APHL 2021 Newborn Screening Virtual Symposium
Poster Abstract List

Adoption and Use of Second-tier Testing

Poster #1

Improved UPLC-MS/MS derivatized method to decrease MSUD false positive results in Newborn Screening

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Background: Maple syrup urine disease (MSUD) is an autosomal recessive inborn error of metabolism caused by deficiency of the branched-chain alpha-keto acid dehydrogenase complex, leading to the accumulation of branched-chain amino acids (BCAAs)- leucine (Leu), isoleucine (Ile), alloisoleucine (Allo-Ile) and valine (Val). Allo-Ile is a specific disease marker for MSUD. The routine FIA-MS/MS screening method produces a combined detection of isobaric species of BCAAs (Leu, Ile, Allo-Ile) and hydroxyproline (OH-Pro). The BCAAs are often elevated in babies born prematurely with low birth weight (< 1000g) who are receiving total parenteral nutrition. To reduce the number of false positive results, we developed a UPLC-MS/MS derivatized method that can reliably separate Allo-Ile from other isobaric species, thus improving the sensitivity and specificity of MSUD detection.
Method: In this method, a 3.2mm disc of newborn dried blood spot (DBS) is punched into a 96-well plate. 100µL of extraction solution containing 5µmol/L internal standards (IS) of each d8-Val,13C6-Leu, 13C6-Ile, d10-Allo-Ile, is added to each well and incubated at 45°C, for 45 minutes. The extract is transferred, evaporated and derivatized into 3N-butanol-HCl solution at 60°C for 30 minutes. The excess HCl is evaporated to dryness. The butyl esters are reconstituted into mobile phase and shaken for 10min at 27°C. The final 5 µL extract of IS, all analytes (Val, Leu, Ile, Allo-Ile, and OH-Pro) is resolved through an UPLC system with a BEH C18 column and analyzed by LC-MS/MS in MRM positive ion mode with a short run time of 5 minutes/sample.
Results: This method is linear across the analytical range (8 levels) with R2 values ≥0.99 for all analytes. Mean recoveries were 80-114% and interday precision was 3.7-9.9% for all analytes. Allo-Ile patient median results observed for normal, false positive, and confirmed positives was 1.07,1.17,17.09µmol/L, respectively. All MSUD confirmed cases showed high level of Allo-Ile compared to false positive and normal patient. The Allo-Ile of confirmed positive patients ranged from 3.90-136.44 µmol/L compared to 0.17-2.69 µmol/L for false positive cases. Additionally, we tested 3 missed cases from our routine screening with Leu value below the cutoff and 2 of them showed elevated levels of Allo-Ile. Our UPLC-MS/MS method has correctly identified all confirmed positives, false positives and normal patient results.
Discussion: The California Newborn Screening Program tests 450,000 to 500,000 newborn specimens every year. Although the true positive MSUD cases average 2.5 cases/year, the current FIA-MS/MS screening method produces an average of 230 MSUD presumptive positive cases/year. The novel UPLC-MS/MS method described here can significantly decrease the presumptive positive flags and efficiently capture true positive specimens for MSUD.

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Poster #2

Comparison of newborn screening performance for Krabbe disease and its implications for selecting an effective screening approach

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Newborn screening (NBS) for Krabbe Disease (KD) has been implemented in New York in 2006 and is currently performed in eight US States. While all these programs rely on galactocerebrosidase (GALC) activity measurement as the primary NBS test for KD, six programs employ molecular genetic analysis and/or psychosine (PSY) measurement as 2nd tier tests. Based on data presented in July 2020 at the Hunter's Hope Global Virtual Leukodystrophy Symposium, the positive predictive value (PPV) of NBS for KD ranges from 1% to 100% (median: 3%), and the negative predictive value (NPV) is 100% because a missed case of KD has not been reported by any state. The prevalence of KD observed by these programs ranges from 1 in 58,000 to 1 in 450,000 live births, and it is 1 in 270,000 in the state that has a PPV of 100% and uses PSY as the 2nd tier test. The prevalence of KD ranges from 1 in 84,545 to 1 in 129,552 with a PPV of 3 to 5% in states that utilize both molecular and PSY analysis as 2nd tier tests. In states that use only molecular genetic analysis as 2nd tier test, the prevalence of KD ranges from 1 in 450,000 to 1 in 139,250, with a PPV of 1 to 2%.

On the one hand, these data indicate that molecular genetic analysis of the GALC gene yields more false positive results than PSY measurement when screening for KD. This discrepancy is due to a higher than expected frequency of GALC pseudodeficiency alleles and variants of uncertain significance. On the other hand, we have previously shown that very rare and unusual cases of later onset variants of KD may have normal PSY values and could go undetected (Guenzel et al. Genet Med. 2020; 22: 1108-1118). In summary, every NBS program should define whether it intends to screen for all variants of KD vs. infantile KD as the core condition and later onset KD variants as secondary conditions. In our laboratory experience, NBS for KD can be performed with highest precision when using PSY as a 2nd tier test.

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Poster #3

Newborn screening for MSUD and the incidental detection of Hydroxyprolinemia

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Hydroxyprolinemia (OMIM 237000) is an autosomal recessive benign condition of amino acid degradation that results from impaired function of the enzyme 4-hydroxy-L-proline oxidase. The neutral loss scan employed for newborn screening (NBS) for amino acid disorders by tandem mass spectrometry does not differentiate between the isobaric amino acids leucine, isoleucine, allo-isoleucine and hydroxyproline (OH-Proline). The former amino acids as well as valine are markers of Maple Syrup Urine Disease (MSUD, OMIM 248600), a potentially lethal autosomal recessive deficiency of the branch-chain ketoacid dehydrogenase complex (BCKDH), included in the Recommended Uniform Screening Panel (RUSP) as a time-critical core condition. Accordingly, Hydroxyprolinemia can be a source of false positive results when screening for MSUD unless a second-tier test is performed to determine which of the isobaric amino acids is elevated (Oglesbee D et al. Second-tier test for quantification of alloisoleucine and branched-chain amino acids in dried blood spots to improve newborn screening for Maple Syrup Urine Disease (MSUD). Clin Chem 2008; 54: 542-9). We present three cases of abnormal NBS in the Hutterite colonies of South Dakota who required rapid follow up of presumptive positive results for MSUD. Confirmatory biochemical testing ruled out MSUD and led to a biochemical diagnosis of Hydroxyprolinemia. These cases highlight the importance of available second-tier testing to avoid unnecessary anxiety and health care costs when screening for MSUD.

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Poster #4

Quantification of Glycosaminoglycan Biomarkers in Dried Blood Spots – Second Tier Testing for MPS-I on a PerkinElmer QSight® 225MD Mass Spectrometer

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The mucopolysaccharisodes (MPS) family of lysosomal storage disorders (LSDs) is caused by defects in the metabolic breakdown of glycosaminoglycans (GAGs). In newborn screening, mucopolysaccharidosis-I (MPS-I) is detected by measuring the amount of α-iduronidase (IDUA) enzyme activity in dried blood spots (DBS). Many of the low IDUA enzyme activity results are due to pseudo deficiencies and the false positive rate can be further reduced when a second-tier analysis of the GAGs in DBS is performed. In this study the IDUA enzyme activity was first determined using a FIA-MSMS assay then the same samples were subject to a GAG mass spectrometry analysis workflow. The samples used in this study consisted of control DBS, presumed normal neonates, as well as presumed MPS-I positive patients.

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Poster #5

The First Year for DNA Analysis of X-Linked Adrenoleukodystrophy by the Texas Newborn Screening Program

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Objective: On August 5, 2019, Texas implemented screening for X-linked Adrenoleukodystrophy (X-ALD). The objective of this study is to discuss the adoption and implementation of Sanger sequencing and analysis as third-tier testing for X-ALD, the results from the first 17 months of screening, challenges since going live and opportunities for the future.

Methodology: DNA testing of X-ALD is performed on samples that meet the cut-off criteria following first-tier screening by tandem mass spectrometry and second-tier liquid chromatography-tandem mass spectrometry (LC-MS/MS) testing. Automated DNA sequencing is performed using the ABI 3500xL Genetic Analyzer to identify the presence of variants within the amplified region of the ABCD1 gene. Sequences are analyzed using the SoftGenetics Mutation Surveyor software and compared to a reference. Electropherograms are independently analyzed by two technicians. Identified variants are interpreted and reported based on recommendations by the American College of Medical Genetics and the Association for Molecular Pathology.

Results and Conclusions: In Texas, each baby is screened at 24to 48 hours and again at one to two weeks of age. Between August 2019 and December 2020, the Texas Newborn Screening Program screened 1,214,028 specimens (628,250 newborns and 585,778 from follow-ups). 248 specimens were reflexed for X-ALD DNA testing; 32 of these were not tested because a previous specimen from that patient had already received DNA testing for X-ALD. Of the 216 specimens DNA tested: 17 were reported as containing at least one pathogenic variant, 13 likely pathogenic, 15 with variants of uncertain significance and 168 as benign, likely benign or with no variants reported. Two specimens were referred for additional testing to confirm results after some regions were unable to be amplified. Since X-ALD implementation, 21 true positive infants were reported to the program from infants screened through December 2020. Of those, 8 had variants identified as pathogenic, 8 likely pathogenic, 4 as uncertain significance and 1 was unable to amplify exon 5 during testing. The complexity of interpreting Sanger sequencing results makes it more sensitive than other processes to fluctuations in staffing. Throughout this time, the DNA Analysis Team has experienced significant turnover of the highly trained technicians needed to perform sequencing and analysis. More detailed information will be discussed including how we have addressed these challenges and are implementing quality improvements to our process. These include adjustments to the cutoffs from second-tier LC-MS/MS, more efficiently utilizing the Mutation Surveyor software package to reduce the need for visual analysis of electropherograms and improving our variant review process.

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Poster #6

Importance of 21-Deoxtcortisol in Congenital Adrenal Hyperplasia Screening: A Retrospective analysis of Missed Cases

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Background: Congenital adrenal hyperplasia (CAH), an autosomal recessive disorder caused mainly by 21-hydroxylase deficiency (21-OHD), is the most common inborn error of the adrenal steroid pathways. The 17-hydroxyprogesterone (17-OHP) accumulates in 21-OHD. The newborn 17-OHP is elevated in other forms of CAH such as, 3β-hydroxysteroid dehydrogenase deficiency, 11-hydroxylase deficiency and P450 oxidoreductase deficiency which makes the diagnosis challenging. The accumulated 17-OHP is converted to 21-deoxycortisol (21-DC) by 11β-hydroxylase in newborns. The California Genetic Disease Screening Program developed an expanded steroid profile as a second-tier test for CAH. The new approach uses tandem mass spectrometry (UPLC-MS/MS) for 21-DC along with 17-OHP, androstenedione, cortisol and 11-deoxycortisol (11-DC). In order to find the absolute level of 21-DC and to demonstrate the importance of 21-DC in improving the sensitivity and specificity of newborn screening, we have retrospectively analyzed CAH true positive and false negative cases using UPLC-MS/MS.
Method: The screening of CAH 2nd tier is done on a single dried blood spot of 3.2 mm punched into a 96 well plate using the UPLC-MS/MS instrument. The specimens are extracted by solution containing internal standards: d8-17-OHP, d7-androstenedione, d4-cortisol, d5-11-deoxycortisol and d8-21-deoxycortisol. The plates are sealed and incubated at 30 °C for 35 minutes for extraction. The extract is dried under nitrogen and reconstituted in mobile phase for analysis. Results from ~8000 CAH 2nd tier specimens are reported in this analysis. For the 449 true positive cases, 412 were reanalyzed by UPLC-MS/MS along with an additional 296 tier 1 negative cases and 16 missed cases. The CAH routine screening cutoffs are birth weight (BW) dependent, so the level of 21-DC is studied for each BW group.
Result: The majority of the 449 true positive cases were found in the >2500g BW group, where the median value of 17-OHP was 11.83 nmol/L, and 98.27 nmol/L in the lowest BW group (< 999 g). For 21-DC, the median value in the low birth weight group was 0.13 nmol/L, compared to 0.09 nmol/L in the highest BW group. For 21-DC, the median value was 24.28 nmol/L which was very high compared to CAH negative cases (0.11 nmol/L).
The 16 missed cases (after 2nd tier screening) were reanalyzed using the more sensitive UPLC-MS/MS instrument and the new algorithm and cutoff for 21-DC. With the new method 69% were diagnosed correctly as positive for CAH. The 21-DC was also able to identify some tier 1 missed cases.
Conclusion: We established that 21-DC is a more specific biomarker for 21-OHD because it is not elevated in premature infants, or in other forms of CAH. Hence the use of the 21-DC marker, in addition to 17-OHP, will improve the sensitivity and specificity of newborn screening of CAH by reducing both the false positive cases and the number of missed cases.

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Conditions under Consideration for Addition or Removal from State Panels

Poster #7

Cytomegalovirus PCR from Saliva in Minutes Using a Disposable Cartridge in a Near-Patient Platform

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Background: Congenital cytomegalovirus (cCMV) is a leading cause of hearing loss and intellectual disability. In the U.S., 1 in 150 newborns are estimated to have cCMV, however, most infants have no clinically detectable symptoms and therefore are not identified. Identification of infants with cCMV can facilitate early detection of CMV-associated hearing loss and provide interventions including antiviral therapy to improve outcomes. States are considering the adoption of CMV screening – either universally or following a failed newborn screening (NBS) hearing test. Saliva and urine currently have superior sensitivity to dried blood spots. Due to infrastructure challenges with the use of saliva in NBS labs, states are actively looking to implement CMV screening in hospitals pre-discharge – similar to bilirubin, hearing screening, and pulse oximetry.
Objective: Evaluation of a new and rapid CMV PCR test and platform
Methods: CMV sequences published by Boppana and custom sequences for internal control (IC) RPP30 were used. Heaters and sensors were integrated into a disposable digital microfluidic (DMF) cartridge to enable rapid amplification of target DNA sequences using polymerase chain reaction (PCR). Reaction droplets (containing sample, master mix, primers, probes, and IC DNA) were subjected to thermal cycling including denaturing and extension (R44DC016576). Saliva samples from babies < 1 yr were run using gold standard methods and on the DMF cartridge. Negative specimen swabs were resuspended in water and positives in viral transport medium.
Results: Sample dispensing, reagent mixing, and thermal cycling were automated on-cartridge. Thermal cycling for PCR was completed in 7 minutes (4 minutes for some positives). Limit of Detection (LOD) was 125 cp/mL. Precision at LOD was 1.3 Ct. We saw good concordance between laboratory and DMF methods. The positive and negative percent agreements for the assay were 95.8% and 94.4% respectively. Further investigation is ongoing to understand the single false positive and negative.
Conclusions: This study demonstrates performance of CMV PCR assays in a disposable cartridge in a completely automated fashion to enable in-hospital screening. All reagents are fully integrated into the cartridge eliminating the need for reagent preparation or loading. Rapid PCR circumvents the necessity to setup infrastructure to transport saliva samples and can accelerate adoption of near-patient testing and rapid return of results. Further testing is required to establish the performance and clinical utility of this device in a clinical setting.

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Poster #8

Newborn Screening for Cytomegalovirus in Dried Blood Spots Using A Novel Quantitative Droplet Digital PCR Assay

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Introduction: Congenital cytomegalovirus is the most common infectious cause of birth defects in the United States, with a prevalence of 1:200. One out of 5 babies with congenital CMV (cCMV) will develop long-term health problems, the most common of which is sensorineural hearing loss (SNHL). As a result of this major public health burden, the states of Iowa and New York, and some healthcare centers, including Mayo Clinic in Minnesota, have begun testing all neonates who fail their newborn hearing screening (NHS) for cCMV. However, limiting cCMV testing to infants who fail NHS will miss most positive cases and hence the window of opportunity for therapeutic intervention, as greater than 50% of neonates may be asymptomatic at birth. At Mayo Clinic, cCMV screening is performed using a real-time PCR (qPCR) method. This test is qualitative and can only be performed on urine or saliva, which are not amenable to many state newborn screening programs. To address these challenges, we have developed a novel droplet digital PCR (ddPCR) method for detecting and quantifying cCMV in dried blood spots (DBS).

Methods: Two DBS extraction methods were evaluated (1) 421 samples were analyzed after direct DNA extraction from a 3.2mm DBS punch using a multistep method and denaturation at 99°C; (2) 326 samples were analyzed after extraction using a commercial QIAamp DNA mini kit. Simultaneous quantification of the CMV U9 gene and human RPP30 (reference gene) was performed using AutoDG and QX200 ddPCR (Bio-Rad). Validation studies included 32 urine and throat swab spiked DNA samples, 22 blinded patient urine samples, 3 positive DBS and 747 residual DBS submitted for supplemental NBS.

Results: Inter-assay imprecision was calculated at 15.6% CV for DBS with 4 CMV copies/µL blood (5 positive droplets). Limit of detection was calculated at 1 CMV copy/µL blood. Concordance using spiked samples as low as 9 CMV positive droplets showed a 95.7% positive percent agreement (PPA) between qPCR and ddPCR. Patient urine samples showed 100% PPA down to a level of 5 CMV positive droplets. Retrospective analysis of the NBS cohort extracted with QIAamp revealed two positive DBS (0.6% prevalence). These two samples, along with another previously identified CMV-positive DBS were submitted for testing by the routine qPCR assay. Two of the DBS with 4 and 12 copies/µL blood (detected as 5 and 17 CMV positive droplets, respectively) did not generate a cycle threshold value, but were positive by melting curve analysis. The third patient DBS was identified by ddPCR with 8 copies/µL blood (11 CMV positive droplets) but was negative by qPCR.

Conclusions: We developed a highly sensitive and accurate multiplexed ddPCR method for absolute quantification of CMV in DBS, urine, or throat swab. The method is cost-effective and suitable for high throughput CMV testing. Benchmark comparison with qPCR suggests that ddPCR may provide greater sensitivity in DBS.

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Poster #9

Prospective Results from a Plate-Based Fluorimetric Screening Assay for MPS II

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Background: Mucopolysaccharidosis Type II (MPS II), also referred to as Hunter syndrome, is an X-linked lysosomal storage disorder (LSD) caused by deficiency of the enzyme iduronate-2-sulfatase (IDS) in the lysosome. MPS II is heterogeneous in terms of disease severity and onset with clinical symptoms that may affect the skeletal, respiratory, cardiac, and neurologic systems. Hunter disease affects an estimated 1 in 100,000 male births in the United States. Recently, Hunter syndrome was nominated for addition to the Recommended Uniform Screening Panel (RUSP).
Methods: The Missouri State Public Health Laboratory (MSPHL) has implemented a fluorimetric enzyme assay for high-throughput screening of IDS activity in newborn dried blood spot (DBS) samples. The previously described method is performed in a microwell plate format and requires two hours of incubation and minimal hands-on time. The method was analytically and clinically validated and MSPHL began prospective newborn screening in November 2018.
Results: This report will summarize the laboratory and follow-up experience using this simple, highly specific assay through testing of more than 200,000 prospective specimens between 11/1/2018 and 6/30/2021. To date, the assay has an extremely low screen positive rate (less than 0.03%); use of second-tier testing, first through molecular and then biochemical methods, has further reduced the false positive rate. Follow up testing has identified three severe cases of MPS II; all three newborns began enzyme replacement therapy (ERT) following the positive screening result. In addition to the positive results, this report will summarize other findings, including pseudodeficiencies and variants of unknown significance observed during follow up testing.
Conclusions: MSPHL has successfully implemented high-throughput screening for MPS II using a specific, sensitive, and easy-to-use fluorimetric enzyme assay. Data from IDS screening and follow-up at MSPHL will be instrumental in support of evidence review for inclusion on the RUSP.

