-derivatized MSMS kit assay only with minor modifications in the sample preparation and MSMS data collection workflow. In this Gal-1-P integrated NeoBase 2 assay, the Gal-1-P measurements were performed simply by adding the stable isotope labelled Gal-1-P internal standard in the dried blood spot (DBS) sample extraction step, whereas all the other NeoBase 2 assay procedure conditions were kept unchanged. This modified assay concept was tested with two different MSMS instruments including Waters® TQD and PerkinElmer QSight® 225MD systems. With both tested MSMS systems, the currently applied positive mode MSMS method to detect other non-Galactosemia markers was kept unchanged, after which additional two Gal-1-P analyte and internal standard transitions (m/z 259->79 and 265->79 respectively) were acquired during the finally added short negative mode function. For Research Use Only. Not for use in diagnostic procedures.


Presenter: Tero Lehtonen, PerkinElmer, Turku, Finland, Email: tero.lehtonen@perkinelmer.com
Detection of Early Onset Carnitine Palmitoyltransferase II Deficiency by Newborn Screening. Should CPT II Be a Primary Disease Target?
R. Mador-House, S. Dyack and Z. Liu, IWK Health Centre, Halifax, NS, Canada

Introduction: Early-onset carnitine palmitoyltransferase II deficiency (CPT II) has severe outcomes which are often fatal in the neonatal to infantile period. CPT II is a secondary target in the USA according to the Recommended Uniform Screening Panel. We report a case of neonatal-onset CPT II deficiency that highlights the ease and impact of detection through newborn screening.

Case Report: The patient was a normal-appearing term female infant. On day 8 of life she was identified as at-risk for CPT II via the expanded Maritime Newborn Screening Program using tandem mass spectrometry. She was clinically assessed on day 9 of life. The parents were educated about risk avoidance recommendations and emergency protocols during illness. She had approximately 15 hospital admissions due to episodes of decreased feeding and/or vomiting during her first 5 years of life.

The diagnosis of CPT II was confirmed through residual enzyme assay and gene variant analysis. Newborn screening prevented a diagnostic odyssey for this family and allowed for personalized management from infancy. We suggest that newborn screening allowed for early treatment and timely intervention during illness and may explain this patient’s normal neurological and cardiovascular development despite having a severe deficiency of CPT II.

Newborn screening for CPT II is highly sensitive and specific with no false positives identified since 2005.

Take Home Message: Newborn screening for early onset CPT II should be considered as a primary newborn screening target. Early diagnosis leads to expedited treatment and improved clinical outcomes while limiting burden on a newborn screening program.

Presenters: Zaiping Liu or Sarah Dyack, IWK Health Centre, Halifax, NS, Canada, Email: zaiping.liu@iwk.nshealth.ca; sarah.dyack@iwk.nshealth.ca
A Systematic Study of Solvent/Buffer Composition for the Extraction of Newborn Screening Biomarkers from Dried Blood Spots

E. Lobo, C. Haynes and K. Petritis, Centers for Disease Control and Prevention, Atlanta, GA

Introduction: Extraction solvent/buffer optimization is required for multiplexing mass spectrometry-based assays. Currently, the US Centers for Disease Control and Prevention’s (CDC) Newborn Screening and Molecular Biology Branch (NSMBB) has a laboratory-developed test multiplexing amino acids (AA), acylcarnitines (AC), succinylacetone (SUAC), and guanidinoacetate methyltransferase (GAMT) analytes for first-tier screening [1]. The current extraction solvent composition is 80:20 acetonitrile/water containing 0.1% formic acid and 3 mM hydrazine hydrate. The objective of this study was to investigate recoveries of existing and additional analytes under various extraction solvent/buffer compositions.

Methods: The samples in this study were quality control (QC) dried blood spots (DBS) created by the CDC. Non-derivatized sample preparation methods were used. Multiple solvent mixtures of methanol/water and acetonitrile/water ranging from 70% to 100% organic solvent, with modifications of salt and acid concentrations, were investigated for optimal analyte recovery. Samples were analyzed by FIA-ESI-MS/MS in positive mode.

Results: Preliminary data indicated adequate extraction of LPCs, SUAC, creatine (CRE), creatinine (CRN), and guanidinoacetic acid (GAA) using 100% methanol and 70:30 methanol/water. The average recovery was 80% for CRE, CRN, and GAA. On the other hand, SUAC recovery was only 20% in 100% methanol, and adding 8% water to the extraction solution increased recovery to 30%; similar to our current method. Percent recovery of LPCs enriched close to X-ALD cutoffs was 80%, using 100% methanol as an extraction solvent. The effect of the extraction solvent on ion-suppression is currently under investigation.

Conclusions: This study aims to determine optimal extraction solvent composition to multiplex different classes of first-tier analytes, ranging from very hydrophobic to very hydrophilic. It is important to balance pros and cons when developing an extraction solvent for a highly multiplexed first-tier screening method.


Presenter: Edgardo Lobo, Centers for Disease Control and Prevention, Atlanta, GA, Email: pvj6@cdc.gov
Newborn Screening: Leveraging Partnerships and Utilizing Key Health Communication Principles to Raise Awareness
J. Loey and N. Bonhomme, Expecting Health at Genetic Alliance, Washington, DC

While every baby born in the United States receives newborn screening (NBS), families are often unaware of this common test. To raise awareness for NBS among new and expecting parents, healthcare professionals, and the public, September has been named Newborn Screening Awareness Month. By harnessing the power of human connection and fostering collaboration across multiple sectors, Baby’s First Test, the nation’s educational resource for NBS, has pushed a national awareness campaign since 2012. To gain a better understanding of effective communication strategies to raise awareness for NBS, Baby’s First Test conducted an analysis of the #2019NBS campaign to share lessons learned and promising strategies to create a successful awareness campaign at the state, regional, and national levels.

Throughout September 2019, Baby’s First Test created NBS graphics to share facts, conducted interviews with key leaders, and shared family stories across our social media platforms, as well as highlighted education and awareness efforts from communities and organizations. To support education and awareness efforts for multiple stakeholders, including state health departments and professional organizations, Baby’s First Test created a variety of resources, such as a social media toolkit and infographic awareness cards, and facilitated a number of activities, including a Twitter Chat and Facebook Live interviews. Baby’s First Test utilized social media analytics, website traffic, and a database of partnerships and requested materials to measure the campaign’s reach.

By raising awareness through multiple modalities and leveraging known dissemination channels, Baby’s First Test reached 2.4 million people and generated 10.4 million impressions. State health departments, including Virginia, Texas, Nebraska, and Michigan, and organizations, such as March of Dimes and Parent Project Muscular Dystrophy, joined the conversation by utilizing Baby’s First Test resources and sharing their own NBS materials to raise awareness. Our findings highlight the importance of multi-sector collaboration and the need for relevant and accessible educational materials.

In this session, we will share data on the different resources various stakeholders of the NBS system utilized, as well as discuss approaches to developing a successful awareness campaign. We will highlight strategies, so organizations can leverage existing work and partnerships in order to build awareness both within and beyond NBS awareness month at the state, regional, and national levels.

Presenter: Jamie Loey, Expecting Health, Washington, DC, Email: jloey@expectinghealth.org
The Challenges and Rewards of Creating Animated Video for Newborn Screening Education
S. Mann and L. Aiyar, Hawaii State Department of Health, Honolulu, HI

Parents want understandable information from a trusted source right after they learn that their baby may have a disorder on newborn screening. To respond to parent requests, the Western States Regional Genetics Network developed the first set of newborn screening fact sheets for parents in 2003. Seventeen years later, current parents and parents-to-be tell us that they prefer to watch videos rather than read the information. This started the journey to convert the newborn screening fact sheets from words and pictures on paper to animated video.

The process to convert the disorder fact sheets from the written word to animated video has been more challenging than expected. Much more consideration must be given to the graphical and oral representation of information including, but not limited to, concepts of race, disability, and personal perception. There is also much more audience testing required and revisions to respond to the testing results.

We will detail the work, cost, and the lessons learned for any newborn screening program considering developing video newborn screening education. We will also share our experiences with the professional and family review process for the videos. Finally, we will show one of our finished animated videos that has completed the full testing and review process.

Presenter: Sylvia Mann, Hawaii State Department of Health, Honolulu, HI, Email: sylvia@hawaiigenetics.org
To Unsat or Not to Unsat, that is the Question; The Process Change of an Increase in Standard of Acceptability of Dried Blood Spots with Facility Education and Outreach in the State of Kansas Education and Outreach in the State of Kansas

D. Caldwell, K. Anderson and M. Mills, Kansas Health and Environmental Laboratory, Topeka, KS

The State of Kansas Newborn Screening planned to onboard four new disorders in 2019 and 2020 which required more punches from the submitted newborn screening (NBS) dried blood spots. We realized there was a need for improvement in collection and facility training in early 2018. Our daily review of specimens received required a more discerning eye and also a change in notifications to facilities to improve communication of unsatisfactory (Unsat) specimens. In 2019, an analyst with four years of phlebotomy experience was hired in the NBS Laboratory, and with the assistance of the Case Management educator, both began to work together to educate facilities regarding proper collection practices. Facilities were officially notified of a laboratory process change in July 2019 regarding an increase in our standards of specimen acceptability.

As part of an outreach to our submitting facilities in Kansas, the laboratory adopted a more comprehensive approach for notification of Unsat specimens. A letter notification and scan of the specimen was sent to the facility to provide a visually observable reason for the Unsat determination. The scan included the designation of Unsat and reasons as to why the specimen may have had an error in the collection process affecting the quality of the specimen. In addition, Unsat specimens were manually marked on the filter paper as to which blood spot circles were acceptable and which were not. Two analysts had to agree to this determination and initial the NBS form. If a facility contacted the laboratory to inquire about the determination, the two analysts would discuss with the facility the reasons behind the determination.

The process of educating submitting facilities has been an on-going team effort between the NBS laboratory and Case Management. The Case Management/Follow-up team continue their process of notifying the provider of the Unsat specimen and request a re-draw of the specimen. In addition, facilities who call with questions about improving their collection process can get advice from the Case Management educator and/or our experienced phlebotomist on staff in the NBS lab. Lab staff are occasionally available to visit facilities for on-site training or video conference with facility nursing and phlebotomy staff. This outreach program has improved communication between facilities and NBS laboratory as well as educated facilities regarding specimen collection. This presentation will outline the increase in standards for satisfactory specimens, the change in notification process, including outreach to and education for submitting facilities in the State of Kansas.

Presenter: Michelle Mills, Kansas Health and Environmental Laboratory, Topeka, KS, Email: michelle.J.mills@ks.gov
Implementation of a Sunday Courier and Decrease of Weekend Transit Time for the State of Kansa  
A. Huber and M. Mills, Kansas Health and Environmental Laboratory, Topeka, KS

The State of Kansas Health and Environment Newborn Screening (NBS) department does not have a daily courier service except for a few larger facilities who provide their own. Birthing facilities must fund their own specimen transport and primarily utilize overnight FedEx or UPS transport of their specimens. Even though the Kansas NBS laboratory has greatly improved turnaround time within the screening laboratory since early 2019 and has increased full operational testing to six days a week by adding Saturday as a work day, we were finding transit times were not improving. Transit times of ≥ 4 days for facilities was at 37%. In addition, birthing facilities recommend inducing on Thursday and Fridays which increases the number of NBS specimen collections over the weekend. A previous grant from 2017 to 2018 through NewSTEPS, called the 360 Timeliness Project, offered seven Kansas submitting facilities the opportunity to receive compensation for their NBS specimens sent through the United Parcel Service (UPS). We were hoping to see a dramatic improvement from the collection time at these facilities to received times at the lab. There was not a marked improvement in turnaround time and one facility could not get consistent UPS pick-up due to location. Therefore, we had to look for another alternative to improve transit times.

The Kansas NBS lab, with the recommendation of a consultant, decided to request a QI grant to fund a Sunday Courier covering the top 29 facilities in terms of their number of NBS specimen submissions. With grant funds, we employed a Sunday Courier to transport the weekend specimens from those specific 29 facilities to the NBS lab in time for testing starting each Monday morning. The courier pickups would cover those NBS specimens collected from late Friday to early Sunday morning. Originally, this large cohort of specimens was not sent by facilities until Monday and not received in the NBS lab until Tuesday morning.

The goal was to reduce the 37% for ≥ 4 days transit time by 50%. This presentation will outline the implementation process of the Sunday Courier along with the results of the decreased transit time and decreased overall turn-around-time for the Kansas NBS system.