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Poster #10

Combined Flow-Injection-Analysis method for MPS II disorder and PerkinElmer NeoLSDTM kit on QSight® Mass Spectrometer

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The mucopolysaccharidoses MPS I and MPS II are lysosomal storage disorders (LSDs) caused by defects in the metabolic breakdown of glycosaminoglycans (GAGs). The PerkinElmer NeoLSDTM IVD kit already includes assay for MPS I screening from dried blood spots (DBS) together with five other LSDs: Gaucher Disease, Niemann-Pick A/B Disease, Pompe Disease, Krabbe Disease and Fabry Disease. The NeoLSDTM kit employs Flow-Injection-Analysis (FIA) approach which has advantage of being fast and simple sample instruction method to a mass spectrometer with no extra chromatographic step and so, the injection-to-injection time of the NeoLSD kit on the PerkinElmer QSight® instrument is only about 55 seconds. This same fast throughput time can be maintained when MPS II is added to be run together with the NeoLSD 6-plex assay. Note: MPS II assay is under development.
The studied assay included separate DBS incubations for MPS II and NeoLSD enzymes but after sample workup, the assays were combined and ran in a single injection to a mass spectrometer. With separate incubations, the FIA-assay performance of MPS II can be enhanced markedly since it allows converting the initial MPS II product to a more stable and ionizable form by using an additional enzymatic reagent. The study demonstrated good performance at different enzyme activity levels and adding MPS II sample didn´t affect the established NeoLSD assay performance.

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Poster #11

Integration of Guanidinoacetate Methyltransferase Deficiency (GAMT) screening markers into the NeoBase 2 Non-derivatized MSMS kit assay

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Guanidinoacetate Methyltransferase Deficiency (GAMT, OMIM 601240) is a severe but treatable Cerebral Creatine Deficiency Syndrome (CCDS), which is currently under the nomination for Recommended Uniform Screening Panel (RUSP) in US. Because the defected GAMT enzyme cannot convert guanidino acetic acid (GAA) to creatine (CRE), accumulated GAA and reduced CRE concentrations in blood provide indicative metabolic markers for the GAMT deficiency screening [1]. Based on the previous findings, a measurement of GAA and CRE markers can be feasibly integrated into the currently used tandem mass spectrometry (MSMS) assays to screen an expanded panel of other metabolic disorders [2,3]. In this work, we similarly demonstrated that these two additional GAMT markers can be also measured with the PerkinElmer NeoBase™ 2 Non-derivatized MSMS kit assay only with minor modifications in the sample preparation and MSMS data collection workflow.
For this GAMT integrated NeoBase 2 assay, the GAA and CRE measurements were performed simply by adding their stable isotope labelled internal standards (IS) 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 studied MSMS systems, additional four transitions for GAA (m/z 118 > 76), CRE (m/z 132 > 90), GAA IS (m/z 120 > 78) and CRE IS (m/z 135 > 93) were acquired during the currently used positive mode MSMS functions, and analytical performance characteristics were compared.

For Research Use Only. Not for use in diagnostic procedures.

References: [1] Longo et al. Disorders of creatine transport and metabolism. Am J Med Genet Part C Semin Med Genet 2011, 157:72. [2] Pasquali et al. Feasibility of newborn screening for guanidinoacetate methyltransferase (GAMT) deficiency. J Inherit Metab Dis 2014, 37:231. [3] De Jesús et al. Non-derivatized assay for the simultaneous detection of amino acids, acylcarnitines, succinylacetone, creatine, and guanidinoacetic acid in dried blood spots by tandem mass spectrometry. Int J Neonatal Screen 2016, 2:13.

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Poster #12

Increasing Efficiency of Mass Spectrometry based Newborn Screening Programs: How will we add Guanidinoacetate methyltransferase (GAMT) deficiency?

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Guanidinoacetate methyltransferase (GAMT) is an enzyme involved in creatine metabolism. This process is initiated by the conversion of arginine to ornithine and glycine to guanidinoacetate. This is accomplished by the enzyme, arginine:glycine amidinotransferase (AGAT). The next step in the cycle is the conversion of guanidinoacetate to creatine by GAMT. Creatine is used to increase adenosine triphosphate (ATP) for cellular energy and is later converted to creatinine through a non-enzymatic reaction. Considering this pathway, individuals with deficient GAMT activity should exhibit an elevation of guanidinoacetate. Reductions in creatine and creatinine may also be observed, however these may not be specific markers from GAMT as creatine can be obtained through diet.

The accumulation of guanidinoacetate can lead to significant neurological deficits. Symptoms of untreated GAMT deficiency include developmental delay, intellectual disabilities, seizures, and behavior disorders. Oral supplementation of creatine is an effective treatment, especially if administered prior to clinical manifestation. For this reason, GAMT deficiency is of high interest to the newborn screening community and will hopefully be added to the Recommended Uniform Screening Panel in 2021.

This work had two goals. First, we wanted to perform a study to investigate the potential of guanidinoacetate, creatine, and creatinine as specific markers for GAMT deficiency. This was accomplished through the development of a stand-alone assay that measured these three markers simultaneously from dried blood spots. The analytical measurement ranges were found to be 100 – 1000 µM for creatine, 20 – 250 µM for creatinine, and 1 – 30 µM for guanidinoacetate. DBS samples collected from two clinically diagnosed GAMT patients and > 500 apparently normal individuals were analyzed. Both patients were clearly distinguished from the normal population based on an elevation of guanidinoacetate. Normal levels of creatine and creatinine were found to be highly variable in the newborn period, therefore measurement of these markers will not differentiate GAMT deficiency patients.

Due to the promising results of the stand-alone assay, the second goal of this project was to add guanidinoacetate to the existing mass spectrometry newborn screening panel. The panel currently measures several amino acids and acylcarnitines from a single 3.1 mm DBS punch. The addition of guanidinoacetate to the panel was successful, with similar performance characteristics to the stand-alone assay. This multiplexing approach allows for the addition of another disorder to the screening panel without significantly increasing sample, staff, or reagent requirements. Therefore, the addition of GAMT to state newborn screening programs will require minimal change for implementation.

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Poster #13

Newborn Screening of Metachromatic Leukodystrophy and Cerebrotendinious Xanthomatosis with Zero False Positives

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Introduction: Newborn screening for metachromatic leukodystrophy (MLD) and cerebrotendinous xanthomatosis (CTX) are expected to be nominated to the Sec. Advisory Comm. for consideration to be added to the RUSP.
Methodology: We carried out a pilot study for MLD and CTX newborn screening using liquid chromatography-tandem mass spectrometry on ~30,000 random newborns.
Results: For MLD and CTX we obtained one newborn each confirmed to have the disease. There were no false positives.
Conclusions: Newborn screening for MLD and CTX have been proven to be feasible in a high throughput newborn screening laboratory. No false positives were observed, and affected patients with both diseases were identified.

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Poster #14

Newborn screening for familial hypercholesterolemia using biochemical markers in dried blood spots

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Background: Familial hypercholesterolemia (FH) is a common genetic disorder characterized by elevated low-density lipoprotein cholesterol (LDL-C) in the blood that accumulates from birth throughout the lifespan. Children with FH develop premature atherosclerosis and are at risk for cardiovascular disease (CVD). Despite widespread availability of cholesterol testing and recommendations for universal childhood screening, over 90% of affected individuals remain undiagnosed. Newborn screening could play a role in detecting newborns at risk for FH, providing an opportunity for early intervention. The objective of our study is to determine if three candidate biomarkers - low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and apolipoprotein B (ApoB) - can be accurately measured in dried blood spots (DBS) for the identification of newborns with FH.

Methods: Clinical laboratories routinely measure TC using step-wise enzymatic reactions, yielding color formation when appropriate substrates are introduced. For this study, we modified Pointe Scientific's kit for TC analysis in DBS specimens. Similarly, we modified Pointe Scientific's LDL-C kit, which incorporates a detergent that isolates LDL particles, followed by enzymatic reactions, for analysis of DBS specimens. The concentration of ApoB in DBS was performed by immunoassay, with modifications from published studies. All three assays were validated according to College of American Pathologists (CAP) guidelines for clinical laboratories.

Results: The performance of TC, LDL-C, and ApoB assays were assessed by evaluation of precision, limit of quantification (LOQ), linearity, and recovery studies. Precision studies yielded coefficients of variation (CV) less than 15% for all three assays, across three control levels. The LOQ and linearity were determined by evaluating progressively lower or higher concentrations of TC, LDL-C, and ApoB in laboratory-created DBS. For all three biomarkers, the reportable range, from LOQ to the upper end of linearity, was comparable to serum-based assays. The recovery of TC, LDL-C, and ApoB from dried blood spots was greater than 75% within all control specimens.

To assess whether TC, LDL-C, and ApoB measured in DBS were comparable to serum assays, 50 research subjects provided both specimen types at a single time point, for analysis. Positive correlations between specimen types were observed for all biomarkers: when a given biomarker was elevated in serum, it was also elevated in the corresponding DBS specimen.

Conclusions: This study reports methods for quantification of TC, LDL-C, and ApoB in DBS. Validation results were within acceptable limits for screening assays, and a positive correlation existed between serum and DBS analyses. These findings suggests that analysis of biomarkers in DBS is feasible. This is an important first step towards the identification of newborns with FH.

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Current Recommended Uniform Screening Panel (RUSP) Conditions in State NBS Panels

Poster #15

Absorbance Based Assay for the Determination of Biotinidase Deficiency

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Purpose: To create a semi-quantitative BTD assay using the chemistry established by Wolf et. al (1984) in tandem with spectrophotometry to improve the reliability of the results and reduce turnaround time.
Introduction: Approximately 4 million newborns are screened each year in the United States and for a plethora of health conditions. One of these conditions is known as Biotinidase deficiency (BTD) and effects 1 in 60,000 newborns. This condition if left untreated has a high rate of morbidity and mortality. The Maryland NBS has screened for BTD for decades using a qualitative visual colorimetric assay. This original method has been considered the gold standard for determining BTD. Since its creation other methods have been developed which are capable of shifting identification of BTD from a qualitative to semi-quantitative or quantitative process.
Design/Method: Dried blood spots (DBS) specimens received (2020-2021) in the laboratory were used for analysis. These samples were separated into four categories based on two criteria. The first being, whether the specimen was a newborn (collected when the infant was less than seven days of age) or subsequent (collected when the infant was equal to or more than seven days of age) and the second being the date (5 days from receipt of specimen by the laboratory, 35 receipt of specimen by the laboratory). Each specimen was analyzed using the current BTD assay and the absorbance was measured using Biotek Spectrophotometers (Model: Synergy H1). Different parameters were tested to evaluate changes in absorbance (pH, temperature, incubation time, shaking, centrifugation).
Results: Several parameters were found to affect the level of absorbance. Incubation time seemed to play a significant role. The study determined that 6-hour incubation time (62% reduction) allows specimens to develop enough color intensity for high confidence results and faster processing.
Conclusion: Data quality improvement for NBS is a continuous process. As technology continues to change and develop so do the methods and techniques utilized by NBS. Evidence collected in this study suggests the spectrophotometric BTD assay under development may provide a fast and reliable alternative to the qualitative and other quantitative methods available.

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Poster #16

Comparison of C26:0- Long Chain Carnitine and C26:0-Lysophosphatidylcholine as Biomarkers for X-Linked Adrenoleukodystrophy in Newborn Screening

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Background: X-linked adrenoleukodystrophy (ALD) is a severe genetic disorder caused by the accumulation of very long-chain fatty acids. The C26:0-lysophosphatidylcholine (LPC) is a biomarker to identify potential affected newborns with ALD disorder. C26:0-long chain carnitine (LCC), a potential new biomarker for ALD, is also elevated in dried blood spots (DBS) from ALD patients. Both 26:0-LPC and 26:0-LCC can be multiplexed into routine analysis of amino acids (AA) and acylcarnitines (AC) in newborn screening. Our routine screening procedure is accomplished by using a two-tier strategy: FIA-MS/MS as 1st tier and LC-MS/MS as 2nd tier analysis of 26:0-LPC. LC-MS/MS is highly sensitive and more specific than FIA-MSMS but hinders high throughput screening. In our study, the use of 26:0-LCC as a secondary biomarker in conjunction with 26:0-LPC could reduce more than 50% the 1st tier false positive cases, thus saving sample preparation and analysis time for 2nd tier testing. We report the development of a new FIA-MS/MS method that simultaneously analyze LPCs (C20:0, C22:0, C24:0, C26:0) and LCCs (C20:0, C22:0, C24:0, C26:0) along with AA, AC, and succinylacetone (SA) from DBSs in a single assay.
Method: In this method, a 3.2mm disc of newborn DBS is punched into a 96-well plate. 100µL of extraction solution containing hydrazine hydrate and internal standards (IS) of AA/AC/SA/C20:0-26:0-LPC/C26:0-LCC, is added to each well and incubated at 30°C, for 45min. The extract is transferred, evaporated, and reconstituted in mobile phase and analyze by FIA-MS/MS in MRM positive ion mode with a total run time of 1.5min per sample.
Results: The new method is linear across the analytical range (6 levels) with R2 values ≥0.99 for all analytes. The recovery of C20:0-26:0-LPCs and C20:0-26:0-LCCs ranged from 76.0 to 99.0%. No significant difference was observed in the recovery of AA, AC and SA between each level of LPCs and LCCs, spiked and non-spiked controls. The %CV ranged for the LPCs and LCCs spiked controls are 4.50-9.8, 4.3-11.2, 4.2-16.2 and 4.5-11.9 for AA, AC, LPCs/LCCs and SA, respectively. The respective 26:0-LPC and 26:0-LCC patient median results (in µmol/L) were observed for: negative cases: 0.250, 0.005; presumptive positives: 0.485, 0.008; confirmed positive – pathogenic cases: 1.085, 0.037; and variants of uncertain significance: 0.530, 0.020.
Discussion: The current routine 1st tier FIA-MS/MS screening for ALD produces many initial positives, and 2nd tier LC-MS/MS reduces the false positives from the 1st tier test. As 2nd tier testing is not a high throughput screening, to reduce 1st tier false positives, 26:0-LCC can be used in the 1st tier as a secondary analyte for ALD screening. This approach can significantly decrease (>50%) the 1st tier false positives thus reducing sample preparation, analysis time and the number of instruments needed for 2nd tier screening.

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Poster #17

First Two Years' Experience of MPS I Newborn Screening in California

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Background: Mucopolysaccharidosis type I (MPS I) is a rare autosomal recessive multisystem lysosomal storage disorder caused by deficiency of the α-L-iduronidase (IDUA) enzyme. MPS I has a wide phenotypic spectrum with the most severe form, Hurler syndrome, resulting in progressive cognitive impairment and reduced life expectancy. Early diagnosis and intervention can improve patients' health outcomes. Newborn screening for MPS I in California started on August 29, 2018. We describe California's MPS I screening algorithm, the classification of cases after sequencing, and the different types of variants identified during the first two years of screening.
Methods: MPS I newborn screening in California uses a two-tiered approach. In tier 1, flow injection analysis-tandem mass spectrometry (FIA-MS/MS) measures IDUA enzyme activity in dried blood spots. Specimens that measure ≤ 18% of the daily enzyme activity median are then sent for tier 2 DNA sequencing at a contracted laboratory to identify variants in the IDUA gene. If at least one pathogenic variant, likely pathogenic variant, or variant of unknown significant (VUS) is found, cases are considered screen positive and referred for clinical follow-up and confirmatory testing at one of the 15 metabolic Special Care Centers (SCCs). SCC staff provide diagnostic information on all referrals and treatment information when indicated. Yearly follow-up is provided by SCCs for confirmed cases through age 5.
Results: As of August 29, 2020, 868,583 California newborns were screened for MPS I. 220 (0.025%) had a tier 1 IDUA enzyme measurement below the cutoff and underwent tier 2 IDUA DNA sequencing. 89 (0.010%) were screen positive after tier 2, all of which were referred to a SCC for follow-up. After clinical follow-up, 3 cases were resolved as severe MPS I (Hurler syndrome), 2 as attenuated MPS I, and 11 as MPS I not otherwise specified, for an overall MPS I birth prevalence of 1/54,286. 181 cases were resolved as MPS I carriers or having pseudodeficiencies, (1/4,799). IDUA activity for newborns diagnosed with MPS I was significantly lower than for those who were carriers or had a pseudodeficiency (% of daily median: 7.6, 12.1, and 11.0 respectively, pA, a pseudodeficiency allele identified in 56% of sequenced newborns.
Discussion: The birth prevalence of MPS I was similar to published estimates. The highest proportion of IDUA variants were pseudodeficiency alleles, followed by VUS. Long term follow-up will be crucial for understanding genotype-phenotype relationships.

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Poster #18

Creating a New Disorder Implementation Checklist - North Carolina's Experience

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Problem: Adding a new disorder to a state's newborn screening panel is a complex process. States need to obtain authority to screen, prepare for laboratory testing, customize laboratory information management systems and follow-up case management systems, coordinate short-term follow-up, and educate multiple audiences. There is a need for a new disorder implementation checklist to guide newborn screening staff and communicate with various stakeholders, such as public health officials and legislators, about the implementation process and program readiness.
Objectives: Our goal was to develop a New Disorder Implementation Checklist for the North Carolina Newborn Screening Program who are adding four new disorders in the next two years. The Implementation Checklist was created while finalizing the SMA implementation and modified during the process of launching statewide screening for X-ALD.
Methodology: Using the NewSTEPs New Disorder Readiness Scale (downloaded from www.newsteps.org 2/16/2021, Kellar-Guenther IJNS 2020) as a foundation, each division of North Carolina's newborn screening program met over several weeks, and detailed concrete "mid-range" steps they needed to take to ensure the program was ready to begin statewide screening for SMA. This process was completed just two months prior to statewide implementation. This tool was then replicated for X-ALD and updated as it was used to guide that process.
Results: Iterative changes made to the North Carolina tool include expanding lab implementation to include sections on validation/verification, LIMS specifications, notifying care providers testing has begun, and ongoing QA. The follow-up section was expanded; specifically, four steps in the NewSTEPs tool were expanded to 22 steps, with the most significant change in creating follow-up protocols. The steps identified to prepare for education also increased from four steps in the NewSTEPs tool to 11 steps for each audience. Finally, the IT implementation section was expanded to include integration of the disorder in the LIMS, Case Management, and Clinical and Environmental Lab Results (CELR) system for testing and reporting and the creation of SOPs, user guides, and staff training to use the test environment. The Implementation Checklist has been used not only to guide the roll-out of new disorders but also to educate external stakeholders on the complexity of and time need to implement statewide screening. The Implementation Checklist's final iteration will be assessed for future implementation of new disorders in North Carolina.
Conclusions: The NewSTEPs Readiness tool was designed to capture larger and more generic steps, making it harder to use as a checklist and a tool to educate stakeholders. The Implementation Checklist developed for North Carolina allows more flexibility in collecting and assessing progress toward implementation. Since 2013 five conditions have been added to the RUSP, and more disorders are being considered. We hope other states will learn from our experiences and take the NC New Disorder Implementation Checklist and edit it for their programs.

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Poster #19

Epidemiology of Congenital Hypothyroidism in Mexico: Data from a National Newborn Screening Program, 2005–2020

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Introduction: Congenital hypothyroidism (CH) is a condition that has an inadequate thyroid hormone production. Patients are usually asymptomatic at birth; however, they may present growth retardation and altered physical and mental development if not properly treated. Despite its high reported frequency in Mexico (2.50 – 3.33 per 10,000 newborns), the fragmented healthcare system and their inefficient operational practice challenge the data reliability.
Objective: To estimate the prevalence of CH from a NBS program in Mexico for the past 16 years, as well as to evaluate the impact of established TSH cutoffs in the aforementioned NBS program.
Methodology: From January 2005 to December 2020, we analyzed 268,950 NBS reports. The DBS were processed by PerkinElmer Genomics, to detect for CH through the examination of the TSH levels, which were measured by fluoroimmunoassay. The TSH cutoff values were < 28.5mU/ml and < 15.0mU/ml for newborns with ages less and older than 7 days, respectively. Follow-up was accomplished for those presumptive CH cases. We assessed the prevalence of CH in this program by regions and nationwide. Furthermore, the impact of the established TSH cutoffs was evaluated through a ROC curve analysis.
Results: The study showed an overall CH prevalence of 5.32 per 10,000 newborns; the highest prevalence was identified in the southeast region (9.54), while the lowest in the west (3.71). Particularly, the prevalence in males and females was 3.86 and 7.28 (ratio of 1: 1.88), respectively. Our analysis also found a general reduction of the birth prevalence; nevertheless, this phenomenon did not occur in all regions. Finally, the ROC curve indicated that this protocol had 100% of sensitivity and a specificity-associated of 98.23%. The area under the curve (AUC) was 0.9995 (P-value=7.67e-08).
Conclusions: This study indicates that the overall prevalence is almost two times higher compared to worldwide reports; however, the prevalence in some regions were found to be similar to the ones reported in the literature. Contrary to the increased incidence rate that most NBS programs present in different countries, the results of this program might be due to ethnicity, sex, number of patients, and birth outcomes. Additionally, the AUC indicates that the adopted protocol is appropriate for detecting CH. More extensive epidemiological studies with RUSP-based programs and timely therapeutic approaches will give a clearer picture of CH distribution and benefits; thus, decreasing the as the morbidity due to CH, as well as the global burden caused by this disease.

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Poster #20

Implementation of a second-tier newborn screening method reduces referrals for evaluation for disorders of methionine, cobalamin, and propionate metabolism

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Background: Newborn screening (NBS) for inborn errors of methionine and propionic acid metabolism relies on abnormal levels of methionine and propionylcarnitine. These analytes are not specific for these conditions and are prone to false-positive results. Implementation of total homocysteine (HCY), methylmalonic acid (MMA), and methylcitric acid (MCA) analysis as a 2nd-tier NBS test when abnormal propionyl-carnitine or methionine concentrations are identified by primary NBS increases specificity and decreases the false positive rate. A review of 10 years of data demonstrates the benefit of this approach.
Methods: Determination of HCY, MMA, and MCA in dried blood spots by liquid chromatography-tandem mass spectrometry.
Results: From November 2011 to April 2021, 6,166 specimens from infants < 3 months of age were tested. Reason for referral was not provided for most specimens though based upon client and age of patient one can presume the majority were submitted for 2nd-tier NBS testing. A total of 279 (4.52%) specimens were reported with abnormal results. Isolated elevations of HCY in 45 specimens suggestive of homocystinuria, methylene tetrahydrofolate reductase deficiency (MTHFR) or another defect in cobalamin metabolism were reported. Elevations of HCY and MMA (+/- MCA) were observed in 77 specimens. An additional 126 specimens exhibited elevated MMA (+/- MCA) with normal HCY. Isolated elevations of MCA were identified in 31 specimens, 12 of these were suggestive of PA while MCA in the remaining 19 was only mildly elevated.
Discussion: Based upon these data, 95% of referrals has the potential to impact the healthcare system by decreasing financial liability and improving overall access to genetics services. In addition, it could prevent the impact to individual families faced with an abnormal NBS result that is later resolved as a false positive outcome.

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Poster #21

A Comparison Study of XALD Screening with NeoBase 2 and LC-MS/MS methods

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Background: Using Neobase 2 (NB2) to successfully screen for XALD would conserve significant resources within our lab. At MDH we routinely use a standalone LC-MS/MS negative ion mode method to screen for XALD (C24:0 and C26:0 LPCs) in newborn dried blood spots. In August 2019 we began using NB2 to screen for amino acids and acylcarnitine disorders. NB2 includes C24:0 and C26:0 LPC markers and this allowed us to do a comparative analysis of patient samples tested using both methods.
Methods: Data were pulled from NB2 and LC-MS/MS (C26:0 LPC only) screening for patients tested within the range of 3/16/20 to 4/25/20 (n=7654). Confirmed positive XALD cases from 2020 that fell outside this range were isolated from both methods and further scrutinized . Evaluation included comparative histograms, correlation measurements, daily rankings and chronological tracking.
Results: Values for C26:0 LPC collected via the NB2 assay yielded a median of 0.220 µlmol/L and a standard deviation of 0.92 while the LC-MS/MS method yielded a median 0.08 µmol/L and a standard deviation of 0.018. The NB2 method showed an average bias of 0.15 µmol/L and a R^2 of 0.049. The NB2 data also showed an unexplained daily oscillation of C26:0 LPC patient averages that was not present with the LC-MS/MS data. Follow up investigations have not identified a cause yet. This instability may contribute to the findings here.
In 2020 MDH identified 15 true positive XALD cases and NB2 could identify 13 of the 15 by selecting the top 3% for 2nd tier analysis. In these cases the positive samples represent the highest daily C26:0 PLC value for only 10 of the 15 patients. There were 2 positive cases missed by NB2 using this selection criteria. These two samples had initial C26:0 PLC values of 0.19 µmol/L and 0.26 µmol/L with the LC-MS/MS method and values of 0.31 µmol/L and 0.32 µmol/L respectively with the NB2 method. The daily NB2 percentile ranks were 79.3% and 77%, and the percent of the daily median were 124% and 128%, respectively. Both cases confirmed with abnormal plasma VLCFA's and a variant of uncertain significance (VUS) in the ABCD1 gene. It is too early to determine if these cases will end up with cerebral X-ALD or not.
Conclusions: NB2 screening for XALD does not appear to consistently identify the same XALD positive results as our current LC-MS/MS method, but extremely elevated LPCs are identifiable with the NB2 method. Newborn screening labs should have a clear definition of what a true XALD case is and set floating cutoffs for NB2 accordingly. While a large majority of the positive XALD cases identified with our LC-MS/MS method were also identified with NB2 using a floating cutoff set at >97%, the data for the 2 positive cases suggests that even with broad selection criteria these samples might have been missed during screening with NB2.

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Poster #22

Examination of Demographic Variables on Lysosomal Enzyme Activities for IDUA, GAA, GBA, and GLA

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Background: Since their addition to the Recommended Uniform Screening Panel (RUSP) in 2016 and 2015, respectively, the lysosomal storage disorders (LSDs) Mucopolysaccharidosis Type I (MPS I) and Pompe disease have been added to the newborn screening panels of more than 20 states. As more states bring these disorders onto their screening panels, often alongside other LSDs, there exists a need for detailed information about the potential correlation of demographic variables including age at collection, birth weight, gestational age, and gender with enzyme activity.
Methods: The Missouri State Public Health Laboratory (MSPHL) prospectively screened more than 475,000 newborns for at least four lysosomal storage disorders between January 2013 and May 2018. This report analyzes the relationship between demographic variables and four lysosomal enzymes: α-L-iduronidase (IDUA), acid α -glucosidase (GAA), acid β -glucocerebrosidase (GBA), and acid α -galactosidase (GLA).
Results: For some enzyme/demographic combinations, the effect is minimal and/or consistent across enzymes; for example, the median enzyme activity is between 3.9% and 5.8% higher for females for each disorder. In other cases, demographic variables cause significant changes in enzyme activity; as another example, GLA activity for very low birth weight (VLBW; < 1,500 grams at birth) newborns is 200-300% higher than larger weight groups. The extremely large dataset allows for control of variables to reduce multivariate effects by limiting the analyses to single variables.
Conclusions: This information will provide a valuable resource for laboratories that implement screening for these conditions, which may otherwise require intensive pilot studies to establish screening cutoffs for these subgroups.

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Poster #23

Investigations of Clinical Outcomes of Idiopathic T-cell Lymphopenia

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The New York State Newborn Screening Program began screening for Severe Combined Immunodeficiency (SCID) and other conditions marked by T cell deficiencies in September of 2010. SCID screening is accomplished by qPCR of T cell receptor excision circles (TRECs), a piece of DNA produced during the formation of T cells in the thymus. While most newborns have >200 average TRECS, newborns with SCID have little to no TRECs. Infants with average TREC counts of less than 125 are referred to one of eleven Pediatric Immunology Specialty Care Centers for clinical evaluation and care. During the first nine years of screening for SCID, from 2010-2019, 2,119,042 newborns were screened for SCID. Of these babies, 1,451 screened positive and were referred to a SCC for diagnostic work-up, and 74 babies were determined to have idiopathic T cell lymphopenia by their Specialist. Although SCID is the primary target of the newborn screen, babies with T cell lymphopenia of other etiologies may screen positive as well. T cell lymphopenia refers to T cell reduction, leading to abnormally low TREC values and T cell deficiency of varying degrees. Idiopathic T cell lymphopenia (ITCL) is a disorder in which T cell lymphopenia has no identifiable genetic or medical causes, such as a metabolic, viral, or chromosomal abnormality. Anecdotal reports from the Immunologists suggest that some cases of idiopathic T cell lymphopenia resolve within the first year of life, whereas others may persist and require monitoring for years. Management and care of these cases is based on individual patient considerations. Physician recommendations may vary on the use of live vaccines, transfusion precautions, and treatment by antibiotic prophylaxis. Of the 74 babies referred to Specialty Care Centers by the NBS Program, specialists submitted a completed Extended Short-Term Follow-Up Form for 75.7% (N = 56) of the referred cases. Of these followed patients 7.1% (N = 4) received antibiotic prophylaxis, 44.6% (N = 25) were recommended to receive live vaccines either on time or with delayed administration, and 1.8% (N = 1) were referred to a transplant specialist of evaluation. Several patients (N = 18) are no longer in care as treatment was no longer clinically indicated. Monitoring and care of these infants has yet to be standardized. Idiopathic T cell lymphopenia is a poorly understood disorder and studying case treatment patterns can help provide a clearer clinical picture for case management going forward.

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Poster #24

An Automated High Throughput Fluorometric Assay for the Determination of α-L-Iduronidase Activity in Dried Blood Spots

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Objectives: Mucopolysaccharidosis type 1 (MPS-1) is a rare autosomal recessive disease caused by a deficiency in α-L-iduronidase (IDUA), required for degradation of dermatan and heparan sulfate. Severe MPS-1 (Hurler syndrome) is a neurodegenerative disease with significant somatic involvement (e.g. bone and connective tissues, viscera, cornea). In attenuated forms (Scheie and Hurler-Scheie syndromes) somatic involvement is lessened and neurodegenerative disease is absent. MPS-1 Hurler is the primary target of newborn screening (NBS) in Ontario. Life expectancy of untreated MPS-1 Hurler is less than 10 years. NBS for MPS-1 commonly involves the measurement of IDUA activity in dried blood spots (DBS). Here we present a novel automated method for the measurement of IDUA activity in DBS, which is the first tier screening method for MPS-1 Hurler at Newborn Screening Ontario.
Methodology: **T**he measurement of IDUA in DBS is achieved through measurement of free 4-methylumbelliferone (4-MU) cleaved from 4-MU-α-L-iduronide substrate coated 96-well plates manufactured by Astoria-Pacific, Inc (Oregon). Automation of the elution and incubation of DBS within substrate coated plates, precipitation, filtration, quenching, and fluorescent measurement is done using a Tecan Freedom EVO platform. Correlation with a commercially available MSMS based assay (Perkin Elmer) was performed for retrospective stored samples (n=383), first tier screen positive samples (n=279), as well as in-house and CDC QC DBS samples. At the time of writing, 105,000 newborns have been screened using this method.
Significant Results: The mean IDUA activity of NBS samples in March 2021 was 7.18 µmol/hr/L blood (SD = 2.27, n = 12,341). Comparison of first tier positive NBS samples (n=279) with the MSMS method showed the following linear relationship: y=1.73x - 0.33 (r= 0.81). Additionally, the measured activity of 25 pseudodeficiency variants shows good correlation between the 4-MU and MSMS methods (r=0.93). Four CDC QC materials with stated mean activity ranging from 0.12 – 8.03 µmol/hr/L blood were distinctly resolved. Further analysis on four in-house heat inactivated DBS samples allowed for separation across an activity range of 0.03- 0.77 µmol/hr/L. Multi-day precision data for DBS samples with activities of 0.7, 2.5 and 5.4 µmol/hr/L blood produced CV results of 19.3, 13.3 and 12.1% respectively (n=35 points per sample). To ensure within sample precision, the first 30,000 samples screened were measured in duplicate.
Conclusions: The implementation of this high-throughput assay with fully automated sample preparation will allow for the screening of over 700 NBS samples for MPS-1 in a 24 hour period on a single instrument. Good correlation with other validated methods for the measurement of DBS IDUA activity supports the adoption of this assay as a reliable, robust, and cost effective first tier screening assay for MPS-1 Hurler.

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Poster #25

A Voluntary Statewide Newborn Screening Pilot for Spinal Muscular Atrophy: Final Results from Early Check

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Early Check is a research study that offers voluntary screening for conditions currently not on the North Carolina (NC) state newborn screening (NBS) panel. Prior to the implementation of statewide newborn screening for spinal muscular atrophy (SMA), we screened infants born in NC enrolled from October 2018 through March 2021. We identified three newborns with 0 copies of SMN1 and two or three copies of SMN2, consistent with severe early onset SMA. We also detected one false positive result, likely stemming from an unrelated blood disorder associated with a low white blood cell count. We evaluated the timing of NBS for babies enrolled prenatally and postnatally and reasons for delays in screening and reporting. Although prenatal enrollment facilitated return of results sooner after birth, results for babies enrolled postnatally were available within a suitable timeframe, such that essential treatment was initiated sufficiently early in life. We evaluated the SMA qPCR screening method at two separate time points, confirming the robustness of the assay. The pilot project provided important information about SMA screening prior to its addition to the NC NBS panel. These results are the final update to the previously published study.

Kucera et al., A Voluntary Statewide Newborn Screening Pilot for Spinal Muscular Atrophy: Results from Early Check, IJNS, 2021

Presenter: Katerina Kucera, RTI International, Email: kkucera@rti.org

Poster #26

Apples and oranges, they can be compared: Harmonization and normalization for Pompe Disease and MPSI newborn screening, among States

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The problem studied and/or objectives: Several states are now screening for Pompe disease and MPS I using either Digital Microfluidics (DMF) or Tandem Mass Spectrometry (MS/MS). Because methodologies differ and cutoffs differ even for the same screening platform, comparison of results can be problematic. Regardless of the platform used for screening, states should achieve the same final outcome: identification of true positives and no missed cases.
Methodology: The Newborn Screening Quality Assurance Program at Centers for Disease Control and Prevention provided contrived samples for analysis which mimicked true-positive cases with low enzyme activity (0% and 5% activity) and samples with normal enzyme activity. Participating states (CA, IL, IN, KS, MD, MI, MN, MO, NY, OH, TN, and VA) analyzed these samples using their respective platform and reported results to Tennessee. States were also asked to submit their enzyme cutoffs, daily median, and monthly median for the 30 day period in which the specimens were analyzed. Enzyme values were analyzed using regression analysis comparing each state's results to a set of reference results. Result comparisons were also made using a log-log transformation of the data.
Significant results, including statistical significance where applicable: Variability was noted in the enzyme values particularly in the low enzyme specimen pools (0% and 5% activity) for states using DMF compared to states using MS/MS. In most cases, this variability was largely diminished or eliminated through either linear or log-log regression analysis of the data.
Conclusions and/or implications: Harmonization is possible across laboratories and testing platforms. Results from this study can help state laboratories facilitate refinements in their own cutoffs and reduce testing variabilities to improve detection of true positives and minimize missed cases.

Presenter: M. Christine Dorley, Tennessee Department of Health: Laboratory Services, Email: m.christine.dorley@tn.gov

Poster #27

Multiplexing Newborn Screening of Spinal Muscular Atrophy and Severe Combined Immunodeficiency in Texas

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Objective: To validate a multiplex assay for Spinal Muscular Atrophy (SMA) and Severe Combined Immunodeficiency (SCID) and implement Multiple of the Median (MoM) cutoff determination for SCID result interpretation for the Newborn Screening Program at the Texas Department of State Health Services.

Background: SMA is a rare genetic disorder affecting approximately 1 in 8,000 to 1 in 10,000 individuals and was added to the Recommended Uniform Screening Panel in July 2018. Approximately 95% of cases are due to a homozygous deletion of exon 7 of the SMN1 gene which results in degeneration of motor neurons in the anterior horn of the spinal cord. The State of Texas currently uses a laboratory-developed, multiplex real-time quantitative PCR (RT-qPCR) method to detect the presence of T-cell receptor excision circles (TREC) and RNase P (reference gene) for detection of SCID. The multiplexing capabilities of RT-qPCR can be further expanded to include detection of SMN1 exon 7 in the same reaction with little to no increase in hands-on and turnaround time.

Methodology: Over 9,000 newborn screening specimens including SCID- and SMA-positive patient specimens, SCID and SMA quality control materials (Centers for Disease Control; Atlanta, GA), and TREC calibrators (Perkin Elmer; Waltham, MA) were used in the validation. Dried blood spots were assayed using an automated DNA extraction method, followed by RT-qPCR on a QuantStudio 12k Flex instrument to detect the presence of TREC, SMN1 exon 7, and RNase P. Study data was analyzed to determine the accuracy, precision, sensitivity, specificity, reportable range, reference range, and potential for carryover contamination of the SCID+SMA multiplex assay.

Results and Conclusion: Multiplexing SMA with the Texas RT-qPCR assay for SCID yielded a robust assay with several advantages. Specifically, multiplexing is cost effective and relatively easy to implement, requiring only minimal changes to existing workflow and instrumentation. Importantly, the assay methodology meets the demand for high throughput newborn screening in Texas without negatively impacting turnaround time. Implementation of TREC MoM for the detection of SCID will improve assay precision, and the development of discrete TREC MoM cutoffs for extremely low birthweight babies (i.e., < 1000g) should reduce the number of false positive screening results for these patients. Based on the evaluation of results from the SCID+SMA multiplex assay validation study, the Newborn DNA Analysis Group at the Texas Department of State Health Services plans to implement the testing system for the detection of SMN1 exon 7 and TREC for Newborn Screening in Summer 2021.