Presenter: Michelle Mills, Kansas Health and Environmental Laboratory, Topeka, KS, Email: michelle.J.mills@ks.gov
Implementation of 2nd Tier Congenital Adrenal Hyperplasia Newborn Screening in Virginia: Rapid Steroid Profile by High Performance Liquid Chromatography-Tandem Mass Spectrometry Using a Biphenyl Stationary Phase Ligand

C. Nixon¹, H. Bryant¹, P. Hetterich¹, L-E. Lion¹, W. Andrews¹, C. Crews², J. MacDonald², S. Rutan³; ¹Virginia Division of Consolidated Laboratory Services, Richmond, VA, ²Virginia Department of Health, Richmond VA, ³Virginia Commonwealth University, Richmond, VA

Background: Virginia has utilized immunoassays targeting 17α-hydroxyprogesterone (17-OHP) for newborn screening (NBS) of congenital adrenal hyperplasia (CAH) since 2002. While sensitive for the detection of classic CAH, immunoassays show poor specificity which can lead to high rates of false positive reporting. Primary causes for false positive reporting of 17-OHP concentrations are cross-reactivity of non-target analytes with immunoassay antibodies as well as stressors such as prematurity, low birth weight, or illness leading to elevated 17-OHP levels in newborn specimens. Despite utilization of birth weight-adjusted cutoffs for 17-OHP, the annual false positive reporting rate for immunoassay CAH screening in Virginia was between 2-3%.

A 2nd tier assay utilizing high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was developed and implemented with the goals of maintaining sensitivity for classic CAH while reducing false positive CAH reporting, maintaining rapid turnaround time for CAH screening, and utilizing existing instrumentation within the laboratory with minimal modifications.

Methods: Five analytes (17-OHP, androstenedione, cortisol, 21-deoxycorticosterone, and 11-deoxycortisol) were eluted from dried blood spots (DBS) and analyzed by HPLC-MS/MS. A core-shell HPLC column with biphenyl stationary phase ligand and a rapid mobile phase gradient separation were utilized to achieve baseline resolution of isobaric compounds at five minutes per injection.

Because CAH is a time-critical NBS condition, CAH screening, including 2nd tier analysis, is performed 365 days-per-year by Virginia’s NBS Program. All specimens showing elevated 17-OHP results by 1st tier immunoassay screening are reflexed to 2nd tier analysis, eliminating confirmatory immunoassay analysis and birth weight-adjusted cutoff considerations.

Results: An interpretation algorithm consisting of individual analyte cutoffs as well as a clinical ratio was used to identify specimens indicative of possible CAH. Results from retrospective 2nd tier analysis of de-identified DBS specimens from cases previously deemed to be true negatives, false positives, and true positives for CAH were used to inform 2nd tier cutoffs. Both retrospective and prospective analyses indicated that the 2nd tier CAH workflow reduced false positive reporting for CAH by as much as 90%.

Conclusions: The reported 2nd tier CAH protocol has improved CAH screening in Virginia without negative impacts to turnaround time. Challenges to implementing the assay included cross-training staff to maintain full coverage for 365 day-per-year analysis and data review and education of primary healthcare providers regarding interpretation of 2nd tier results. The assay and cutoff algorithm will continue to be monitored to determine areas for future improvement.

Presenter: Christopher Nixon, Virginia Division of Consolidated Laboratory Services, Richmond, VA, Email: christopher.nixon@dgs.virginia.gov
Building an SMA Newborn Screening Implementation Pilot Project Management Plan Using the New Conditions Checklist

K. Noble Piper\textsuperscript{1}, S. Berberich\textsuperscript{2}, A.R.U.L. Calhoun\textsuperscript{3}, T. Henry\textsuperscript{2}, C. Johnson\textsuperscript{4}; \textsuperscript{1}Iowa Department of Public Health, Des Moines, IA, \textsuperscript{2}State Hygienic Laboratory at the University of Iowa, Coralville, IA, \textsuperscript{3}Stead Family Department of Pediatrics, University of Iowa Health Care, Iowa City, IA, \textsuperscript{4}University of Iowa Stead Family Children's Hospital, Iowa City, IA

The Iowa Department of Public Health (IDPH) Iowa Newborn Screening Program (INSP) is undertaking a newborn screening implementation pilot for Spinal Muscular Atrophy (SMA).

**Problem:** With so many moving parts and so many stakeholders, there needs to be a project management plan in place to monitor and assess the processes and progress of the SMA implementation pilot project. The project plan should include all activities necessary to ensure a successful pilot.

**Methodology:** In 2019, the APHL and the Newborn Screening Technical assistance and Evaluation Program (NewSTEPs), through the efforts of the New Disorders Work Group, developed a New Disorders Checklist. This checklist outlines four phases for newborn screening programs to consider when implementing screening for a new disorder. The Executive Team of the Iowa Newborn Screening Program used this framework to build an SMA newborn screening implementation pilot project management tool (SMA PM tool.) The SMA PM tool expands on the New Disorders Checklist by outlining eight categories: SMA Pilot Planning; Authority to Screen; Laboratory; Follow-up; Information Technology; Education; Partner Notifications; and Screening. Each category contains tasks to be completed; responsible person(s); a timeline, including start and end dates; and a measurement of progress.

**Significant Results/Conclusions:** The SMA PM tool has proven to be a useful tool for managing the Iowa SMA implementation pilot project. In addition to allowing tracking of progress on activities, it assures that all activities required for successful SMA newborn screening are outlined. The SMA PM tool also provides a means to report progress to stakeholders. The SMA PM tool has been modified for use in other programs, both in- and out-of-state.

**Presenter:** Kimberly Noble Piper, Iowa Department of Public Health, Des Moines, IA, Email: kimberly.piper@idph.iowa.gov
Newborn Screening Outcome Review

The implementation of a new laboratory information system gave the Iowa Newborn Screening Program the opportunity to update and improve newborn screening case management outcomes. Case management outcomes are assigned by medical consultants and follow-up staff once an infant is diagnosed with a newborn screen condition or a newborn screen condition has been ruled out after an abnormal newborn screen result. Outcomes are fundamental in assessing the effectiveness of the screening process and provide a wealth of data to improve and refine cutoffs and other program metrics.

Previous program outcomes included 16 designations with poor definitions and inconsistent usage. The new outcomes will include 28 designations with clear definitions and specific case examples. The new outcomes differentiate between whether the specified disorder is currently on the RUSP, how the diagnosis was made (via genetic confirmation or by clinical presentation) and options for false negative results. Outcomes were developed with input from Iowa laboratory staff, follow-up staff and medical consultants.

Once there was a consensus on the new outcomes and how they would be used, practice cases were developed representing different newborn screening scenarios. The practice cases were distributed to follow-up staff, genetic counselors and lab staff. Staff were asked to independently assign an outcome to each case using the new definitions. Overall agreement between staff on assignment of the new outcomes was high, and inconsistencies in how the outcomes were used were discussed and resolved as a group. These refined outcomes and definitions were then shared with Iowa’s state partners (North Dakota, South Dakota, and Alaska), and based on their input, additional revisions were made to ensure the outcomes would work across state lines.

These outcomes will be implemented in Iowa’s new laboratory information system. The system will use built in logic to help ensure accurate outcome capture. Definitions and case examples will be visible in the laboratory information system for ease of usability. Future projects involve developing outcomes for baby matching cases and long-term follow up case management.