Presenter: Derek Seidel, Texas Department of State Health Services, Email: derek.seidel@dshs.texas.gov

Poster #28

A highly specific and sensitive Applied Biosystems™ TaqMan SCID/SMA multiplex assay optimized for dried blood spots

S. Baral[1], K. Gill[1], A. Huang[1], N. Kimitoshi[2], S. Takaki[2]; [1]Thermo Fisher Scientific, Santa Clara, CA, [2]Kumamoto University, Kurokami, Kumomoto, Japan

Intensive research in early detection of Severe Combined Immunodeficiencies (SCID) and Spinal Muscular Atrophy (SMA) is essential to discover prevention methods for infants' permanent disabilities or death. In addition to SCID, 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 TREC and/or KREC. We have developed two versions of the TaqMan SCID/SMA multiplex assay (with and without KREC), that permit concomitant detection of both SMA and SCID targets, and each assay includes RNase P gene as an internal genomic control. We designed the SMA assay to target exon 7 of SMN1 gene and effectively eliminated non-specific detection of the highly similar SMN2 gene. This high SMN1 target specificity of SMA assay limits both ambiguous calls and removes the need for retesting. We also confirmed the SCID assay's high sensitivity with TREC and KREC copy number detection capability of as low as 10 copies per reaction. Additionally, a simple and robust DBS sample preparation method was developed using DNA Extract All Lysis solution. Furthermore, we demonstrate the performance of TaqMan SCID/SMA Plus assay (contains KREC assay) with healthy neonatal DBS samples, and the assay's high analytical accuracy was evaluated using confirmed SMA positive samples and SCID positive samples containing low levels of TREC/KREC. 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, Email: sonu.baral@thermofisher.com

Data Analytics and Bioinformatics

Poster #29

Evaluation Medium-chain Acyl-CoA Dehydrogenase (MCAD) Deficiency using Additional Analytes and Ratios

J. Voudren, M. Morrissey and M. Caggana, Wadsworth Center, New York State Department of Health, Albany, NY

Medium chain acyl-CoA dehydrogenase (MCAD) deficiency is a caused by a defect in the ACADM gene. This enzyme deficiency leads to an inability to metabolize medium chain fatty acids causing an accumulation medium chain acylcarnitines (C6 to C10). MCAD is the most common fatty acid oxidation disorder. In the United States the incidence is estimated to be approximately 1:16,000 live births. The symptoms of MCAD may appear in early infancy and include vomiting, lethargy, and hypoglycemia. In times of stress or fasting, babies with MCAD are at risk of seizures, brain damage, coma, and sudden death. The primary markers for MCAD screening include the C6, C8, C10, C10:1 acylcarnitines. There is some overlap of activity with the short chain and long chain acyl-CoA dehydrogenases. In most cases the C8 acylcarnitine (octanoylcarnitine) will show the most accumulation. However, the severity of the disease is not necessarily indicated by the concentration of C8 in the newborn screening sample. Secondary markers include the ratios C8/C2 and C8/C10. In New York State, the primary markers for MCAD screening are C8 and the ratio of C8 to acetylcarnitine (C2). In this presentation, we report of the use of additional markers to reduce the number of false positive cases reported for MCAD.
Random, anonymous, screen negative newborn screening (NBS) specimens (348) and 79 screen positive cases were re-analyzed with the inclusion of the new analytes C10:1, C10, C12:1, C12, C16:1OH. Samples were prepared using a derivatized method for amino acids and acylcarnitines.
When the positive cases were evaluated for the C10 analyte and the C8/C10 ratio as well as the primary C8 analyte, the number of positive cases was reduced from 79 to 17. All 13 true positive cases of MCAD were correctly identified. The number of false positive cases was reduced from 62 to 4 and all borderline cases were eliminated. The use of the C10 marker and C8/C10 ratio along with the primary C6, C8, and C8/C2 markers significantly reduced the number of false positive results reported. The impact of additional markers will also be presented. Importantly, utilization of a spectrum of results from medium chain acylcarnitines for assessment of results will reduce costs to the medical system and decrease stress and expense experienced by parents who wait for confirmatory testing results.

Presenter: Mark Morrissey, New York State Department of Health, Email: mark.morrissey@health.ny.gov

Poster #30

A Cost-Effective and Intuitive Tandem Mass Spectrometry Method for Inborn Errors of Metabolism Research

Y. Zhou, K. Van Natta, S. Samra and B. Hart, Thermo Fisher, San Jose, CA

The problem studied and/or objectives: Currently more than 50 Inborn errors of metabolism (IEMs) in the neonatal period are treatable to prevent serious clinical consequence if early diagnosis and treatment are implemented. IEMs refers to a group of diseases that caused by the defect of an enzyme, its coenzyme, or a transporter leading to the accumulation of its substrate and/or the insufficiency of its downstream products. Tandem mass spectrometry (MS/MS) simultaneously analyzes those substrates and metabolites in a single dried blood spot. MS/MS has been implemented in the majority of developed countries. However, there are still some technical barriers such as assay affordability and the lack of trained technical staff.

Methodology: We developed a fully automated and cost-effective MS-based analytical method for the determination of amino acids, acylcarnitines and succinylacetone in dried blood spots (DBS). Samples were extracted from dried blood spot cards. Internal standards (IS) were added during the extraction procedure, and extracted samples were injected onto an LC-MS system. Flow injection analysis (FIA) was performed on a UHPLC system. Detection was performed on a triple-stage quadrupole mass spectrometer with heated electrospray ionization (HESI) by selected reaction monitoring (SRM). Quality control (QC) samples from CDC Newborn Screening Quality Assurance Program (NSQAP) were used for assay performance evaluation. A single software package was used for instrument control, data acquisition, data review, and automated data processing and reporting, which included custom report plug-in templates that performed data meta-calculations.

Significant results, including statistical significance where applicable: When the instrument method, processing method, reporting template, and injection sequence are defined and ready, one click will initiate a seamless start-to-finish workflow, including data acquisition, data processing, data review, and automated reporting. This simple and intuitive method addressed the challenges of the cost effectiveness and the learning curves for the complicated instrument operation, meta dataset analysis, reporting and interpretation, by eliminating the manual process and removing transcription errors in the post-analytical phase. We compared quantitative results of our FIA MS/MS method, with CDC NSQAP QC controls mean values. The comparison yielded an $R^2 > 0.9$.

Conclusions and/or implications: We developed a seamless start-to-finish FIA MS/MS workflow and data analysis strategy, including data acquisition, data processing and automated reporting. The method proved to be robust and reliable, and met the required sensitivity requirements typically demanded by clinical research laboratories.

Presenter: Yu Zhou, Thermo Fisher, Email: lotusyzy@gmail.com

Poster #31

Generation of in Silico Read Data for Use In CLIA Validation And Algorithm Development

B. Assay, N.M. Ruiz-Schultz and A. Rohrwasser, Utah Public Health Laboratory, Taylorsville, UT

Newborn screening (NBS) uses a variety of molecular and biochemical tests in the early detection of disorders that may not be clinically or phenotypically evident during the prenatal period. Because of the advantages that genetic variant-based analysis provides, they are increasingly being employed as a secondary or tertiary tool for differential diagnosis to existing pipelines. This includes identifying complex disorders or novel pathogenic variants that have not been previously reported in the literature. However, genomic sequencing bioinformatics pipelines used in the analysis are limited by the variant call methodology and tools that have been employed.

Within NBS, there are no CLIA validated tools in which to verify bioinformatic pipelines. This is of concern because there are a variety of variables that can influence performance and there needs to be a mechanism in which to benchmark. Variables that can influence the output are the preprocessing of raw data, quality assessment, reads alignment, and software libraries used in variant identification. Using actual de-identified patient data to benchmark would be optimal, however many of the disorders are rare, and using actual patient health information is problematic. These issues and limitations underline a need for the development of tools and variant read sequences to measure the accuracy of the analysis and provide a benchmark for current and newly-developed NBS pipelines. At Utah Public Health Laboratories, we are developing a library of in-silico genomic reads containing disease-specific variants that can be used to verify and benchmark bioinformatic pipelines. The development of tools is comprised of the following steps:

- Generation of variant call files (VCF) representing genes underlying disorders currently on the newborn screening panel
- Utilization of simulated read sequencing library Neat-genreads to create disease-specific in silico generated read sequences
- Verification of the data/toolset using currently implemented pipelines.

This tool can be used to check:

- The current hardware used to run the pipeline
- Measure the computational analysis time to run a sample.
- The accuracy of identifying variants

We will share the software code as well as any in-silico generated files.

Presenter: Bryce Asay, Utah Public Health Laboratory, Email: aphlnbsba1@utah.gov

Emergency Preparedness & Contingency Planning

Poster #32

Newborn Screening Response to Covid-19 Pandemic: Interventions to Reduce Burden on Hospital Systems and Increase Compliance with Timely Repeat Screening

J. Carmona, K. Tullis and M. Cellucci, Nemours Newborn Screening Program, Nemours/AI duPont Hospital for Children, Wilmington, DE

The Delaware Newborn Screening Program has historically relied on birth hospital outpatient laboratories for collection of the majority of second or repeat screens. With the advent of the Covid-19 pandemic in March 2020 and imposition of state restrictions, the Newborn Screening Program was faced with the closure or limited availability of these traditional repeat screening sites. Furthermore, families were hesitant to bring their infants to hospital outpatient laboratories for fear of Covid-19 exposure. In order to address these concerns the program reached out to pediatric practices throughout the state to recruit them as repeat screening sites. A total of 4 new pediatric practices were recruited to join the 2 existing practices that were collecting repeat screens prior to the Covid-19 pandemic in our area (March 2020). Realizing the need and concerns of parents several (3) of the practices extended the service to all infants needing screens. The shift away from hospital collections sites was significant: prior to Covid-19 (March 2020) 9% of repeat screens were collected at primary care practices the addition of the 4 new sites resulted in 22% of repeats being collected at primary care practices. The time-to-repeat collection was also reduced in the infants with repeats collected at these practices. The addition of non-hospital collection sites during the Covid-19 pandemic presented parents with a non-hospital and increased the likelihood of obtaining a repeat screen on infants with abnormal results.

Presenter: Kathryn Tullis, Nemours AI duPont Hospital for Children, Email: kathryn.tullis@nemours.org

Financial, Legal, Ethical, Policy & Social Implications (FLEPSI)

Poster # 33

Parental Depression and Stress Associated with Newborn Screening for Complex Disorders

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Introduction: There has been growth of disorders on newborn screening (NBS) panels, including some with broad phenotypic spectra, variable ages of onset, and prognostic uncertainty. NBS for lysosomal storage disorders (LSD) or X-linked adrenoleukodystrophy (X-ALD) has renewed focus on the ethical issues associated with screening for complex disorders. We compared psychosocial experiences of parents whose children were diagnosed with LSDs, X-ALD, and RUSP conditions via NBS or other means.
Methods: Parents of children born between 2013 and 2018 were recruited via providers and advocacy groups. 65 parents had a child diagnosed with LSDs or X-ALD (Group 1, or G1), either through NBS (G1 NBS, n= 21) or not through NBS (G1 other, n=41). One control group was children diagnosed via NBS with a RUSP condition (G2, n=52). A second control group (G3, n=62), was parents whose children had normal NBS results. A survey included the Hospital Anxiety and Depression Scale (HADS) and Parental Stress Scale (PSS). HADS scores of ≥8 indicate considerable depression. PSS measures parenthood stressors. Higher scores represent higher stress. Using SPSS, scores were compared based on means of diagnosis.
Results: Parents who experienced symptoms of depression was 70.8% for G1 Other, 67.3% for G2, 33.4% for G1 NBS, and 16.4% for G3. Kruskal-Wallace demonstrated an association between all groups and mean depression scores. G3 (M=3.95) had lower depression that differed significantly across groups. There was a significant difference between G1 NBS and G1 Other (M=8.24), and between G1 NBS and G2 (M=8.35). On average, parents in G1 NBS had lower odds of being depressed (OR = 0.207; 95% CI = 0.067-0.640), compared to parents in G1 Other. Members of G1 Other were 2.417 more likely to exhibit clinically significant levels of depression than G1 NBS.
G1 Other reported higher PSS scores than G1 NBS across all subscales: stressors, satisfaction, rewards, and lack of control. Kruskal-Wallace demonstrated an association between all groups and mean stress scores. G3 (M=31.81) had lower stress that differed significantly across all groups. G1 NBS (M=37.76) and G2 (M=42.94) differed significantly.
Discussion: Although there are concerns about the impact of NBS for complex disorders, there is limited data assessing its benefits and harms. This study, the first of its kind to evaluate the psychosocial impact of NBS for LSDs and X-ALD, suggests that parents of children diagnosed via NBS have lower depression than those diagnosed through other means, as well as lower depression and stress than those diagnosed with “routine” disorders. Although the study was small, the data is reassuring that the current practice of NBS for LSDs and X-ALD is not causing undue psychological harm. These trends may challenge assumptions regarding NBS for later onset conditions and prompt re-examination and modernization of NBS’s traditional focus on early onset disease.

Presenter: Niamh Mulrooney, Albert Einstein College of Medicine, Email: niamhmulrooney@gmail.com

Genetic Counseling

Poster #34

Utilization of a Telephone-based Hemoglobin Trait Counseling Program by Caregivers of Infants Identified with Hemoglobin Trait by the California Newborn Screening Program

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Background: Hemoglobin (HB) disorders are a group of autosomal recessive disorders characterized by the presence of abnormal hemoglobin. Individuals who inherit one abnormal allele and one normal allele are carriers of a hemoglobinopathy trait (HBT). Knowledge of trait status can help caregivers understand the clinical implications of HBT. The California Genetic Disease Screening Program (GDSP) identifies infants with HBT in newborn screening and refers them to the Hemoglobinopathy Trait Follow-up Program (HTFP). The program consists of free telephone education, counseling, and voluntary hemoglobin parent testing. In order to evaluate the reach and effectiveness of HTFP, we conducted a survey among the caregivers of newborns with HBT identified by GDSP.
Methods: A 10-question survey was mailed to 3,000 caregivers of infants who were identified with HB S, C or D Trait through newborn screening between January 1, 2017 and June 30, 2018. Descriptive analysis was performed to examine how caregivers received HBT information, their self-described level of HBT-related knowledge, and for caregivers who received services from the HTFP, their satisfaction with the program.
Results: Of 3000 surveys sent, 12% surveys were undelivered, and 84% received no responses. A total of 132 survey responses (4%) were returned (46 online responses and 86 mail responses). African Americans reported greatest understanding about HBT (57.4%) and Hispanics reported being least aware about the trait (33.6%). 47.7% of the respondents reported receiving trait information from their doctors, whereas only 18.9% reported that the HTFP was the source of trait information. Of the caregivers who responded that they called the HTFP program, 81.2% reported they were satisfied with the services provided.
Conclusion: Based on survey findings, although customer satisfaction among respondents was high, HTFP counseling services were underutilized. Further assessment is needed to understand possible barriers to access, including obtaining appointments, transportation, concerns regarding costs or health literacy. A comprehensive communications approach could improve the outreach, enrollment, and utilization of the HTFP program.

Presenter: Anubhuti Charbe, California Department of Public Health, Email: anu.charbe@cdph.ca.gov

Health Equity

Poster #35

Examination of Newborn Screening Program's Data Collection on Race and Ethnicity

J. Taylor and A. Brower, Newborn Screening Translational Research Network, Bethesda, MD

Problem: Newborn screening (NBS) is available to every infant born in the United States regardless of geography, race, or socioeconomic status. Each year millions of newborns are screened for up to 80 disorders by state-based public health agencies that collect a variety of data including: demographics, birth-related data, screening results, and sometimes information about diagnostic results. These data collection efforts enable an accurate and complete picture of screened newborns but does not provide detailed information about the longitudinal health outcomes of screen positive infants. Prospective, longitudinal health information is sometimes collected in patient registries operated by disease-specific advocacy groups, by clinical researchers, and/or by an individual state-based NBS program. These stakeholders may also use the information collected by the public health program in their prospective studies. A population-based data collective that harmonizes and aggregates data from diagnosed cases could improve our understanding of diseases and interventions. Accurate representation of race and ethnicity could be used to assess health outcomes and disparities across all racial groups, and biological variables such as genetic ancestry should be considered to help advance the understanding of the etiology of certain conditions. However, inaccuracies or misuse of non-biological variables such as race or ethnicity can lead to social harms and unvalidated conclusions.
Results: We examined demographic information collected from NBS programs using information reported to the NewSTEPs state profiles website and found that 32 states (67%) collect information on race and ethnicity. When further examining the data collected, different NBS programs used various and different terminology to describe their population. We compared the terms used with the standards developed by the Newborn Screening Translational Research (NBSTRN) and identify areas where standardization could improve data collection and use.
Conclusions: Newborn screening captures nearly the entire population of babies born each year, and this offers the potential for researchers to examine race/ethnic prevalence for screened conditions. The majority of state NBS programs collect information on race and ethnicity but use varied terminology. While race and ethnicity can be informative, the NBS community must be careful when identifying race as a risk for any disease because race is a social construct and not a biological variable. To facilitate and improve data collection efforts, the NBSTRN has developed consensus standard language that can be used to collect demographic information, and this could improve the usability of the data to advance disease understanding and improve care for all newborns.

Presenter: Jennifer Taylor, American College of Medical Genetics and Genomics, Email: jtaylor@acmg.net

Health Information Technology

Poster #36

The Current State of Interoperability in Newborn Screening Programs

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Newborn screening programs rely on timely and accurate data exchange to ensure infants receive critical and often life-saving care. Electronic data exchange between providers and newborn screening programs can increase data quality and timely care. While some states have made considerable advancements towards interoperability, including implementing electronic blood spot test orders and results and electronic reporting of pulse oximetry screening and hearing screening results from birth hospitals, many programs continue to rely on manual processes.

The HRSA-sponsored Innovations in Newborn Screening Interoperability (INBSI) program completed an environmental scan to document the current vision and strategy, technical capacity, and workforce capacity of newborn screening programs to implement electronic data exchange as well as capture existing lessons learned from early adopters and ongoing barriers. The environmental scan included a review and synthesis of published documentation and interviews with 17 newborn screening programs with representatives from blood spot screening programs, early hearing detection and intervention (EHDI) programs, and critical congenital heart defects/disease (CCHD) programs. To collect information from each program, a semi-structured interview guide was developed. 17 programs were interviewed to represent diversity across programs related to several characteristics, including electronic interoperability experience, lab testing arrangements, number of required dried blood spot screens, population characteristics, and geographic diversity.

The results from this environmental scan indicate that newborn screening programs have varying capacity, experiences, and understanding of interoperability. While newborn screening programs have access to national format and vocabulary standards, the adoption rates of these standards by health agencies and birth hospitals remain low. Common barriers continue to challenge programs and data exchange partners including the lack of a national strategy for achieving interoperability, competing priorities for critical staff, the need for increased workforce capacity, and the absence of sustained funding. Projects supported by public health informatics staff have had the most positive experiences and many programs communicate the need for strengthening workforce capacity. This poster will review the findings from our environmental scan, how these findings will inform INBSI's support of newborn screening programs, and our recommendations for harmonizing interoperability across stakeholders.

Presenter: Kaitlin Houlihan, OZ Systems, Email: khoulihan@oz-systems.com

Poster #37

Use of Electronic Health Record Patient Portals to Recruit for the Early Check Newborn Screening Research Study, April 2020 – July 2021

L. Gehtland[1], R. Paquin[1], S. Andrews[1], A. Lee[2], E. Pfaff[2], A. Gwaltney[1], M. Duparc[1], D. Bailey[1]; [1]RTI International, Research Triangle Park, NC, [2]University of North Carolina, Chapel Hill, NC

Patient-facing electronic health record applications ("patient portals") are increasingly being used to send research recruitment invitations to eligible patients. The ability to accurately identify and send invitations to large numbers of patients makes patient portal messaging particularly valuable for studies with large target sample sizes. For the Early Check research study, which seeks to offer expanded newborn screening to all newborns in North Carolina each year, queried the electronic data warehouses of the University of North Carolina and Duke University health systems to identify and send research invitations to women in their 2nd or 3rd trimester who were eligible to enroll their babies in the study prenatally. Research invitations included a description of the study and a link to the study's online permission portal. We repeated the query every two weeks to identify women who were newly eligible to receive a research invitation. An analysis of invitations sent between November 2019 and March 2020 showed invitations increased the likelihood a mother would enroll her newborn in the study, but there were disparities in enrollment by race and urban/rural status (manuscript under review). In this poster we will report data from patient portal invitations sent over the subsequent 15 months, since the onset of the COVID-19 pandemic. We will report on the effect of invitations on enrollment and report on any disparities by race, ethnicity, urban/rural status, or age.

Presenter: Lisa Gehtland, RTI International, Email: lgehtland@rti.org

Poster #38

A comprehensive newborn screening data interface for Utah providers, parents, and guardians

M. Watkins[1], A. Au[1], K. Eilbeck[1], N. Ruiz-Schultz[2], A. Rohrwasser[2], K. Hart[2], M. Williams[3]; University of Utah, Salt Lake City, UT, [2]Utah Department of Health, Taylorsville, UT, [3]Geisinger Health System, Pittsburgh, PA

An application, ResultsMyWay, has been developed to address several pain points in the current newborn screening (NBS) workflow. It has three interfaces, each designed for one of the three main participants in a typical NBS workflow: screening laboratory, clinicians, and parents/guardians of the infant being screened. Each of these participants requires different services and functionality. The screening laboratory interface provides data translation from in-house custom formats to standardized HL7 FHIR Genomics Reporting resources and facilitates the exchange of those resources to the appropriate clinical ecosystem. The clinician interface utilizes the SMART on FHIR specification to provide an interactive display of the screening results. This medium allows the results to be enhanced with clinical decision support previously incompatible with a traditional PDF report. This study pioneers the use of the HL7 Clinical Quality Language (CQL) specification to computationally provide clinicians with standardized care plans and other recommendations as formulated in the ACMG ACT sheets. The parents/guardians interface provides access to educational resources, online support networks, and relevant clinical trials that are all carefully curated for the disease/disorder for which their infant received an abnormal screen result. The interface also facilitates communication between those parents/guardians and any relevant clinical providers. This ensures that communication gaps that have previously been a source of anxiety and distress for parents/guardians are replaced by instant access to empowering and pre-filtered informational resources. While NBS is a complex process with many participants, this proof-of-concept application demonstrates how several pain points in the traditional workflow can be addressed using interoperable and modern informatics methods.

Presenter: Karen Eilbeck, University of Utah, Email: keilbeck@genetics.utah.edu

Molecular Technology: Methods & Utility

Poster #39

Reflex Test to Determine the Genetic Phase of Multiple CYP21A2 Variants Detected by a Molecular Second-Tier Congenital Adrenal Hyperplasia Assay Using a Dried Blood Spot Specimen

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CAH due to 21α-hydroxylase (21-OH) deficiency is a recessive disease caused by pathogenic variants in CYP21A2. When two or more pathogenic variants are detected, there variants may be present in each copy of the gene on the two chromosomes, also called trans-phasing, or the variants could be present on only one chromosome, cis-phasing. When CYP21A2 pathogenic variants are inherited in trans-phase, both copies of CYP21A2 are affected and that person is at high-risk for CAH. When pathogenic variants are inherited in cis-phase, a functional copy of CYP21A2 remains, so the person is a low-risk carrier for CAH. CDC developed a molecular test for second-tier CAH newborn screening in collaboration with the University of Minnesota the Mayo Clinic in Rochester, MN, Children's Hospital of Minnesota, and the Minnesota Department of Health's (MDH) newborn screening program. Minnesota families with one or more children diagnosed with CAH were recruited to identify pathogenic variants in the MN population. In 13% of families, a CYP21A2 gene with 2 or more pathogenic variants in cis-phase was transmitted from parent to child. In an MDH one-year retrospective study using the CAH molecular assay, 2 or more pathogenic variants in 67 DBS specimens from individuals without CAH were identified, suggesting cis-configuration. Together, these findings suggest that multiple pathogenic variants in cis in CAH appears to be relatively common. Thus, it was determined that CAH molecular test interpretation requires phase data to more accurately determine risk. Unfortunately, chromosome phasing using parental inheritance patterns is not feasible for most newborn screening programs, so a reflex test based on allele-specific PCR (AS-PCR) was developed to manually determine chromosome phase when two or more pathogenic variants are present in a DBS specimen.

AS-PCR reactions were developed to separate the parental chromosomes containing the CYP21A2 gene based on the pathogenic variantsIVS2-13 A/C>G, p.Ile172Asn, p.Ile236Asn, p.Val281Leu, and p.Gln318X. The choice of AS-PCR reaction for phasing is based on the pathogenic variants present in CYP21A2. Each AS-PCR was analyzed for the MN panel of pathogenic variants using the CDC-developed ASPE assay and gene sequencing to determine phase. DNA sequencing and the pathogenic variant panel ASPE assay results were compared to parent to child inheritance patterns for accuracy. These results suggest that a viable algorithm to screen for CAH could include a 1st tier 17-OHP with a conservative cutoff, followed by 2nd tier molecular interrogation using a pathogenic panel of variants, and a 3rd tier reflex to AS-PCR genotyping to determine phase when 2 or more variants are identified. This algorithm would help eliminate both false positives and false negatives while still maintaining specificity.

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Poster #40

Molecular Resource for Newborn Screening - Providing Online Tools for Molecular Testing

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The Newborn Screening (NBS) Molecular Resources Website, launched in 2013, was created to share molecular NBS best practices, quality improvements strategies and educational resources to enhance and support molecular testing. The website's content is created and vetted by APHL's NBS Molecular Subcommittee and CDC's Newborn Screening and Molecular Biology Branch. At the time of its debut, the website featured three sections: a summary of ongoing molecular laboratory assays; information and request forms for the Molecular Assessment Program (MAP) site visit program; and NBS molecular training resources. These sections remain and have been continually updated with new information. Over the years, new sections have been added including a sequencing decision matrix to help laboratories assess their needs for incorporating gene sequencing into their testing; resources related to incorporating automation into molecular testing; Frequently Asked Questions (FAQs) hot topics; and NBS Molecular Assay Validation resources.

The Sequencing Decision Matrix is a user-interactive tool to help laboratories evaluate the many considerations when needing to incorporate gene sequencing methods into routine laboratory workflow. This tool takes the user through four sections covering the utility of sequencing, laboratory readiness, outsourcing and general sequencing considerations using skip logic to generate custom recommendations unique to a laboratory's current situation.

The Automation Methods has four sections including a summary of instrument capabilities and considerations for differing laboratory throughput including semi-automated and highly automated instruments, automation methods currently in use for molecular testing, considerations when selecting a new liquid handler, and methods used to verify pipetting volumes. Each automation section provides a wealth of detailed information to assist newborn screening laboratories to select automation appropriate to their testing volume and laboratory setup.

In the Frequently Asked Questions, two new hot topics, Spinal Muscular Atrophy (SMA) and Expanding Lab Space for Better Workflow, have been added. Each topic covers some of the most common questions asked of the Molecular Subcommittee member laboratories and CDC's Newborn Screening and Molecular Biology Branch.

The new NBS Molecular Assay Validation section contains examples of validation plans and criteria used for molecular assays in newborn screening laboratories. The information in this section includes real-life strategy and approach used in validation of Real-time qPCR assays and other commonly used genotyping methods.

The NBS Molecular Resources Section of the APHL Website will continue to expand on topics and information as the need grows in the newborn screening community. Please contact the authors with suggestions and thoughts on additional needed topics.

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Poster #41

Retrospective sequence analysis of Medium Chain Acyl-CoA Dehydrogenase Deficiency and Very Long Chain Acyl-CoA Dehydrogenase Deficiency referrals in NY: Correlation with carnitine levels and diagnosis

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Background: The New York State Newborn Screening Program (NSP) began screening for medium chain acyl-CoA dehydrogenase deficiency (MCADD) in 2002 and very long chain acyl-CoA dehydrogenase deficiency (VLCADD) in 2004 using tandem mass spectrometry (MS/MS) to screen for elevated levels of octanoylcarnitine (C8) and tetradecenoylcarnitine (C14:1) respectively. These fatty acid oxidation disorders are caused by pathogenic variants in the ACADM and ACADVL genes. Screening for MCADD initially included a second-tier targeted genotyping assay for the c.985A>G (p.Lys329Glu) variant, reported to be the most common disease-causing variant. Although this variant was common in MCADD referrals, many infants confirmed with disease had 1 or 0 copies p.Lys329Glu. Molecular analysis was not included in our VLCADD screening algorithm. In this work, we investigated the spectrum of sequence variants in infants referred for MCADD or VLCADD to determine if there was a correlation with acylcarnitine levels and final diagnosis.
Methods: Sanger sequencing assays were developed and validated to identify sequence variants in the coding exons and intron/exon borders of the ACADM and ACADVL genes. We retrospectively sequenced 119 specimens from infants referred for MCADD and 77 for VLCADD. Of the infants sequenced, 47 had a clinical diagnosis of MCADD and 14 of VLCADD.
Results: For MCADD, we observed a trend towards higher C8 levels when 2 ACADM variants were detected and disease was confirmed. C8 levels were typically lower in infants with 1 or 0 variants identified and for whom disease was not diagnosed. We identified 2 potentially disease-causing variants in all but 3 of the infants with a confirmed MCADD diagnosis; all three had only 1 variant identified. We did not observe a strong correlation between C14:1 levels and specific ACADVL variants or a VLCADD diagnosis. We did, however, identify 2 potentially disease-causing variants in 10 of the 14 infants confirmed to have disease and 1 variant was identified in each of the remaining 4 infants.
Conclusion: Sequence analysis of infants referred for MCADD or VLCADD revealed a wide spectrum of variants in the ACADM and ACADVL genes in our population. We were able to identify at least 1 potentially disease-causing variant in all infants with confirmed disease, and 2 variants in most cases. New York implemented 2nd tier whole gene Sanger sequence analysis of MCADD referrals in June 2020 and VLCADD in May 2021 as supplementary tests and to further investigate the genotype-phenotype correlations in these fatty acid oxidation disorders.

Presenter: Matthew Nichols, New York State Department of Health, Email: matthew.nichols@health.ny.gov

Poster #42

Reduction in MPS I referrals in New York by 2nd tier IDUA sequence analysis

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Background: Mucopolysacchardosis type I (MPS I) is a rare lysosomal storage disorder caused by a deficiency of α-L-iduronidase, an enzyme required for the degradation of two mucopolysaccharides, dermatan sulphate and heparan sulphate. The accumulation of these mucopolysaccharides in various organs and tissues of MPS I patients causes multi-organ damage which, in severe cases, results in death during childhood. MPS I is an autosomal recessive disease caused by pathogenic variants in the IDUA gene. Benign pseudodeficiency variants, that lower enzyme activity but do not cause disease, may lead to first-tier false positive results. Due to approval of a recommendation from the US Secretary of Health and Human Services Advisory Committee for Heritable Disorders in Newborns and Children, the New York State (NYS) Newborn Screening program began screening for MPS I in October of 2018.
Methods: The MPS I first-tier screening test measures IDUA enzyme activity in dried blood spots using tandem mass spectrometry reflexing any sample below 6% of the daily mean to Sanger sequencing of the full IDUA gene. Interpretation of results is enhanced using the post-analytical tool Collaborative Laboratory Integrated Reports (CLIR).
Results: From October 1, 2018 to March 31, 2021, 537,398 newborns were screened for MPS I with seventy-nine reflexed to second-tier molecular testing due to low IDUA enzyme activity. Fifty-three of those were reported as pseudodeficiency only and twenty-six were referred to Specialty Care Centers for diagnostic evaluation. The twenty-six referrals include fifteen carriers of either a variant of uncertain significance or a pathogenic variant combined with a pseudodeficiency allele(s), one carrier of a pathogenic variant, four cases lost to follow-up, two cases where the variants identified in the newborn screening sample were not confirmed in a repeat sample and determined to be a sequencing artifact, and four possible attenuated MPS I cases that are still being followed.
Conclusion: The two-tiered algorithm used in NYS for MPS I screening reduced referrals by 67.1% by excluding false positive results for infants with only known pseudodeficiency alleles (p.Ala79Thr, p.Thr99Ile, p.Asp223Asn, p.Val322Glu) from the referral group.

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Poster #43

Improved CMV Dried Blood Spot PCR for Universal CMV Screening

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Congenital cytomegalovirus (cCMV) is the most common infection in newborns, affecting between 0.5 – 2.3% of births worldwide and is the leading cause of neurodevelopmental impairment and non-genetic sensorineural hearing loss in the developed world. Of the 20,000 to 40,000 infants infected annually in the U.S., only 10-15% are symptomatic at birth, with symptoms ranging from mild to severe multi-organ dysfunction. Of those asymptomatic at birth, up to 20% will have neurodevelopmental impairment by 2 years of age. Additionally, many infected children will have a fluctuating and progressive hearing loss that may not develop until 6 years of age.

Currently, there is no national standard for universal screening of cCMV in newborns. The gold standard for CMV testing is to perform CMV PCR on urine or saliva samples. Neither urine nor saliva samples have been collected by NBS programs to date, and the collection of either sample type would require a significant paradigm shift. Dried blood spot (DBS) specimens are currently collected for NBS, and a highly sensitive and specific DBS cCMV test would enable faster and more efficient inclusion of cCMV in NBS programs. Initial studies for CMV detection from DBS resulted in unacceptably low sensitivities, but Mark Schleiss (University of Minnesota) and Sheila Dollard (CDC) have recently shown improved sensitivity (75%).

Although sensitivity for cCMV was improved, it is still much lower than any other screening test in NBS programs and would result in many false negatives. We are working to improve the sensitivity of the CDC assay using two approaches: 1) digital droplet PCR and 2) direct PCR. DBS will be made from infant blood containing known amounts of CMV either added in the laboratory or naturally infected. We anticipate these methods will improve the sensitivity and reduce the number of false negatives and the number and size of the DBS punches needed for the detection of cCMV.

Presenter: Jean Kim, RTI International, Email: jeankim@rti.org

New Methodologies

Poster #44

Implementation of a High Throughput Microsphere Based Assay for HIV Antibody Screening in Infant Dried Blood Spots

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Background: In New York State (NYS), mother-to-child transmission of HIV has effectively been eliminated, due to regulations that require women be offered HIV testing during pregnancy and expedited HIV testing at labor/delivery for women without a record of a negative prenatal HIV test. However, infants born to women who become infected late in pregnancy after an initial negative prenatal HIV test are at high risk for infection and may continue to be exposed through breastfeeding. In 1997, NYS implemented universal newborn HIV screening to identify all HIV-exposed infants, including infants born to women with previously undetected HIV infection. Initially screening was performed on infant dried blood spots (DBS) using a modified FDA-approved HIV immunoassay. In 2018, a low cost, high-throughput microsphere immunoassay was developed and implemented to detect maternal HIV antibodies in DBS collected from all newborns born in NYS.
Methods: DBS from all babies born in NYS are collected and sent to the newborn screening laboratory. Elution buffer is added to a 3-mm DBS punch and the eluate is combined with magnetic microspheres covalently coupled to HIV-1 gp120/41 antigens, in a 384-well microplate. After a wash, a phycoerythrin-labeled secondary anti-human IgG antibody is added to bind the microsphere-HIV-1-antibody complex. After a second wash, the samples are analyzed on a Luminex FlexMAP 3D instrument. Reactive samples are retested in duplicate and, if consistently reactive, tested using the Bio-Rad Geenius HIV-1/2 supplemental assay under a modified protocol.
Results: Between December 1, 2018 and December 1, 2020, 434,031 babies (511,869 specimens) were screened for HIV-1 antibodies. Of those, 684 newborns (0.16%) screened positive. One infant was detected whose mother was not previously known to be HIV infected, thereby proving the efficacy of this assay. There were 4 false positive and 5 false negative results. All false positive samples had low reactivity and an unusual Geenius banding pattern. All false negative results were in infants of mothers who received antiretroviral treatment and had suppressed antibody levels, indicating a limitation of screening for HIV antibodies in infants, especially when maternal HIV treatment is initiated before full seroconversion is achieved.
Conclusion: Initial testing results show the benefits of screening all newborns for HIV exposure even if the mother was previously screened for HIV. In addition to its low cost and ease of use, this assay has the flexibility to be modified for additional targets, such as hepatitis C virus and SARS-CoV-2, making it a valuable assay to employ in the newborn screening program.

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Poster #45

SCID-SMA Triplex Assay Validation and Population Study in North Carolina Newborns

K. Blake, K. Chao, S. Shone and D. Pettit, North Carolina State Laboratory of Public Health, Raleigh, NC

Purpose: The North Carolina State Laboratory of Public Health (NCSLPH) began screening for Severe Combined Immunodeficiency (SCID) using the CDC on-spot real-time PCR assay to measure the levels of T-cell receptor excision circles (TREC) in 2017. A 3-year screening review showed a larger than expected proportion of babies with low or borderline TREC levels. NCSLPH has validated the CDC's Newborn Screening and Molecular Biology branch triplex assay that measures TREC, SMN1, and RNase P from nucleic acid extracts derived from single dried blood spot specimen punches. TREC levels in the NC preterm population were also evaluated, to improve the reporting algorithm and to reduce false-positive abnormal SCID call-outs due to prematurity. This assay replaces the previous SCID assay and adds screening for Spinal Muscular Atrophy (SMA) at NCSLPH. The Research Triangle Institute's (RTI) Early Check program has provided free, voluntary SMA testing and essential follow-up services to the NC newborn population since October 2018.
Methods: SMA screening uses an SMN1-specific probe in a real-time PCR assay to detect homozygous deletions of SMN1 exon 7, while simultaneously evaluating the TREC levels to detect elevated risk for SCID. RNase P evaluation are included in the assay as a quality control to ensure the success of the DNA extraction and to evaluate for PCR inhibition. A preterm and full-term normal population study compared TREC levels obtained at various gestation ages (GA). The difference of the mean TREC quantification cycle (Cq) values obtained for each GA group were evaluated using an analysis of variance method. Cumulative population percentages were applied to determine screening cutoff values.
Results: Assay accuracy assessments showed an absence of TREC levels in two confirmed classic SCID specimens, in one leaky RAG1-related SCID specimen, and in one adenosine deaminase deficiency SCID specimen. Thirty-two babies with other diagnoses presented with borderline to no TREC levels. Absence of SMN1 exon 7 was detected in two babies with confirmed SMA. One previous SMA false positive case demonstrated a low SMN1 exon 7 level. The population study consisted of a total of 3467 NBS specimens. TREC levels were analyzed between full-term (GA≥37 weeks, N&#3f2591) and preterm (GA < 37 weeks, N&#3f876) specimens and significantly different means (P < 0.001) were derived for the GA groups. Mean TREC Cq values decreased with increasing gestation age.
Conclusion: The North Carolina NBS program has validated the CDC triplex PCR screening assay and accurate classifications for abnormal SCID and abnormal SMA were achieved. The new TREC cutoff values for full-term abnormal and borderline SCID were determined after evaluating gestational age groups. Preterm babies with low TREC levels may be lymphopenic which is associated with prematurity; however, lymphopenia due to prematurity often resolves with age.

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Poster #46

Verification and Implementation of NeoBaseTM 2 Non-derivatized MSMS kit: A Systemic Overhaul of NCSLPH MSMS Newborn Screening

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Objective: The North Carolina State Laboratory of Public Health (NCSLPH) MSMS Laboratory verified the performance of the NeoBaseTM2 Non-Derivatized MSMS kit to replace a derivatized MSMS Laboratory Developed Test (LDT) for the measurement of amino acids, acylcarnitines, succinylacetone, free carnitine and lysophospholipid concentrations to screen for metabolic disorders. The implementation of this method will allow NCSLPH to add three additional disorders not screened for by the LDT, improve sample identification, reduce specimen preparation time, revise disorder logic, and improve overall quality assurance.