Presenter: Emily Phillips, University of Iowa, Iowa City, IA, Email: emily-phillips@uiowa.edu
A Fully Automated Assay for Quantification of NT-proBNP Level from DBS Samples
H. Appelblom, H. Polari and M. Sairanen, PerkinElmer Inc., Turku, Finland

Critical Congenital Heart Disorder (CCHD) is the leading cause of birth-defect associated deaths affecting ca. 1 in 400 births in the US but is often treatable if detected early. Currently all states in the US screen for CCHD using point-of-care pulse oximetry testing, but around 25%-30% of newborns with CCHD remain undetected.

To potentially improve the efficiency of CCHD newborn screening, an automated immunoassay based on the DELFIA® technology was developed on GSP® instrument (PerkinElmer, Turku, Finland). The assay detects amino-terminal fragment from prohormone of brain-type natriuretic peptide (NT-proBNP) using monoclonal antibodies. The natriuretic peptides are synthesized as pre-prohormones and cleaved to prohormones. At release from cardiac myocytes to blood circulation, biologically active BNP is formed along with biologically inactive NT-proBNP. Increases in both BNP and NT-proBNP concentrations in plasma have been associated with adverse outcomes in heart failure patients. However, only few studies have reported distribution of the natriuretic peptides during infancy and childhood. All these studies have been done by analysing venous blood specimens, which is challenging especially with newborns.

The developed assay is able to measure NT-proBNP from dried blood spot specimens, which is known to be an important biomarker in cardiac diseases in adults. In the future, measuring of NT-proBNP levels may improve the screening of CCHD in newborns.

Presenter: Hanna Polari, PerkinElmer Inc., Turku, Finland, Email: hanna.polari@perkinelmer.com
Quality Initiative Reveals Room for Improvement

V. Ragland, A. Porter, A. Ingram, Y. Li, H. Fryman, G. Dizikes and C. Dorley, Tennessee Department of Public Health, Nashville, TN

Problem: Issues that remain and warrant further efforts for quality improvement in the Tennessee Newborn screening program include extremely long transit times, lost specimens, and delayed reporting due to incomplete or inaccurate information submitted on specimen forms and no recollection when specimens are unsatisfactory.

Methodology: We evaluated quarterly reports compiled to track timeliness for DOL 5 and DOL 7 reporting and weekly reports sent to birthing hospitals to ensure dried blood spot (DBS) specimens have been collected.

Significant Results: Our analysis revealed we had an increase in missed specimens (in 2018 a total of 162 infants were identified, excluding expired babies, ranging from six to 27 in a particular month), a number of specimens that were unsatisfactory that had no recollection (in 2018, 188 infants with an initial unsatisfactory specimen had no specimen recollected, so were never retested) and delayed demographic entry impacting DOL5 and DOL7 reporting times (time trend for abnormal results reported out DOL5 reached 74% in Q4 of 2018 and time trend for abnormal results reported out DOL 7 was 69% for Q4 of 2018). We also realized that we do not currently have the ability to hold our providers accountable for their role in achieving timeliness of newborn screening benchmarks.

Conclusion: Timeliness is not met when specimens are lost or delayed and when hospitals do not collect the specimens on babies who meet criteria for collection at 24 hours and 1 minute, they miss the courier pickup thereby delaying testing by a full day. Hence, we are targeting these hospitals and birthing centers monitoring their amount of specimens sent per month, how many of these specimens were inaccurate, and the transit times. These targeted hospitals will be prioritized for site visits and will have the OZ system installed first. The OZ system provides a customized electronic form prepopulated with baby’s details from the hospital electronic health record which should eliminate manual data entry, eliminate missing information, decrease human error and provide a tracking system to decrease lost or delayed delivery of specimens to the laboratory.

Presenter: Valerie Ragland, Tennessee Department of Public Health, Nashville, TN, Email: valerie.ragland@tn.gov
Information Needs Among Providers Related to SCID Newborn Screening and Patient Care
M. Raspa¹, A. Gwaltney¹, K. Porter¹ H. Peay¹, A. Huston², B. Fitzek², J. Boyle²; ¹RTI International, Research Triangle Park, NC, ²Immune Deficiency Foundation, Towson, MD

Objective: Severe Combined Immune Deficiency (SCID) is a collection of genetic disorders that cause profound defects in cellular and humoral immunity. As of December 2018, all 50 states have implemented newborn screening for SCID. Despite this success, significant challenges remain for patients, families, clinicians, and public health professionals, including information needs for all stakeholders. The purpose of this session will be to present results from a provider survey which sought to understand the information needs related to SCID newborn screening and patient care.

Methodology: As part of the SCID Compass program, funded by the Health Resources and Services Administration, we conducted an online survey of two types of providers: those who had treated a patient with SCID and those who had not. For both groups, we asked how familiar they were with SCID and in meeting the needs of patients with SCID, what types of resources they would need, where they turned to for resources, how they rated currently available resources, and what their preferred format of resources were. Providers were recruited through multiple sources, including listservs managed by the Immune Deficiency Foundation and SCID Angel for Life Foundation, as well as an online provider panel. In total, 313 providers responded to the survey. On average, providers were 45 years old, predominantly White (72%), were from a variety of specialties (e.g., allergists/immunologist, pediatrician, family medicine, genetic counselor, nurse practitioner), and mainly practiced at an academic institution or in private practice.

Results: About two-thirds (67%) of providers had seen a patient with SCID. Not surprisingly, providers who had seen a patient with SCID were more knowledgeable about SCID and more comfortable in meeting the needs of patients with SCID (6.27 versus 3.38) than those who had not. Providers who had seen a patient with SCID were most likely to seek information in the peer-reviewed literature, and the Clinical Immunology Listserv whereas those who hadn’t seen a patient with SCID were most likely to turn to professional organizations. Top rated information needs for provider who had seen a patient with SCID were (1) understanding all available treatment options, (2) understanding child’s specific type of SCID, and (3) understanding what to expect over the SCID lifespan. For providers who had not seen a patient with SCID, their top-rated needs were (1) understanding child’s specific type of SCID, (2) knowing where to find specialists, and (3) knowing how to talk to families about test results. The most preferred format for materials was reading a web site.

Implications: These findings highlight the different types of information needs for providers regarding SCID newborn screening and patient care. With these results in mind, SCID Compass is designing a web site for providers to easily access SCID information.