Method: Analytes were extracted from dried blood spots (DBS) with a solution containing labeled internal standards and analyzed using Waters Acquity TQD tandem mass spectrometry (MSMS) systems in Multiple Reaction Monitoring (MRM) mode. Method verification studies performed included accuracy, precision, reportable range, relative percent difference, carryover, kit lot verification, instrument comparison, reference ranges, and cutoff analysis using kit QC materials, CDC QC, CDC PT specimens, and specimens from confirmed cases. Precision analyses were performed to assess variation within run, between run, between day, and between operator. Accuracy studies included 76 confirmed patients and 14 PT specimens, tested using the NeoBaseTM 2 Non-Derivatized MSMS kit. 12,183 specimens were analyzed to determine population cutoffs. Histograms created from population data for each analyte were overlayed with specimens from patients with known conditions and abnormal PT samples to support to cutoffs.

Results: For accuracy studies, QC was acceptable for all plates. 85 specimens reflected the correct interpretation. 5 specimens produced discrepant results which showed specimen degradation. Precision analyses showed %CV ≤ 15% for all analytes above the biologically significant range. Reportable Ranges were determined based on the lowest and highest QC concentration. Cutoffs were determined by identifying natural breaks in the patient population and compared against percentile calculations for each analyte.

Conclusions: NeoBaseTM2 Non-Derivatized MSMS kit showed acceptable performance in identifying increased risk for metabolic disorders. By implementing this method, the NCSLPH will add M/SCHAD, CPTI, and a first-tier screening method for ALD to the Newborn screening panel. Specimen preparation time was reduced from 7 steps to 2 steps. Method-specific QC for all primary analytes and the addition of QC software modules for consistent monitoring of QC trends improved quality monitoring. The NeoBaseTM2 Non-Derivatized MSMS kit will allow the NCSLPH Newborn Screening MSMS Laboratory to provide a more robust screening system for detecting babies with an increased risk for metabolic disorders in North Carolina's newborn population.

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Poster #47

Steroid Profiling from Dried Blood Spot Samples Using PerkinElmer QSight® Mass Spectrometer

R. Bozic, L. Bacci, A. Fabegat and T. He, PerkinElmer, Turku, Finland

Inborn errors of metabolism include a wide variety of conditions with abnormal steroidogenesis caused by mutations affecting the enzymatic activity in the steroid biosynthesis pathways. E.g. 21-hydroxylase (21-H) deficiency and 11-β hydroxylase (11β-H) deficiency impact the normal synthesis of glucocorticoids and mineralocorticoids.

Measurement of increased 17-hydroxyprogesterone (17-OHP) concentration in dried blood spot (DBS) by immunoassays (IA) is associated to analytical interferences arising from cross-reactivity of the reagent antibodies with other structurally related steroid metabolites, particularly 17-hydroxypregnenolone.

To allow more specific steroid marker profiling from dried blood spot (DBS) samples, we report here a gradient UHPLC-MS/MS method on the PerkinElmer QSight®, which provides simultaneous specific determination of 17-OHP, androstenedione, cortisol, 21-deoxycortisol and 11-deoxycortisol. Therefore, this multiple steroid profiling method enables an improved distinction between 21-H and 11β-H deficiencies, and it can be also potentially used as a second-tier test for 17-OHP immunoassays suffering from cross-reactivity interferences.

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Poster #48

LC-MS/MS measurement of isobaric C5 acylcarnitines in dried blood spots using PerkinElmer QSight® 225 MD Mass Spectrometer

R. Bozic, T. Lehtonen, A. Meierjohann and T. He, PerkinElmer, Turku Finland

In isovaleric acidemia (IVA) deficiency, a defected Isovaleryl-CoA dehydrogenase enzyme causes an accumulation of isovaleric acid, which is toxic to the central nervous system.
The first-tier IVA screening is commonly based on the measurement of increased isovalerylcarnitine (C5) concentration in blood. By direct flow injection analysis tandem mass spectrometry (FIA-MS/MS) assays without liquid chromatography (LC) separation, this specific biomarker cannot be however distinguished from other possibly existing three C5 isobars including valeryl-, pivaloyl-, and 2-methylbutyrylcarnitine. Especially, pivaloylcarnitine interference commonly causes false positive C5 screening results because it can be present in newborn blood due to use of pivalic acid ester containing antibiotics or nipple creams. To allow more specific measurement of these isobaric C5 markers, we report here a gradient LC-MS/MS method on the PerkinElmer QSight®225 MD, which is capable to more accurately quantify isovalerylcarnitine (C5) concentration in dried blot spot (DBS) samples, and therefore provides potential analytical tool for the second-tier testing.

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Poster #49

LC-MS/MS measurement of methylmalonic acid and 3-hydroxypropionic acid in dried blood spot using PerkinElmer QSight® MD Mass Spectrometer

R. Bozic, L. Bacci, T. Lehtonen and T He, PerkinElmer, Turku, Finland

Elevated propionylcarnitine (C3) is a commonly applied first-tier screening marker for propionic acidemia and methylmalonic acidemia, which are caused by defected Methylmalonyl-CoA mutase or Propionyl-CoA carboxylase enzymes.
More specific indicative biomarkers for these enzymatic deficiencies are however methylmalonic acid (MMA) and 3-hydroxypropionic acid (3OHPA), which cannot be detected by a direct flow injection analysis tandem mass spectrometry (FIA-MS/MS) assays without liquid chromatography (LC) separation. To improve this situation, we report here more specific isocratic LC-MS/MS method on the PerkinElmer QSight® MD, which is capable to measure MMA and 3OHPA concentrations in dried blot spot (DBS), and therefore provides potential analytical tool for the second-tier testing.

Presenter: Roberto Bozic, PerkinElmer, Email: roberto.bozic@perkinelmer.com

Poster #50

LC-MS/MS measurement of alloisoleucine and branched-chain amino acids in dried blood spot using PerkinElmer QSight® MD Mass Spectrometer

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Maple syrup urine disease (MSUD) is caused by a defected branched chain alpha-keto acid dehydrogenase multienzyme complex (BCKDH), which results in a toxic accumulation of the branched chain amino acids (BCAAs): leucine (Leu), isoleucine (Ile) and valine (Val), and their corresponding alpha-keto (i.e. 2-oxo) acid by-products in the blood, urine and body tissues.
Even if the first-tier screening of MSUD is commonly based on the measurement of increased BCAA concentrations in blood as a sum of Leu, Ile and Val, the most specific pathognomonic marker is D-alloisoleucine (Allo-Ile). By a direct flow injection analysis tandem mass spectrometry (FIA-MS/MS) assay without liquid chromatography (LC) separation, this important biomarker cannot be however distinguished from other isobaric amino acids like Leu, Ile and 3-hydroxyproline (3-OH-Pro).
To allow more specific measurement of these isobaric markers, we report here a gradient LC-MS/MS method on the PerkinElmer QSight® MD, which is capable to quantify Allo-Ile concentration in dried blood spot (DBS) and therefore provides potential analytical tool for the second-tier testing.

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Other

Poster #51

Validation of Real-Time Quantitative PCR Workflow for Spinal Muscular Atrophy Newborn Screening in California

P. Bhattacharjee, C. Wu, C. Aznar, L. Shih, L. Wu, S. Diaz, J. Kurosaka, L. Tom and R. Koupaei, California Department of Public Health, Richmond, CA

Introduction: Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder caused by the deletion of the exon 7 region of the Survival Motor Neuron (SMN1) gene. The California Department of Public Health (CDPH) Genetic Disease Screening Program was mandated to begin newborn screening for SMA as of July 2020. The objectives were to optimize and validate a CDC-developed multiplexed real-time quantitative polymerase chain reaction (qPCR) assay at the CDPH Genetic Disease Laboratory, and verify performance of instrumentations that would be used in routine SMA screening of ~500,000 specimens per year.
Methods: The qPCR assay detects the presence or absence of SMN1 from DNA extracted from newborn dried blood spots (DBS), and uses the Ribonuclease P protein subunit P30 (RPP30) gene as endogenous control. Laboratory workflow includes using two Hamilton® custom liquid handlers for DNA extraction, and four Thermo Fisher® QuantStudio7™ (QS7) Flex Real-Time PCR instruments in 384-well format for quantifying gene targets. Method validation was performed on one QuantStudio7 instrument to establish method accuracy, precision, reference range and reportable range. Verification of performance specification of the other QS7 instruments was performed.
Results: All instruments performed within acceptable quality control criteria. Clinical validation was performed by testing SMA positive and carrier specimens using the qPCR method, followed by droplet digital PCR (ddPCR™) to verify SMN1 and SMN2 copy numbers. Based on these results, qPCR cutoff thresholds for SMA disease determination in the California population were established. Finally, pre-launch capacity testing for three consecutive days established the feasibility of the SMA screening workflow in processing up to ~2500 newborn specimens per day. SMA newborn screening was launched in California on June 24, 2020.

Presenter: Preeti Bhattacharjee, California Department of Public Health, Email: preeti.bhattacharjee@cdph.ca.gov

Poster #52

Validation of Liquid Handler Workflow for Spinal Muscular Atrophy Screening in California

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Introduction: Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by deletions of the Survival Motor Neuron gene (SMN1). The incidence rate is 1 out of 10,000 to 20,000 newborns. The California Department of Public Health (CDPH) Genetic Disease Screening Program was mandated to begin screening for SMA as of July 2020. The objective was to design, optimize and validate the liquid handler workflow for SMA screening of ~500,000 newborn specimens per year.
Methods: The CDPH Genetic Disease Laboratory customized the Hamilton® STARplus™ liquid handler in order to perform DNA extractions from dried blood spots (DBS) and enable downstream processing with a quantitative polymerase chain reaction (qPCR) assay in the 384-well plate format. The liquid handler includes on-deck shaking heating/cooling plates, centrifuge, plate peeler and plate sealer. Each customized liquid handler can process up to sixteen 96-well plates in an eight-hour period. Programmable parameters for DNA extraction protocol sequences were optimized. Validation and verification of the instruments were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.
Conclusion: Criteria for instrument accuracy and precision were met allowing for high through-put automated processing of ~2,500 specimens daily. The validation of the liquid handler workflow allowed for the implementation of SMA screening in California.

Presenter: Lifan Shih, California Department of Public Health, Email: Lifan.Shih@cdph.ca.gov

Poster #53

Laboratory Workflow Validation for Spinal Muscular Atrophy Screening in California

C. Wu, C. Aznar, G. Kuntamallapanavar, L. Shih, P. Bhattacharjee, L. Wu, S. Diaz, J. Kurosaka, L. Tom , S. Sciortino and R. Koupaei, California Department of Public Health, Richmond, CA

Introduction: The California Health and Safety Code for Genetic Prevention Services mandates that new disorders, once adopted to the federal Recommended Uniform Screening Panel (RUSP) must be added to the Newborn Screening Program (NBS) testing panel within two years from the inclusion date. Following the addition of Spinal Muscular Atrophy (SMA) to the RUSP, the California NBS Program had to implement screening for this new disorder by July 2020. This effort required designing the laboratory workflow and modifications of a facility at the California Department of Public Health Genetic Disease Laboratory (GDL), which began in 2019. The objective was to establish, optimize and validate a screening assay workflow that can process ~500,000 newborn dried blood spot (DBS) specimens per year.

Methods: The SMA screening workflow at GDL utilizes four Perkin-Elmer DBS Punchers®, two customized Hamilton® STARPlus® liquid handlers, four ThermoFisher Scientific quantitative polymerase chain reaction (qPCR) instruments, one Bio-Rad® droplet digital PCR (ddPCR™) instrument, and Perkin-Elmer Specimen Gate® for data acquisition. The daily workflow was designed to process up to thirty-two 96-well plates, or ~2500 specimens in an eight-hour work period. The workflow is as follows: dried blood spots (DBS) are punched into 96-well plates, DNA extraction is performed by the liquid handlers, DNA extracts are aliquoted into 384-well plates by the liquid handlers, and quantified by the qPCR instruments. Screen-positive specimens are analyzed for copy numbers of the target genes with ddPCR. Validation and verification of the method and instruments were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. High throughput liquid handler accuracy and precision were established first before validating the qPCR workflow. Method validation was performed to establish reference instruments, followed by instrument performance verification, and clinical specimen testing. Method accuracy, precision, reference range, and reportable range obtained during the validation and verification met established criteria.

Outcome: SMA screening of newborn specimens in California was successfully implemented on June 24, 2020. As of March 2021, 19 positive SMA cases were identified in California.

Presenter: Cindy Wu, California Department of Public Health, Email: cindy.wu@cdph.ca.gov

Poster #54

Validation of Droplet Digital PCR™ Workflow for Spinal Muscular Atrophy Screening in California

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Introduction: Spinal Muscular Atrophy (SMA) is a neuromuscular disorder that causes a progressive loss of motor neurons. In many severe cases without treatment or respiratory aid, death will occur within two years. The most common underlying cause of SMA is the deletion of the SMN1 gene in the exon 7 region. Newborn screening for SMA was added to the Recommended Uniform Screening Panel in July of 2018. Under California's statutory mandate, the California Department of Public Health (CDPH), Genetic Disease Screening Program was required to start SMA screening by July of 2020.
Methods: The CDPH Genetic Disease Laboratory (GDL) optimized and validated a laboratory screening workflow in order to screen ~500,000 newborn specimens per year. A real time qPCR (qPCR) assay is used as the primary screening method for the presence and absence of SMN1. The Bio-Rad droplet digital PCR™ (ddPCR) workflow is used to determine SMN1 and SMN2 copy numbers in order to provide quality assurance of specimen processing. This presentation describes the validation of dried blood spot SMN1 and 2 copy number quantification workflow using ddPCR™ at GDL. During this validation, quantification of SMA positive, SMA carrier, in-house references, and negative clinical specimens were quantified. The Center for Disease Control and Prevention (CDC) provided the SMA positive and carrier samples. Additionally, a set of negative SMA and positive SMA samples were also selected and tested for clinical validation. All samples tested were dried blood spots spotted on a filter paper.
Conclusion: The results from the clinical samples were as expected. Method accuracy, precision, and reference range were obtained during this validation and were found to be acceptable under the acceptance criteria.

Presenter: Lawson Wu, California Department of Public Health, Email: lawson.wu@cdph.ca.gov

Poster #55

Where do we begin? A review of publicly available RUSP nomination evidence reviews

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Background: The Recommended Uniform Screening Panel (RUSP) currently includes 35 core conditions. However, there are many more childhood onset conditions that could benefit from early detection through newborn screening. The RUSP nomination process requires a collaborative approach from multiple newborn stakeholders to develop the evidence required to submit a RUSP nomination package. The federal Advisory Committee on Heritable Disorders in Newborns and Children (Advisory Committee) releases a limited number of public reports on each condition nominated to the RUSP, both successful and unsuccessful. These reports can include a Letter from Committee, an Executive and/or Full Summary, and an External Evidence Review Report and/or Letter. To our knowledge there has not been a systematic review or centralized discussion of these reports across conditions. While reviewing individual reports may be helpful in modeling a new nomination package, a better understanding of the commonly included elements (or elements commonly asked for by the Advisory Committee) is needed to help patient organizations anticipated potential evidentiary requirements and more effectively and efficiently prepare new nomination packages.

Methods: To begin understanding these elements we reviewed all publicly available Full and Executive Summary documents for the conditions that have been added to the RUSP through a nomination process (N&#3f6). Next, we reviewed the published Letters from Committee (N&#3f10) and the Full and Executive Summaries when available (N&#3f3) for those conditions not recommended for the RUSP. From these documents we identified common themes across conditions. We then reviewed all available External Evidence Review Reports from successful nominations to further refine and understand the common elements, as well as look for ways that these packages addressed the issues that came up in unsuccessful nominations.

Results: We identified 7 common elements across successful nomination packages: test logistics, test performance, prevalence data in the United States, prospective population-based data, clinical utility of screening, public health impact, and strong, clear case definitions. In addition, we found that many of these elements were noted by the Advisory Committee as missing from unsuccessful nomination packages.

Discussion: This study represents the first known review of successful and unsuccessful nominations to the RUSP. While the full decision-making process of the Advisory Committees on Heritable Disorders in Newborns and Children are not publicly documented, understanding the commonly included elements may serve as a starting point for organizations seeking to create a new nomination package.

Presenter: Dylan Simon, EveryLife Foundation for Rare Diseases, Email: dsimon@everylifefoundation.org

Pre-analytical Aspects

Poster #56

Sixty-day Assessment of Creatine-Kinase MM Stability in Newborn, Contrived and Patient Dried Blood Spots Used in Screening for Duchenne and Related Muscular Dystrophies

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Duchenne muscular dystrophy (DMD) is a rare X-linked neuromuscular disease affecting approximately 1 in every 3,000 – 6,000 males worldwide. DMD is associated with severe progressive muscle loss and premature death. Diagnosis of DMD typically occurs between ages 4-5 years and patients are often wheelchair bound by age 7-13 years, with death occurring usually before age 30. Elevated levels of the enzyme Creatine-Kinase isoform MM (CK-MM) in the bloodstream in DMD patients can be used as a secondary marker for screening newborns using dried blood spots (DBS). In November 2020 our laboratory implemented newborn screening (NBS) for DMD and related muscular dystrophies as part of the RTI International Early Check (EC) NBS study. EC offers optional screening to parents whose infants have NBS in North Carolina using leftover specimens from the North Carolina Laboratory of Public Health (NCSLPH) after all routine NBS is complete. To screen for elevated CK-MM as a part of the EC study, we selected and verified the FDA approved Neonatal Creatine Kinase -MM kit (Perkin Elmer) that runs on the genetic screening processor (GSP) commonly found in many NBS public health labs. The stability of CK-MM in DBS is affected by high temperatures and humidity, particularly when exposed for an extended period of time. The FDA-approved CK-MM kit is appropriate for specimens stored at dry conditions and ambient temperature up to 20 days post collection. While the turnaround time for state provided newborn screening typically spans a few days to a week, our research study utilizes the state's DBSs after all state screening is complete and allows for infant enrollment up to 4 weeks after birth. As a result, one third of EC study samples are tested after the 20-day timeframe. We have devised two strategies to improve the reliability and understanding of CK-MM testing results in stored DBSs: 1) We implemented dry storage conditions for DBS specimens at the NCSLPH and 2) Designed a 60-day stability study to assess CK-MM levels in DBS specimens stored at dry and ambient humidity. In this study we compared CK-MM stability in: 1) DBSs from presumed healthy newborns, 2) DBSs collected from older patients with known neuromuscular conditions affecting CK-MM levels, and 3) contrived DBS specimens spiked with commercially available CK-MM. The results of this study will determine: 1) the effectiveness of the dry storage condition for the three types of specimens and 2) whether separate cut-offs are needed for specimens stored for an extended period of time.