Presenter: Melissa Raspa, RTI International, Research Triangle Park, NC, Email: mraspa@rti.org
SCID Newborn Screening: Validation of a 55-Gene Next Generation Sequencing Panel

Severe combined immunodeficiency (SCID) is a rare disease characterized by lack of functional T cells, B cells and/or Natural Killer cells. Deficiencies can result in impairment of cellular and humoral immune response, causing recurrent severe infections. If not diagnosed and treated during the neonatal period, SCID can become fatal. In May 2010, the Secretary of the US Health and Human Services (HHS) endorsed the addition of SCID as a core condition to the Recommended Uniform Screening Panel (RUSP). Variants in more than 40 genes have been shown to cause SCID and other immunodeficiencies. Mutations in the cytokine-receptor genes (IL2RG, JAK3, and IL7R), antigen receptor genes (RAG1, RAG2, DCLRE1C, and CD3E), and others such as ADA and PTPRC have been linked to SCID. Knowledge of the molecular defect can influence treatment however, molecular testing done via a clinical laboratory has slow turnaround times and requires prior authorization, causing increased stress and cost to families. This can potentially delay diagnosis and treatment. The New York State Program offered a multi-gene next generation sequencing (NGS) test for newborns, who screened positive for SCID as part of a CDC-funded project. We hypothesized that implementation of a second tier SCID gene panel via newborn screening could improve outcomes for infants and their families.

A 39-gene SCID panel was designed using TruSeq Custom Amplicon (TSCA) NGS technology. A custom bioinformatics pipeline was implemented, and Sanger sequencing was used to verify detected variants. The American College of Medical Genetics and Genomics (ACMG) guidelines were used to interpret the clinical significance of identified variants, and reports were provided to ordering physicians at Specialty Care Centers. When TSCA production was discontinued, we designed a new custom 55-gene ArcherDx SCID panel.

The 55-gene panel will be validated using DNA extracted from dried blood spots previously tested with the TSCA panel. Results from cases and reference samples with known genotypes will be compared to assess accuracy, sensitivity, specificity, false positive and false negative rates. Each gene will be assessed to ensure that no clinically relevant regions are missed due to poor coverage or quality. Reproducibility will also be assessed by testing replicates within and between runs. A bioinformatics pipeline will be implemented, optimized and validated to ensure high quality and accurate detection of variants using Archer Analysis, a comprehensive bioinformatics suite provided by ArcherDx that performs standardized analysis of Archer NGS data and allows for custom scripts to be incorporated into the analysis and report generation. A major goal of this project is to provide NBS programs with an easy and practical protocol for SCID variant identification, to quickly and efficiently provide a molecular diagnosis for infants who screen positive.

Presenter: Jessica Respress, Wadsworth Center, New York State Department of Health, Albany, NY, Email: jessica.respress@health.ny.gov
Global Newborn Screening of Common, Treatable Conditions Using Digital Microfluidics
P. Roesch, R. Sista and V. Pamula, Baebies, Durham, NC

Of the approximately 100 million babies born worldwide each year, an estimated 80% do not receive any testing through newborn screening (NBS), including disease risk identification, disease confirmation, or follow-up testing. Countries that have instituted and expanded newborn screening are able to identify and diagnose newborns with diseases that can be treated through medicinal or nutritional therapies. Specifically, the pre-symptomatic identification of several of these disorders in newborns enables the rapid initiation of treatments and reduces the significant health consequences associated with these diseases.

A platform has been developed to investigate the use of digital microfluidic testing (DMF) to help minimize potential barriers to newborn screening in regions where challenges exist in implementing central laboratory-based testing programs. The platform utilizes whole blood to simultaneously measure total serum bilirubin (TSB), glucose-6-phosphate dehydrogenase (G6PD) activity, galactose-1-phosphate uridylytransferase (GALT) activity, and thyroid stimulating hormone (TSH) to screen for hyperbilirubinemia risk, G6PD deficiency, classic galactosemia and congenital hypothyroidism, respectively. These diseases represent four of the most common, treatable neonatal conditions in the developing world with a combined incidence of approximately 1 in 10. The tests for TSB, G6PD, GALT and TSH are run together on disposable single-use cartridges using < 50 μl of whole blood. After sample loading onto the cartridge, all sample processing steps (including plasma separation) and assay reaction steps for all assays are automated by the platform with a combined run time of less than 2 hours.

Each of the assays was developed to span the clinically relevant range for each analyte with commensurate limits of detection. Preliminary testing of analytical assay performance including precision, interference and limit of detection were completed and assay performance met established requirements. Initial method comparison analyses showed strong correlation to currently utilized methods. These results demonstrate that inexpensive, low blood volume assays for these common disorders are possible using a DMF platform. NBS of these conditions via whole blood sample has the potential to enable screening in parts of the world that currently do not screen for these common conditions at birth.

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Poster #42

A Year in Review: Summary of Results from the First Year of Screening for Six Lysosomal Storage Disorders in New Jersey

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The New Jersey Newborn Screening (NJ NBS) Laboratory implemented screening for six Lysosomal Storage Disorders (LSDs), Krabbe, Pompe, Gaucher, Fabry, Niemann-Pick and Mucopolysaccharidosis I (MPS1), in July 2019. Using Customer Contracted Manufacturing Service (CCMS) products from PerkinElmer, enzyme activity is measured in all initial specimens utilizing a Waters TQD tandem mass spectrometry system. Screening cutoffs were established based on results of a blinded pilot study that included over 8,000 patients and defined as a percent of the daily patient median. Additionally, second-tier Sanger sequencing is performed for any newborn with enzyme activity below the retest cutoff for three of the LSDs, Krabbe, Pompe and MPS1. In the first six months of screening, 57,246 newborns were screened for the six disorders. For the three disorders where second-tier sequencing is not performed, 8 screened positive for Gaucher, 4 for Fabry, and 0 for Niemann Pick. For the three disorders where in-house sequencing is performed, 18 screened positive for Krabbe, 1 for Pompe, and 15 for MPS1. For Krabbe, all 18 specimens contained pseudodeficiency alleles. 8 of the 18 also had variants of uncertain significance, with one specimen containing 2 different variants of uncertain significance. 2 specimens were heterozygous for the most common pathogenic variant, the 30 kb deletion. For Pompe, the 1 sample sequenced was homozygous for the well-characterized late-onset variant c.-32-13T>G. For MPS1, 14 of the 15 samples sequenced showed pseudodeficiency alleles. 6 of the 14 also contained a variant of uncertain significance. 2 specimens had a heterozygous pathogenic variant. To date, 6 of the screen positive cases have been confirmed as true disease cases and 2 were confirmed as carriers. 14 cases are still pending, and the remainder have been cleared as no disease. Data will be updated to include the full first year of screening.