Presenter: Brooke Migliore, RTI International, Email: bmigliore@rti.org

Public/Community Engagement/Experiences in Newborn Screening

Poster #57

The Pivotal Role of Parents in Newborn Screening Research

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Purpose: For the past sixty years, the parents of children with rare genetic conditions have been strong advocates for research. And while advancements in screening and treatments have been essential to advance newborn screening (NBS), the tireless efforts of families and advocacy groups have been pivotal to the expansion of newborn screening from one condition to over thirty-six conditions. NBS research depends on the involvement of parents and families, and in the case of prospective pilots, relies on the recruitment of parents who may have little or no experience with rare diseases. To identify successful strategies and create content for an online parent-focused NBS research resource, we undertook an effort to learn more about the role of parents and families in NBS research.
Methods: A review of the literature was conducted to identify parent and family involvement in NBS research. The search engines PubMed, OVID Nursing Database, and Google Scholar were queried for English language peer-reviewed articles published in 2018-2020. Keywords included newborn screening, research, parent engagement, and ELSI (ethical, legal, social implications). Reference lists were scrutinized for additional relevant publications.
Results: The search returned 213 articles, and the findings from 13 were. Two distinct themes emerged from our review: "recruitment," defined as a single aim defined by parental informed consent to participate in the study, versus "engagement," described as multifactorial, longitudinal participation with the research team in all study phases. Engaging parents in the planning and conduct of research and disseminating findings was identified as a successful strategy. We will present a parent-focused NBS research resource that highlights our findings.
Conclusion: The advancement of NBS relies on the involvement of parents and families, who may have little experience with a rare disease. NBS research efforts that engage families in all aspects of the research process have been the most successful in translating research to public policy and clinical practice. Understanding the best approach to parent engagement in NBS research will inform future efforts.

Presenter: Amy Brower, American College of Medical Genetics and Genomics, Email: abrower@acmg.net

Quality Improvement and Assurance Activities

Poster #58

Using an Evidence-Based Collaborative Approach to Champion Quality Improvement in Newborn Screening

C. Norman and K. Walters, Association of Public Health Laboratories, Silver Spring, MD

There can be many barriers to beginning a quality improvement initiative. Barriers include a lack of resources, support, and access to subject matter experts. These barriers often discourage staff and teams from initiating improvement activities resulting in band-aid solutions instead of a systems approach to addressing process issues and challenges.

The Institute for Healthcare Improvement's (IHI) Breakthrough Series (BTS) is an evidence-based collaborative model that reduces the initial barriers to starting an improvement initiative by providing a structured approach to learning and applying quality improvement methods in a team-based environment. The Association of Public Health Laboratories (APHL) Quality Improvement (QI) Projects collaborative is modeled after the IHI BTS and has used this framework across 3 cohorts since its inception in 2019.

Although still in its infancy, applying the BTS framework to the QI Projects collaborative has resulted in early success and measurable improvements among participating newborn screening programs. This includes improvements to metrics such as timeliness, turnaround time, percent satisfactory specimens, as well as an overall improvement to tracking the prevalence of and reporting of newborn screening conditions. Additionally, the structure provided by the BTS framework has resulted in increased collaboration, knowledge sharing and improved understanding and application of common improvement tools such as the Plan Do study Act (PDSA) cycle and run chart.

The early gains observed by programs participating in the QI projects collaborative is encouraging. The initial success has the potential to inform program design for future collaborative activities within the newborn screening community as well as further underscore the importance of structured evidence-based quality improvement programming as one approach to improving newborn screening systems.

Presenter: Kayana Walters, Association of Public Health Laboratories, Email: kayana.walters@aphl.org

Poster #59

The Mystery of Malonic Acidemia False Positives: An Investigation

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Background: Malonic acidemia (MAL) is a rare metabolic disorder that affects about 1 in 750,000 California (CA) newborns. A deficiency of malonyl-CoA decarboxylase can cause complications that are life-threatening in the neonatal period but can be managed through diet and supplementation. The CA Newborn Screening (NBS) Program observed an increase in MAL false positives (FP) in January of 2019, which remained elevated through March of 2021. The increase in FPs has led to several negative consequences, including parental anxiety, concern about NBS methodologies by clinicians, and overburdening of follow-up care providers. This report describes the investigation into CA's increase in MAL FPs as well as the interventions to reduce FP rates.

Methods: To screen for MAL, the CA NBS Program evaluates C3DC, the ratio of C3DC to C10 (C3DC/C10), and the ratio of C5DC to C3DC (C5DC/C3DC) in dried blood spots (DBS) using PerkinElmer's NeoBase™ Non-derivatized MSMS kit. We took a collaborative approach to investigate the rise in MAL FPs, examining the issue through epidemiological, laboratory, and clinical lenses. Procedural interventions were implemented at hospitals to increase awareness of collection workflows and at regional laboratories to retest screen positive specimens. We analyzed screening data for specimens accessioned between January 1, 2017 and December 31, 2020 to determine the magnitude and cause of the issue, and to formulate a new screening algorithm.

Results: MAL FP rates rose from 0.01% in 2017-2018 to 0.05% in 2019-2020. The 99.9 percentile for C3DC increased from 0.43 to 0.46, and the 0.1 percentile of C5DC/C3DC decreased from 0.48 to 0.37, respectively. No changes were observed in C3DC/C10. FPs were primarily concentrated at 5 hospitals which collected DBS for 75% of FP but only 5% of CA's DBS. No significant changes in workflows were identified at hospitals with large volumes of FPs. A laboratory investigation into the effect of sanitation wipe contamination on DBS yielded insignificant results. Only minimal improvements were observed following procedural interventions at hospitals and laboratories. Adjusting C3DC and C5DC/C3DC in our current algorithm was modeled to decrease 2019-2020 MA FPs by 11%, from 426 FPs to 379. Adding C16/C8 to our adjusted algorithm provided a reduction of 64%.

Conclusions: An investigation into the rise in MAL FPs indicates that contamination of DBS may be the source of the issue, though it is unclear where the contamination is originating. We implemented several workflow changes at hospitals and laboratories, but modeling suggests that adjusting our screening algorithm will provide the greatest reduction in MAL FPs. Continuous monitoring of MAL positives will be key to evaluating our success and determining if further intervention is warranted.

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Poster #60

Addressing the Impact of the COVID-19 Public Health Emergency on Newborn Screening: Creating the Virtual Site Visit

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Background: The COVID-19 public health emergency suspended the ongoing Newborn Screening Program (NBS) Area Service Center (ASC) mandated in-person site visits. These site visits are regularly performed by the ASCs to assess birthing facility compliance with California NBS regulations and the integrity of the NBS specimen chain of custody processes. APHL Public Health Emergency funding was used to support the Virtual Site Visit model development.
Methods: A Virtual Site Visit quality improvement plan and detailed timelines were developed in January 2021 that outlined the overall project plan, including the creation of tools for the Virtual Site Visit and an evaluation plan. First, a readiness survey was designed and conducted to measure facilities' ability to participate in a proposed Virtual Site Visit. A specimen tracer tool was created to measure regulatory compliance and to document the NBS specimen chain of custody. Key areas included: specimen collection processes including handling and shipment, documentation of specimens not collected, specimen tracking, and facility changes due to the COVID-19 pandemic. A pre and post Virtual Site Visit questionnaire was designed for facility completion, in addition to a post site visit evaluation for ASC completion. The current plan is for ASCs to conduct a pilot of six virtual site visits across the State by June 30, 2021. The final project evaluation will be completed by July 2021.
Conclusion: Evidence supports the use of a Virtual Site Visit model to continue essential California ASC NBS education and quality monitoring at birthing facilities. The ASC-administered Virtual Site Visit can improve the NBS program by assessing birth facility process performance indicators. Despite not being able to conduct in-person site visits due to the COVID-19 pandemic, the Virtual Site Visit will allow ASCs to identify processes that need further improvements at individual birthing facilities, and to provide the additional support and improved education in a virtual format that would otherwise not occur.

Presenter: Ressie Jane Ascueta, The Lundquist Institute at Harbor UCLA Medical Center, Email: ressie.ascueta@lundquist.org

Poster #61

Delaware Newborn Screen Program and Nemours Cystic Fibrosis Center: Quality Improvement in Abnormal Newborn Screens

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Background: Newborn Screening (NBS) for Cystic Fibrosis (CF) accounts for the majority of new diagnoses since mandated by all 50 states by 2010. CF NBS measures immunoreactive trypsinogen (IRT) as the first indicator of positive screening. When elevated, a second tier of testing is triggered. Subsequent utilization of DNA testing improves sensitivity and specificity of diagnosing CF patients (IRT/DNA). The goal of CF NBS is to achieve early CF diagnosis so that comprehensive medical and psychosocial therapies can be implemented in infants prior to the onset of clinical symptoms to improve disease outcomes. Thus, infants require diagnostic testing by < 4 weeks of age.
Objective: The Delaware (DE) NBS Program transitioned from IRT/IRT/DNA to IRT/DNA testing strategy in January 2018. DNA testing has always included a 39+4 CFTR mutation panel. The DE NBS program partnered with the CF team at Nemours/A.I. duPont Hospital for Children to develop a reliable method to notify them promptly of abnormal results and notify the primary care doctors so the infants could be scheduled for confirmatory sweat testing and a clinic visit to review results.
Methods: In this quality improvement initiative, the medical records of infants with abnormal CF NBS referred by the DE NBS Program to the CF clinic were reviewed from January 2017 – December 2020. Charts were reviewed for: infant date of birth, age at referral to schedule diagnostic sweat chloride testing (ST), family contact and age at ST completion with discussion of results. A t-test was used to compare data obtained in 2017 compared to 2018-2020, with a $p \leq 0.05$ considered significant.
Results: The number of infants referred increased from 16 in 2017 to 64 in 2020. Since these changes the average age at completion of the CF NBS improved from 7.6 ± 5 days in 2017 to 2 ± 3 days in 2018 and was maintained at 1.4 ± 1 day through 2020. The average age an infant was identified and referred for diagnostic testing decreased from 21.4 ± 11.3 days in 2017 to 12.4 ± 13.8 days in 2018 ($p=0.02$) and maintained at 11.3 ±13.9 days in 2020 ($p=0.009$). The percentage of caregivers contacted to schedule diagnostic testing when the infant was less than 21 days of age improved from 37.5% in 2017 to 86.8% in 2018 and 92.2% in 2020. The percentage of infants with successful sweat chloride testing completed at less than one month of age improved from 57.1% in 2017 to 62.2% in 2018 and 72.1% in 2020.
Conclusions: Transition from an IRT/IRT/DNA to IRT/DNA CF NBS strategy improves time to referral for diagnostic ST. Close collaboration between the state NBS program and the CF clinics helps improve communication with families to schedule and complete diagnostic ST in a timely manner.

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Poster #62

Prioritizing Newborn Screening (NBS) Education: Engaging Hospitals to Improve NBS Quality and Reduce Unsatisfactory Rates in Newborn Screen Blood Specimens

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In Georgia, the average unsatisfactory specimen rate from 2014 to 2019 was 3.42%, meaning that out of the average number of specimens collected (147,688) annually during that period, approximately 5,051 specimens were deemed unsuitable for testing by the State Lab. Recognizing that quality issues in specimen collection and documentation contributed to this high unsatisfactory rate and potentially delayed adequate screening, confirmatory testing, and linkage to necessary specialist care, the Georgia Newborn Screening (NBS) Program designed and implemented a quality improvement (QI) project from December 2020 to May 2021, supported by funding from the Association of Public Health Laboratories. In this project, the Program sought to assess whether an increase in the dissemination and implementation of educational tools and webinars reduced the number of unsatisfactory specimens received by the State Lab, thus improving the quality of NBS specimens in the state. Project methodologies included recruiting a cohort of 13 birthing facilities and hospitals across the state with either unsatisfactory specimen rates above the Program's historical target of 1% or high numbers of specimens delayed in transit greater than 5 days. Those selected hospitals were required to designate NBS Champions who were tasked with attending monthly conference calls with the Program, accessing the NBS state database on a monthly basis to monitor their respective facility's NBS performance data and complete monthly data forms, and working with their facility staff to implement strategies for ameliorating NBS quality at their respective facility. Other project methodologies included implementing a series of monthly educational webinars open to all hospital staff and distributing QI educational tools, such as visual pocket aids that illustrated proper specimen collection (i.e., "badge buddies"), to cohort facilities. As of March 2021, the Georgia NBS Program has seen improvements in specimen collection quality, as reflected in the February 2021 average state unsatisfactory rate of 2.23%, compared to the February 2020 average state unsatisfactory rate of 3.65%. This improvement in NBS quality can be attributed to actions taken at the facility level. For example, by analyzing NBS data, NBS Champions identified NBS quality errors and performed targeted education to staff exhibiting issues in collection. In conclusion, although the average unsatisfactory specimen rate for the state remains higher than the Program's historical target of 1% when considering February 2021 data, the Georgia Newborn Screening Program has seen improvements from several hospitals within the cohort. By continuing to share educational resources with healthcare staff who perform newborn screening, the Program aims to achieve further reductions in the number of unsatisfactory specimens received by the State Lab.

Presenter: Kristina Parkins, Georgia Department of Public Health, Email: kristina.parkins@dph.ga.gov

Poster #63

Enzyme Activity Stability in Stored Dried Blood Spots for Various Lysosomal Storage Disorders

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Background /Objective: The newborn screening (NBS) of Lysosomal Storage Disorders (LSD) is becoming more common in screening laboratories due to the additions of Pompe and Mucopolysaccharidosis type I (MPS I), also known as Hurler Syndrome, to the Recommend Uniform Screening Panel (RUSP). In addition to these disorders, various state labs are also screening for Fabry and Gaucher disease. The screening of these specimens is done by measuring the lysosomal enzyme activities of specific markers for the LSD disorders. This has typically been achieved by using one of two testing platforms. The first uses tandem mass spectrometry (LC-MS/MS). The second, with which this study was carried out, uses Digital Microfluidics Fluorometry (DMF). These methods both utilize the standard matrix for NBS blood collection by way of an FDA-cleared dried blood spot (DBS) specimen collection card. Depending on each state's regulations and protocols, NBS programs across the country vary greatly on how long and at what temperature they store their residual (leftover) DBS's. It is helpful to know how these protocols effect the results for each new disorder that is added to a state's panel so that the stored specimens can be successfully utilized for continuous quality assurance (QA), quality control (QC), quality improvement (QI) and method development for ongoing screening of the disorders.
Methods / Results: In this study, we tested the stability of these LSD enzyme activities over the course of a year while blood spots were stored at -20 degrees C, which is the recommended temperature in the CLSI Standards NBS01-A6 for long-term freezer storage for DBS's. Previous research has showed decreased enzyme activity when stored at refrigerator or room temperature. The decreased enzyme activity would occur in as little as 10 days of cool storage. Previous studies also showed stable activity when stored at -80 C.
Conclusions and/or Implications: This study has shown that DBS's can be stored for up to a year with little to no degradation of the four LSD enzyme activities that we measured when stored in a -20 C freezer. This provides a significant advantage in terms of ongoing QA/QC/QI and for the verification new reagents, testing platforms and/or testing methodologies.

Presenter: Casey Guccione, Kansas Department of Health and Environment Laboratory, Email: casey.m.guccione@ks.gov

Poster #64

Positive impacts of updated quality assurance activities and training on Critical Congenital Heart Disease (CCHD) newborn screening outcomes in Minnesota

J. Laine and R. Marino, Minnesota Department of Health, St. Paul, MN

Minnesota's Newborn Screening program worked to redesign quality assurance practices, improve adherence to CCHD screening protocols, and develop a quality report for all CCHD screeners. We began a new process of consistently monitoring quality reports in Spring 2020, which led to direct contact to Minnesota CCHD screeners every six weeks via email. Through these direct communications we provided improvement feedback about missing or outstanding CCHD screening results and protocol adherence. While reviewing previous metrics for Minnesota babies born in 2018 and 2019, we found that with our new quality assurance practices implemented in Spring 2020 that there was a 27.2% in 2018 and 34.7% in 2019 reduction in patients with missing or not screened CCHD results. After reviewing our 2018 and 2019 CCHD screening data, we found that consistent data quality review and additional education was needed to ensure proper adherence to protocols. This allowed for training opportunities and increased communication with our hospital and out of hospital screening provider partners.

This increase in communication assisted with resolving technical issues, and allowed for targeted virtual training. CCHD quality assurance efforts have led to foundational relationships with both external and internal partners, including our internal birth defects and newborn screening teams. Additionally, we were able to provide birth screening providers, who met our criteria of a specific birth rate, with their first CCHD quality report in the Spring of 2021 detailing measures such as completed CCHD screenings, missing results, and overall protocol adherence for 2020. The reports provided a specific screening provider or hospital's results and protocol adherence as compared to other similar providers. Through refining our processes, we made ongoing adjustments to the monthly quality assurance reports and determined appropriate frequency of contact to CCHD screeners. Several small changes were made throughout the revision process to ensure accurate data was being shared and to offer screening provider specific training. We hope to continue to refine our CCHD newborn screening quality assurance practices and continue to strengthen our relationships with CCHD birth screening providers. Our goal is to do this through continued education, quality reports, training, and consistent, timely, and helpful communication.

Presenter: Jenna Laine, Minnesota Department of Health, Email: Jenna.Laine@state.mn.us

Poster #65

Development of a Comprehensive Inventory System Optimised for Newborn Screening and Genetic Diagnostic Laboratories

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The management and upkeep of a medical laboratory's supplies and reagent inventory is key to ensuring smooth and continuous operations of the laboratory. Why then, is inventory management so often seen as drudge work and a task that most labs attempt to maintain using spreadsheets or whiteboards? At the Children's Hospital of Eastern Ontario (CHEO), two laboratories; Newborn Screening Ontario (NSO) and Genetics Diagnostic Laboratory (GDL) set out to find a solution. CHEO'S GDL and NSO worked together to implement similar comprehensive systems that would efficiently manage inventory in both labs using as few manual processes as possible.

Historically, NSO's laboratories have experienced a number of preventable shortages of consumables due to the lack of a proper inventory management system. CHEO's GDL has experienced cost-related issues due to staff inadvertently over-purchasing stock without realizing an order was already in progress. In addition, the separation of procurement and laboratory departments in a large organization like CHEO results in siloed workflows, lacking in communication. In all cases, inventory-related issues present risks, which have been amplified by the COVID-19 pandemic. Without supplies, labs may need to put testing on hold which could result in a delayed critical result.

The solution to these issues was an electronic inventory management system, based in Microsoft Access, and conforming to both CHEO's procurement and operational workflows and ISO/IQMH (Institute for Quality Management in Healthcare) requirements. NSO benefitted from CHEO GDL's previous work in the initial design and contributed to further development and the expansion of the system. Today, the system is used across both laboratories to track supplies and reagents, solutions prepared in-house, and orders from the purchase requisition stage to delivery. Staff are now automatically notified of expiring products, low stock, and orders that have been placed and not yet received. More recently, the system was expanded to help manage the storage of screen positive samples and specimens for NSO's newly created biobank for metabolic research. Future uses include equipment inventory and timely triggers for the renewal of maintenance service agreements.
With the implementation of a comprehensive inventory management system, the practice of keeping an accurate inventory of supplies and reagents is no longer considered a time-consuming chore. Our labs have shifted from a reactive model of inventory management to a proactive model. They are now alerted when supplies are running low, they can track outstanding orders, and place new orders in a timely manner. In times like these when global supply chains of scientific and medical supplies are in jeopardy, it has never been more important to keep an accurate inventory to ensure continuity of Newborn Screening and related laboratory operations.

Presenter: Philippe Morin, Newborn Screening Ontario, Canada, Email: pmorin@cheo.on.ca

Poster #66

Implementation of a Sunday Courier Service for Newborn Screening Samples

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Newborn screening (NBS) quality improvement (QI) plays an integral role in the South Carolina (SC) newborn screening program. The primary focus of NBS QI is to improve the quality of blood spots collected and reduce the turn-around time for blood spot specimens from SC birthing hospitals and pediatrician offices to the SC Public Health Laboratory (PHL). To achieve these goals, NBS QI staff regularly train hospital staff on specimen collection, packaging, and shipping with the goal of getting specimens to the PHL as quickly as possible to improve health outcomes for SC babies.