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30 Years of Newborn Screening for Hemoglobinopathies in Ohio
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Background: Since March 1990, all infants born in Ohio are mandated to be tested with newborn bloodspot screenings for sickle cell disease (SCD), sickle cell trait (SCT), and other hemoglobinopathies. The primary purpose of is to identify infants with SCD in order to initiate penicillin prophylaxis, which substantially reduces mortality from pneumococcal sepsis. The landmark penicillin study by Dr. Marilyn Gaston in Cincinnati in the 1980’s, led to a partnership with the Ohio Department of Health (ODH) to begin newborn screening (NBS). The ODH funds a regional network of six sickle cell projects based in pediatric hospitals (Cincinnati, Dayton, Columbus, and Akron) and community-based agencies (Toledo and Cleveland). Projects are funded to provide coordination and follow-up of abnormal NBS results; hemoglobinopathy counseling for patients and families; referrals to specialized medical teams, and outreach education activities for healthcare providers and the public. The ODH Public Health Laboratory notifies the sickle cell programs of all presumptive positive abnormal hemoglobin results for follow-up services in partnership with the newborn’s physician of record. In recognition of the 30th anniversary of newborn screening for hemoglobinopathies in Ohio, a retrospective data analysis was done to analyze trends.

Methodology: NBS was obtained from the Ohio Public Laboratory. Due to changes in data systems over time, only data from the last nine years was analyzed.
Results: From 2010 - 2019, there have been 1,247,232 babies tested in the NBS program; with 31,694 newborns having an abnormal hemoglobinopathy alert; 30,528 of those cases having presumed hemoglobin trait. There were 1,168 newborns with a presumed hemoglobin disease alert: (361 presumed cases of hemoglobin SS; 185 presumed cases of hemoglobin SC; and 61 cases of sickle beta thalassemia). Lost to follow-up for presumed disease cases was less than 3%, including babies that expired.

Conclusions: The ODH Newborn Screening Program is a complex system that provides diagnosis of hemoglobinopathies, particularly to ensure newborns with SCD receive early intervention with appropriate medical care and enables confirmatory testing of SCT to ensure that unusual or unstable hemoglobin variants are detected. As a next step, further analysis of this data will identify other trends, such as growing incidence in outlying areas, which will provide the opportunity to use targeted strategies to educate healthcare providers and general public, about hemoglobin disorders.

Presenter: Lisa Shook, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, Email: lisa.shook@cchmc.org
How to Prepare for the Incoming Influx of New Conditions on the RUSP
D. Simon, A. Kennedy and J. Jenkins, EveryLife Foundation for Rare Diseases, Washington, DC

Currently there are 35 RUSP conditions, with 50 states screening for at least 31 of the RUSP conditions. While all states are working towards screening for all 35 conditions, we know that the number of conditions on the RUSP will continue to grow. With multiple new disease communities currently collecting evidence to support RUSP nomination, the burden on state public health laboratories will only continue to increase. It is understood that state programs are committed to implementing newly added RUSP conditions, yet experience significant gaps in resources, equipment, personnel, and training when compared to the emerging need. To help address this issue, the EveryLife Foundation sought to find ways to ensure that public health laboratories could focus primarily on the screening methods itself, rather than whether they had funding to support adding each new condition. In California and Florida, we worked with the states to help pass RUSP alignment legislation that ensured that the state public health laboratories could add RUSP conditions to their state newborn screening panels with the long-term funding source to pay for it. Both states will test for all 35 RUSP conditions by the end of 2020 with the funding they need.

RUSP alignment legislation recognizes the need for a long-term funding source, while also ensuring that once a condition is added to the RUSP, it is added to state newborn screening panels nationwide. This allows for states to focus on the new screening methods themselves, follow-up services, and their workforce, as opposed to having to focus on how they are going to secure funding. State newborn screening programs must continue to work with their local governments and patient organizations to properly prepare for the new diseases added to their state screening panels. All three of these major stakeholders have unique perspectives on the needs of a successful newborn screening program and the disease communities they serve. By not having to focus on long-term funding with the implementation of RUSP alignment legislation, state newborn screening programs can instead remained focused on saving as many lives as possible through newborn screening.

Presenter: Dylan Simon, EveryLife Foundation for Rare Diseases, Washington, DC, Email: dsimon@everylifefoundation.org
Newborn Screening Metabolites Provide Important Insights on Lower Respiratory Tract Infection Pathogenesis

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Rationale: As metabolism plays an important role in susceptibility to infections, assessing metabolic products may provide critical insights on lower respiratory tract infection (LRTI) pathogenesis and may aid in the identification of those at risk and targets for prevention and treatment. We aimed to leverage existing newborn screening (NBS) metabolic data to identify metabolic profiles at birth associated with LRTI during infancy.

Methods: Our study populations were drawn from the Environmental influences on Child Health Outcomes (ECHO) Program’s Children’s Respiratory and Environmental Workgroup (CREW) consortium. We included children enrolled in INSPIRE, MAAP, and WISC cohorts with linked NBS, demographic, and clinical data. Existing NBS metabolic data, including targeted measurement of organic acids, amino acids, and acylcarnitines quantified, stored, and selected for NBS panels by each state’s public health department, were provided by the Tennessee, Michigan, and Wisconsin NBS programs for cohort participants. To reduce the risk of potential participant identification, NBS metabolic data were restricted to newborns with normal NBS values. LRTI during the first year of life was ascertained by parental report of an LRTI event or physician diagnosis of bronchiolitis or pneumonia. In the discovery phase, we used the least absolute shrinkage and selection operator (LASSO) technique to identify leading metabolites associated with LRTI in the first year of life within the largest cohort (INSPIRE). The identified set of metabolites were then included in multivariable logistic regression. The findings were replicated using MAAP and WISC.

Results: Existing NBS metabolic data were successfully linked to ≥95% of infants in the INSPIRE, MAAP, and WISC cohorts (n=1923, n=134, and n=165). 25%, 5%, and 5% of these infants had an LRTI during the first year of life, respectively. In the discovery phase, increased levels of citrulline and acetylcarnitine (C2) at birth were associated with decreased odds of LRTI in infancy (citrulline: adjusted odds ratio (aOR) 0.81, 95% confidence interval (CI) 0.68-0.97; C2: aOR 0.81, 95% CI 0.66-0.99). The inverse relationships for both metabolites were replicated within WISC, and citrulline findings replicated within MAAP.

Conclusions: In this discovery analysis, we identified novel relationships between two targeted metabolites measured at birth and subsequent infant LRTI. We speculate that as higher citrulline levels are associated with increased nitric oxide production, a promoter of antiviral activity, and as higher short-chain acylcarnitine (e.g., C2) levels are associated improved host immune response, these metabolites represent plausible pathways related to LRTI risk in infancy. These findings support the feasibility and power of using metabolites routinely captured on NBS to define novel pathways in childhood disease pathogenesis.

Presenter: Brittney Snyder, Vanderbilt University Medical Center, Nashville, TN, Email: brittney.m.snyder@vumc.org
Developing a Novel CFTR Panel to Improve CF NBS Sensitivity in Washington State


Background: The Washington State Newborn Screening Program (NBS) added cystic fibrosis (CF) to its mandatory panel in 2006 using an IRT/IRT method. Four years later, we added second-tier DNA testing for F508del to help expedite the diagnosis of CF in babies that were too small or too sick for a timely sweat test. Following that change, the pediatric pulmonology consultants in Washington State have advocated for an expanded CFTR panel to further improve screening sensitivity of CF.

Objective: We will tell the story of developing and implementing a novel CFTR panel. This includes findings from our research, shopping among the vendors, performing an epidemiological evaluation on babies with CF in Washington State, coordinating stakeholder work with our consultants and arriving at the panel’s final composition. We will share challenges and lessons learned from the method validation process and data from almost one year of screening experience.

Highlights: The technology we chose allowed us to select among a large set of targets within the CFTR gene. Analyzing the genotypes of CF cases identified by IRT/IRT was critical in the evaluation of this new testing platform as we chose-our-own targets for the Washington State specific panel. We will discuss the rationale behind choosing to exclude the R117H variant. We will explain the impact of this change to the laboratory workflow and the timeliness of follow-up.

Presenter: John Thompson, Washington State Public Health Laboratories, Shoreline, WA, Email: john.thompson@doh.wa.gov
Poster #47

Data Leads Changes without the Regulatory Mandates
J. Carmona, K. Tullis and M. Cellucci, Nemours AI duPont Hospital for Children, Wilmington, DE

In January 2018, the Delaware Newborn Screening Program was contracted outside of the Delaware Division of Public Health to Nemours/AI DuPont Hospital for Children, which is the only free-standing children’s hospital in the state. The laboratory portion of the program was subcontracted to PerkinElmer Genetics. This transition required the birth facilities, who previously had a state courier pick up samples from the maternity floors Monday – Friday and deliver to the state Public Health Laboratory, to adopt protocols to send samples overnight through UPS to PerkinElmer in Pittsburgh. The Newborn Screening program collaborated with each birth facility to develop procedures and protocols around shipping changes. Initially there was resistance to the implementation of Saturday shipping to PerkinElmer and none of the seven birth facilities adopted the available Saturday shipping option. At the time, Delaware regulations did not address shipping and the Newborn Screening Program birth facilities to implement shipping on Saturdays.

In order to encourage the adoption of Saturday shipping, the Newborn Screening Program collected NewSTEPs timeliness data on births at each of our hospitals individually. This data confirmed a significant delay in receiving results on infants born on Thursday’s and Friday’s. On average, infants born on Thursdays were resulted on day 7 or 8 of life, where infants born Saturday thru Wednesday were averaged to be resulted on day 5 or 6 of life. This data was presented to the newly appointed Newborn Screening Advisory Committee in March 2019 at which the Committee voted to recommend changes to the Delaware Newborn Screening Regulations to require shipment six days per week (Monday - Saturday), which would be effective January 2020.

Due to the delay in the regulatory process, the Committee encouraged the program to present the data to the birth facilities individually, which was conducted through a series of site visits during the spring of 2019. The program found that all birth hospitals were interested in the data and several worked quickly to implement Saturday shipping. By September 2019, the majority of Delaware’s birth facilities had successfully implemented Saturday shipping and saw significant improvements of more than 80% in their NewSTEPs timeliness data. Delaware’s new regulations took effect January 1, 2020 with six out of seven birth facilities actively shipping six days per week.

Presenter: Jessica Carmona, Nemours AI duPont Hospital for Children, Wilmington, DE, Email: jessica.carmona@nemours.org
Creation of an Online Newborn Screening Educational Program Using Adaptive Learning

C. Hinton¹, C. Cuthbert¹, A. Gaviglio²; ¹Centers for Disease Control and Prevention, Atlanta, GA, ²G2S Corporation/Centers for Disease Control and Prevention, Minneapolis, MN

Newborn screening is a public health program that identifies conditions that can affect a child’s long-term health or survival. Early detection, diagnosis, and intervention can prevent death or disability and enable children to reach their full potential.

A well-trained workforce is essential to ensuring that public health laboratories have the capacity to conduct the critical activities of newborn screening to ensure timely detection of affected newborns. High turnover of staff, emerging technologies, implementation of new complex conditions, and policy changes that impact newborn screening practice all point to the need for a comprehensive educational program. Such a program should be targeted at the needs of the evolving newborn screening community to deliver consistent communication of best practices and new content.

One of the challenges in designing educational resources for a community with diverse educational and technical capabilities is how to present content that engages both the novice and the expert learner. To address these needs, CDC’s Newborn Screening and Molecular Biology Branch has created an online educational product. CDC has contracted with an educational vendor to develop the product. The educational product uses an adaptive learning platform that adjusts in real-time to the needs of each learner and constantly tailors the lesson content and teaching approach based on the responses of each individual learner. As such, both the novice and the expert can take the same course and have different learning experiences, with the aim of moving both learners towards greater mastery of the course material. The initial release of the educational product includes three modules that cover: 1) Amino Acid Disorders, 2) Severe Combined Immunodeficiency (SCID), and 3) Molecular Methods. These modules will provide Continuing Education credits and will be available free of cost through the CDC TRAIN Learning Network, a comprehensive catalog of online public health training opportunities.

The online newborn screening product will educate public health professionals from US newborn screening programs about the newborn screening system, current and emerging technologies, current and anticipated screened conditions, and policies and issues that impact practice. The product will be on-demand and free with material curated by the CDC. An adaptive learning platform has been shown to increase learner motivation and competency in other biomedical and clinical fields. It is anticipated that this online educational product will reinforce learning newborn screening professionals, incorporate learner self-evaluation, be responsive to variations in learner response, and provide real-time adaptive response to individual learners.

Presenter: Cynthia Hinton, Centers for Disease Control and Prevention, Atlanta, GA, Email: ceh9@cdc.gov
The Emergence of Short-term Follow-up in Newborn Screening

The APHL Short-term Follow-up Workgroup - D. Freedenberg¹, C. Ingham², C. Johnson³, J. Thompson⁴, C. Crews⁵, S. Denniston⁶, A. Gaviglio⁷, M. Kleyn⁸; ¹Texas Department of State Health Services, ²New England Regional Genetics Group, ³University of Iowa Stead Family Children's Hospital, ⁴Washington Newborn Screening Program, ⁵Virginia Department of Health, ⁶Oregon Department of Health, ⁷Association of Public Health Laboratories, ⁸Michigan Department of Health

The Short-Term Follow-Up (STFU) Workgroup was established by the Association of Public Health Laboratories (APHL) Newborn Screening Technical assistance and Evaluation Program (NewSTEPs) in 2013. The workgroup supports the Newborn Screening (NBS) community in efforts related to STFU, and it developed a position paper to define STFU. On-going activities include quarterly webinars which provide resources and education to NBS program staff, in-person meetings to address universal STFU challenges, and peer network support for technical assistance. Key goals include:

- Strengthening the NBS system by providing input, guidance, and technical assistance on follow-up activities in NBS.
- Offer a forum for communication in which follow-up staff from regional and state NBS follow-up programs can network and collaborate on quality improvement efforts.
- Identify needs and offer NBS programs technical assistance related to Short-Term follow-up.

Three in-person meetings for the NBS STFU community occurred, resulting in the exchange of state operational information and the development of five task forces to address universal STFU challenges:

- Continuity of Operations Planning
- Long Term Follow-Up
- New Hires
- Succession Planning
- Molecular Literacy

Since 2013, there have been 28 webinars each averaging 100 participants from more than 40 states. Participant evaluation of these webinars noted the effectiveness, utility, and practical application of the information presented. Presentations by the NBS staff from 24 US states and territories, giving in-depth looks at commonalities and differences among programs throughout the country were included.

Webinars included:
Operational Concerns: Establishing cut-offs for STFU Programs; Emergency Preparedness; Ensuring all babies are screened; Data Management systems; HIPPA issues
Quality Improvement: False Negatives/missed cases; COIIN projects for timeliness in NBS; Quality Improvement initiatives
Condition-specific: CPT1A; SMA; CF; X-ALD; CAH; SCID; hemoglobinopathies
Education and family centered communication: Collaborating with Families and Providers in Birth Setting; The Power of Case Studies; Genetic Counseling and NBS; Linkage to care: communication and collaboration; infographics for data and parent materials; HIPAA Issues in NBS.
Emerging issues: Implementing Direct to Patient Laboratory Test Report Legislation; NBS Education and Long-Term Follow-Up Systems and Perspectives

This presentation will venture more deeply into the work that the STFU Workgroup has done and will highlight the accomplishments. The STFU Workgroup exemplifies the value and productivity of a true collaboration.
Presenter: Debra Freedenberg, Texas Department of State Health Services, Austin, TX, Email: debra.freedenberg@dshs.texas.gov
When a Birthing Facility’s Newborn Screening Process Derails: How to Help Them Get Back on the Tracks

Facility X has been underperforming for the last 3 years and has received technical assistance from program staff multiple times. In October 2019, the newborn screening system at Facility X derailed. The issues at this facility included:

- Newborn screening reports were not getting to PCPs
- 10-day old specimen received in the lab
- Failed to obtain a newborn screen for a baby in the NICU.
- 403 samples from January-September 2019 could have been received a day earlier if recommended collection practices were followed
- Timeliness data showed that they were the worst performing facility in the state.

Facility X took the following actions while working with the NBS program:

- Developed an action plan
- Escalated problems to executive leadership to obtain buy in
- Called a meeting with key stakeholders to review data and identify barriers/solutions
- Set a date for a corrective action plan
- Identified quick fixes such as web portal access and increasing staff awareness
- Identified other items that needed changed, such as order set priority
- Collect screen at 25 hours of life regardless of the time of day
- Instituted a daily 7:00 am check in to make sure all eligible babies were screened
- Developed a daily report to identify all babies needing to be screened
- Implemented an EMR fix so PCPs could see screening results in a timely manner

This presentation will include more detailed information of the events that took place as well as the solutions to the identified problems. We will report on the sustainability of the interventions and the lessons learned by both parties on how to work together to improve newborn screening practices.

Presenter: Carol Johnson, Iowa Newborn Screening Program, Ankeny, IA, Email: carol-johnson@uiowa.edu
Screening the Premature Infant for SCID in Iowa: Making Changes Based on Stakeholder Feedback and Data Review

D. Bayer¹,²,³, MB Fasano¹,²,³, P. Ferguson¹,²,³, T. Henry²,³, A. Atkins²,³, V. Van Zee²,³, E. Phillips¹,³, C. Johnson¹,³; ¹University of Iowa Stead Family Children’s Hospital, Coralville, IA, ²State Hygienic Laboratory at the University of Iowa, Iowa City, IA, ³Iowa Newborn Screening Program, Des Moines, IA

The Iowa Newborn Screening Program started an implementation pilot for SCID screening in June 2013 and fully implemented SCID screening in July 2014. We utilized a protocol for premature infants of repeat screens every 2 weeks until normal or until 37 weeks adjusted gestational age. From 6/3/2013 to 10/24/2014 we screened 43,930 babies and 92 (0.22%) had abnormal SCID results. Forty-three of the 92 abnormal screens (47%) were indeterminate due to prematurity (IND). Thirty-two of the 43 abnormal screens (74%) were reported as within normal limits with further screening up to 6 times in some babies. No cases of SCID or T-cell lymphopenia were detected. The number of repeat screens in premature babies prompted a review of our data and a protocol change on 10/25/2014.

The new algorithm for premature infants with an initial IND result included repeat screens be collected every two weeks until normal or until there were a total of 3 IND results. Once a premature baby had 3 IND results, flow cytometry was obtained. In late 2019, stakeholders questioned the utility of serial screens due to the number of false positives, blood volume required for screening and flow cytometry, and parental anxiety. This prompted another data review. From 2015-2019, 105 babies of 22-26 weeks gestational age (GA) had an abnormal SCID screen. Eighty-three (79%) of these abnormal screens were resolved by repeat screen or were closed as false positive following flow cytometry. One hundred and fifteen infants of 27-30 GA had abnormal SCID results. One case of CHARGE was identified; and 109 were resolved by repeat screen or as a false positive. One hundred and twenty-one infants of 31-36 gestational age had abnormal SCID screens. One baby had a 15q11.2 deletion, one ADA SCID, one was a false positive and 108 had repeat screens that were normal. Based on the data we again revised our algorithm for preterm babies.

Beginning 12/18/2019, for babies < 34 weeks with an initial IND, we rescreen at 36 weeks gestation. If 2 additional screens are abnormal, we request flow cytometry. For babies <=34 but < 36 weeks, we screen at 36 weeks. If that is abnormal, we rescreen and if it is still abnormal, we request flow cytometry. For babies =>36 weeks, we repeat the screen in 7 days and if abnormal, we request flow. For babies =>37 weeks with a presumptive positive result, flow cytometry is requested. So far, the feedback is positive. We will continue to review the data and review at SCID NBS team meetings.

The goal of our presentation is to initiate discussion on a national level about how best to screen for SCID in the premature infant population.

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