In 2019 the SC PHL NBS Program received funding from the Association of Public Health Laboratories (APHL) for a Continuous Quality Improvement (CQI) grant to improve turn-around times for NBS specimens. The primary goal for the funding was to improve weekend specimen delivery from SC birthing hospitals to the PHL. This was accomplished by implementing two separate transit services. One of these services was to have all SC birthing hospitals send Friday specimens using priority overnight shipping for Saturday morning delivery from the weekday commercial courier. The second service was implementing a Sunday courier pick up of specimens for delivery early Monday morning to the PHL. To facilitate the onboarding of a Sunday courier service, a field coordinator was hired to implement and manage the Sunday courier service and assist NBS QI staff with education and outreach of hospitals on the importance of collecting satisfactory specimens and shipping specimens promptly after drying.

In September 2020, the Sunday courier service started with a total number of 36 out of 38 birthing hospitals participating. To date, the Sunday courier service has improved turn-around times by an average of 12% across all SC birthing hospitals. Additionally, 90% of SC hospitals are seeing at least a 5% decrease in turn-around time. The efficiency and success of the Sunday courier service has resulted in the solicitation of a 6-day per week courier with a target start date of Spring, 2022.

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Screening for Hemoglobinopathies

Poster #67

Iowa Newborn Screening Program experience with hemoglobinopathy screening over the last 20 years

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The Iowa Newborn Screening (NBS) Program has been in place since 1966 to screen all babies born in Iowa for inherited diseases. Hemoglobinopathy screening was implemented in Iowa since 1989 and its focus is to diagnose sickle cell disease (SCD). Currently, all 50 states and the District of Columbia perform newborn screening for SCD as recommended by the American College of Medical Genetics (ACMG) and endorsed by the March of Dimes, given the positive public health impacts.

In the recent years, immigration to the United States has increased and as a result, newborn screening programs are identifying non-sickling hemoglobinopathies which are more prevalent in Asian populations, such as alpha and beta thalassemia. Certain subgroups of these non-sickling hemoglobinopathies lead to non-transfusion dependent thalassemia (NTDT), such as Hemoglobin H disease (HbH), beta-thalassemia intermedia and Hemoglobin E/beta thalassemia (HbEβ thalassemia). Although NTDT do not require regular blood transfusions for their daily function, it is increasingly recognized that this entity leads to significant morbidities such as growth retardation, pulmonary hypertension, gallstones, leg ulcers, splenomegaly, liver disease, thromboembolic events, extramedullary hematopoiesis and iron overload state.

This is a retrospective, observational, quality improvement study which aims to evaluate all newborn screens and determine the prevalence of hemoglobinopathies detected by the State Hygienic Laboratory for the state of Iowa from 1st January 2010 to 31st December 2020. Genotype-phenotype correlation of patients who were referred by the short-term follow-up NBS team to the respective pediatric hematology-oncology units was collated to evaluate the efficacy of the workflow and to determine the feasibility of a standardized long-term follow up process for patients with molecularly determined hemoglobinopathies.

Our results show a prevalence of 151 positive newborn screens for sickle cell disease and 179 positive newborn screens for non-sickling disease detected through the Iowa NBS program during this period. There is an increasing trend of SCD since 2017 which may be due to a recent increase in migrants from West Africa. Molecular testing was performed for 49.50% of patients with non-sickling disease and showed a variety of alpha and beta globin mutations, such as beta-thalassemia major, homozygous E, deletional and non-deletional Hb H disease. Compound heterozygotes for both alpha and beta globin mutations were confirmed through molecular testing.

Based on these results, we conclude that the Iowa NBS Program plays a significant role in screening and confirming the diagnosis of sickling and non-sickling hemoglobinopathy in the United States. As migration trends shift over time, public health programs need to continuously monitor these changes to determine the health needs of the individuals we serve.

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Screening of Special Populations

Poster #68

Analytical performance evaluation of NeoBase 2 and MassChrom MS/MS kits for high-throughput flow injection analysis-tandem mass spectrometry on the RenataDX Screening System in a routine newborn screening laboratory

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The introduction of Tandem Mass Spectrometry (MS/MS) to clinical laboratories and the advent of expanded Newborn Screening (NBS) has been one of the major changes to public health programs worldwide. Speed, robustness, accuracy, selectivity and specificity of analysis are all requirements of expanded NBS. These test attributes are needed to minimize the risk of false positive results, to possibly eliminate false negative results and to improve the positive predictive value (PPV) of NBS. In this study, we evaluated the RenataDX Screening System, a fully integrated flow-injection MS/MS (FIA-MS/MS) IVD system for high-throughput dried blood spot (DBS) analysis in a routine NBS laboratory. A choice of several commercial MS/MS NBS kits is available, and we sought to compare two such kits on the RenataDX platform. We evaluated precision, accuracy and carry-over for NeoBaseTM 2 (PerkinElmer®) and MassChrom® (Chromsystems) non-derivatized kits. Moreover, we also compared clinical measurements from the two different sample preparation approaches by analyzing greater than 500 newborn DBS samples extracted with the two kits. Precision and accuracy were evaluated using QC materials provided by each kit. Precision established for NeoBase™ 2 and MassChrom® kits, respectively, were directly compared based on the lower %CV per analyte for each QC level. In particular, intra- and inter-day %CV values were found to be less than 15% for all QC levels using either kit. Moreover, the accuracy of the two methods was also compared by highlighting the lower %bias per analyte at each QC level. Again, with the exception of some acylcarnitine species, the calculated % bias showed good accuracy for both the methods (%bias < 15% for all QC levels). In order to establish the best solution to minimize cross-contamination during FIA-MS/MS, the carryover values obtained by MassChrom® and NeoBase™ 2 kits were compared. All carryover values were found to be less than 0.4 % for all analytes using either kit, but MassChrom® was associated with less carryover. To verify the degree of correlation between data obtained by NeoBase™ 2 and MassChrom® kits, a correlation analysis was provided by Passing-Bablok regression on data derived by FIA-MS/MS of 543 neonatal DBS samples. The use of NeoBase™ 2 and MassChrom® kits in NBS analysis by RenataDX suggests that the two methods are in agreement with one another, having clinically insignificant differences that did not impact the screening result. In conclusion, both kits were deemed suitable for routine MS/MS analysis of newborn DBS. While the NeoBase™ 2 kit has an easier and faster sample preparation, the MassChrom® kit provides a cleaner sample extract which empirically should improve instrument reliability.

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Poster #69

Sulfatide Analysis for Screening of Metachromatic Leukodystrophy in Dried Blood Spots on a PerkinElmer QSight® 225MD Mass Spectrometer

J. Trometer and T. Au Yeung, PerkinElmer, Waltham, MA

Arylsulfatase A, or cerebroside-sulfatase, is an enzyme that breaks down sulfatides, namely cerebroside 3-sulfate into cerebroside and sulfate. In humans, arylsulfatase A is encoded by the ARSA gene. Metachromatic Leukodystrophy (MLD) is an autosomal recessive disorder caused by the deficiency of Arylsulfatase A (ARSA). Direct measurement of ARSA activity in Newborn Screening (NBS) is made difficult by the relative instability of the ARSA enzyme in dried blood spots (DBS). Sulfatides have been found to be natural substrates for the ARSA enzyme and levels present in MLD patients are significantly increased when compared healthy individuals. C16:0 Sulfatide is among a group of substrates for ARSA enzyme that can be detected in blood. Endogenous levels of C16:0 Sulfatide in blood can be elevated making the development of DBS controls for NBS difficult. This work demonstrates the development of novel DBS to mitigate the difficulty for the use of DBS in newborn screening. Using a single 3.2 mm dried blood spot punch and incubation cocktail, followed by a short incubation time our assay has the ability to identify samples with low concentrations of C16:0 Sulfatide. Sample-to-sample time using a PerkinElmer QSight® 225MD MS/MS analysis is less than 2 minutes allowing this to be used in a high-throughput screening program.

Presenter: Joe Trometer, PerkinElmer, Email: Trometjd@perkinelmer.com

Poster 70

An Eight-plex assay to measure I2S, SGSH, NAGLU, GALNS, GLB1, ARSB, GUSB, and TPP1 enzyme activities in DBS on a PerkinElmer QSight® 225 MD Mass Spectrometer

T. Au Yeung, M. Timmons, M. Lancaster and J. Trometer, PerkinElmer, Waltham, MA

The mucopolysaccharisodes (MPS) family of lysosomal storage disorders (LSDs) is caused by defects in the metabolic breakdown of glycosaminoglycans (GAGs). This work demonstrates a novel MS/MS assay that simultaneously monitors the activity of eight different disorders. Using a single 3.2 mm dried blood spot (DBS) punch and incubation cocktail, our assay has the ability to identify samples with low enzyme activities for I2S (MPS II), SGSH (MPS IIIA), NAGLU (MPS IIIB), GALNS (MPS IVA), GLB1 (MPS IVB), ARSB (MPS VI), GUSB (MPS VII), and TPP1 (CLN2). The eight-plex is incubated overnight in the presence of incubation cocktail at 37°C followed by a derivatization step required for MPS IIIA and a fully automated workup. Enzyme activities are measured by LC-MS/MS on a PerkinElmer QSight® 225 MD triple-quad Mass Spectrometer. Sample-to-sample time using MS/MS analysis can be as low as 2 minutes, which allows the possibility to measure up to 3600 results per day on a single instrument.
Method performance studies show good linearity for each enzyme in their respective activity range. Furthermore, a study consisting of several hundred presumed healthy neonates, confirmed low I2S, SGSH, NAGLU, GALNS, GLB1, ARSB, GUSB, and/or TPP1 activity and CDC control DBS showed excellent resolution and clear distinctions between the different enzyme activity levels.

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Poster #71

Who is refusing dried blood spot newborn screening in Iowa and why?

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Iowa state law requires that all newborns undergo dried blood spot newborn screening unless parents/guardians refuse. Parents/guardians have the right to refuse for any reason. Birth providers are required to complete and return a refusal form anytime the dried blood spot newborn screen is refused. Refusal forms are returned to the newborn screening program and matched to vital record data. This birth match process allows for tracking of refusal rates and assists in follow up of instances where an infant's dried blood spot newborn screen was not received by the State Hygienic Laboratory at The University of Iowa.

On average, 0.286% of parents/guardians in Iowa opted out of newborn screening over the last three years. Although this is a small percentage, this translates to over 200 infants who could potentially have an undiagnosed newborn screen condition. In 2018-2020, 64% of refusals were community-based births. Eleven different midwives in the state of Iowa participated in these deliveries.

Seventy-six percent of refusal forms state a reason for refusal. The reasons listed include a variety of responses such as cost, lack of family history of newborn screening conditions, and religion. Some families who initially refuse newborn screening change their minds and go on to have their infant screened. When the newborn screening program receives a signed refusal form, the infant's primary care provider is contacted notifying them of the refusal and educational materials are provided. The newborn screening program also mails parents/guardians a letter explaining they can still have their child screened if they desire. Newborn screening educational materials are provided to families as well as contact numbers for further questions. Over the last three years, 25.7% of families who signed a refusal form go on to have their child screened.

Iowa's current process for birth matching facilitates tracking of refusal rates and reasons for refusal. Knowing the reasons and provider type for the majority of the refusals allows the Iowa Newborn Screening Program to provide targeted interventions and educational materials that speak to concerns expressed by Iowa families. The process of providing parents/guardians with newborn screening education at the time of refusal assists them in making informed decisions.

Presenter: Emily Phillips, University of Iowa, Email: emily-phillips@uiowa.edu

Short-term and Long-term Follow-up

Poster #72

The Current Landscape of Phenylketonuria in Greece through the eyes of the National Neonatal Screening Program

D. PlatisA. Skouma, E. Petropoulou and P. Girgnoudis, Institute of Child Health, Athens, Greece

Phenylketonuria Neonatal Screening was the first screening program initiated in the 60's and it based on the fortuitous realization that phenylalanine (Phe) – restricted diet prevents serious neurological and physiological problems if the disorder is diagnosed early on. In Greece, PKU screening was initiated in 1974 with the voluntary participation of the national and private maternity units and has been entrusted since then to the Institute of Child Health. The program is being run by a single laboratory, the Department of Neonatal Screening, which tests the dried blood spot cards from all neonates born in maternity units in Greece (over 80,000 neonates/annum). Over the last 45 years, more than 4,000,000 neonates have been screened and on average 1 in 5-10,000 neonates will present with some form phenylalanine disorder (Phenylketonuria or Hyperphenylalaninemia). Since the initiation of the Screening Program, metabolic disease experts, nutritionists, lab technicians, biologists and psychologists at the Institute of Child Health, have been responsible for treatment initiation and clinical and laboratory follow-up of these patients. Since, 2018 the Hellenic Neonatal Screening Program has been upgraded in multiple ways in an effort to improve services and provide more effective screening procedures. This effort has allowed us to design a more efficient framework for the screening, analysis and follow up of Phenylketonuria patients, as well as, the accompanying data. In this report, we present an overview of these upgrades as well as our experience over the last 10 years regarding the management of Phenylketonuria in Greece.

Presenter: Dimitrios Platis, Institute of Child Health, Greece, Email: dimitris_platis@hotmail.com

Poster #73

Remote access to Electronic Health Records of Minnesota health systems for chart abstraction: A Minnesota newborn screening follow-up project

J. Laine and J. Hauser, Minnesota Department of Health, St. Paul, MN

In early 2020 and amidst the start of the COVID-19 pandemic, the Minnesota Newborn Screening program embarked on an effort to gain remote access to the Electronic Health Records (EHR) of several Minnesota health systems for the purpose of improving the efficiency and quality of newborn screening (NBS) follow-up. Through collaborations across several areas within the Minnesota Department of Health, we learned from past remote EHR access request experiences. We used that information to inform planning of our remote EHR access project and sent a letter of introduction to several health systems regarding our follow-up practices and how HIPAA interacts with Minnesota statutes and allows for the ability of EHR access for state newborn screening follow-up purposes.

Out of the 12 health systems to which we sent initial letters requesting remote EHR access, we heard back from all of them. To date, out of the 12 we originally requested access from, we are able to remotely access the EHR for 6 Minnesota health systems. We are currently in the active review process for 4 additional health systems, and only two health systems have denied our remote access request. We will also share additional methods of documentation, tracking, and consistent communication to the health systems that are still in the process of granting us remote access. We will discuss the initial barriers in response and agreement from health systems due to the COVID-19 pandemic and the lack of staffing resources to support or assist with our request. With many of these health systems we made direct calls and arranged meetings with their security, privacy, legal, and medical records departments to describe our practices and how our goal of having remote access to their system's EHR could improve NBS follow-up practices.

We made several changes to our contact practices and methods throughout this past year and will share ongoing communication challenges and lessons learned. We will also share our future goals for this project, including continuing to gain remote EHR access to additional Minnesota health systems, border state health systems, and developing tracking mechanisms for improved internal workflow efficiencies and timeliness of follow-up. Finally, we will share our goals for using remote EHR access to improve public health newborn screening longitudinal follow-up of infants and children identified with conditions through Minnesota newborn screening.

Presenter: Jenna Laine, Minnesota Department of Health, Email: Jenna.Laine@state.mn.us

Poster #74

Regional differences in lost to follow-up cases during COVID-19 pandemic in New York State

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New York State (NYS) has one of the highest COVID-19 case counts in the nation, and was particularly hard hit in Spring 2020, during the early days of the pandemic in the United States. On March 22, 2020, Governor Andrew Cuomo ordered the implementation of the "NY Pause," effectively locking down the entire state, although there were significant regional differences in COVID case infections during that first wave. The NYS Newborn Screening Program (NBSP) continued to function throughout this time, but many birth hospitals around the state faced significant disruptions in services and staffing, with some closing their birth facilities to free up beds for COVID patients. In addition, many families refused to have repeat screens or follow-up labs drawn. This created substantial challenges when following screen positive and unsuitable cases, and in response NBSP Follow-up staff created an additional lost to follow-up diagnosis code to distinguish these cases. To better understand the impact of the COVID-19 pandemic on follow-up in NYS, the NBSP compared data from borderline and/or unsuitable cases that required either a repeat newborn screen or independent testing from the 6-month period immediately prior to the pandemic, to the 5 initial months of the pandemic and looked for regional differences. The statistical significance of differences in lost to follow-up rates pre-COVID and during COVID were evaluated using Chi Square analysis. From June to the end of November 2019, 9,631 babies required follow-up due to a borderline or unsuitable sample; from December 2019 to early May 2020, 8,776 babies required follow-up. Of the pre-COVID cases, 2.8% (N = 267) were eventually closed as lost to follow-up, whereas 6.0% (N = 530) were closed lost to follow-up during the first months of the pandemic; this was a statistically significant difference ($p < .00001$). Of the ten geographic regions that make up NYS, all experienced increased rates of lost to follow-up cases during the first few months of the pandemic, but statistically significant differences in rates were seen in the Capital Region ($p = .023553$), Long Island ($p < .00001$), Mid-Hudson ($p = .000113$), New York City ($p < .00001$) and Western NY ($p = .002697$). New York City and its surrounding locations (Long Island and Mid-Hudson) were most impacted by the first wave of the pandemic in NYS and as a result, NBS stakeholders in those regions faced the most disruptions. For maximum effectiveness and reach, the newborn screening system relies on close partnerships with birth hospitals, pediatric offices, midwives, and parents. Although the NBSP remained operational throughout the pandemic, these data illustrate the profound impact COVID-19 had on entities critical to the success of this public health program.

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Poster #75

Arkansas Newborn Screening Long-Term Follow-up Database Study – ANGELS Newborn Screening 2019 Annual Report

J.A. Bolick[1], J. Nick[2], K. Heard[1], B. Spray[2], D. Dubois[1], G. Schaefer[1]; [1]University of Arkansas for Medical Sciences/Arkansas Children's Hospital, Little Rock, AR, [2]Arkansas Children's Research Institute, Little Rock, AR

Problem/Objectives: Every year approximately 38,000 Arkansas newborns receive a newborn screen for 31 conditions (includes hearing loss and Critical Congenital Heart Disease); approximately 100 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.

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 Arkansas Children's Hospital (ACH) and its Research Institute (ACRI). The funding for the Database Study was provided by 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 Database was developed using REDCap (Research Electronic Data Capture) hosted by the UAMS Translational Research Institute (NCRR/NIH 1 UL1 RR02988).

Significant Results: After receiving Institutional Review Board approval 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 2019 marked the eighth full year of implementation of the Database. The 2019 Annual Report provides a summary of the data gathered between 01-01-2019 and 12-31-2019 along with some cumulative data. Calendar Year 2019 marked the second full year of collecting data on kept/missed clinic visits to the ACH primary specialist. With 120 new subjects/cases in 2019, the Database contained 1,212 subjects representing 1,219 NBS cases at the end of 2019.

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

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Training/Education/Communication

Poster #76

Benefits and Challenges of the Family Contact Call Initiative in the Iowa Newborn Screening Program
G. Younger, University of Iowa, Iowa City, IA

Newborn screening for phenylketonuria in Iowa was first recommended in 1965, and became available to all infants in 1981. Over time, this public health program has expanded to include many more conditions and has been described as one of the ten greatest public health achievements in the United States by facilitating earlier life-saving treatment and intervention for newborns with certain genetic and endocrine disorders. In 2020, Iowa newborns were screened for over 50 genetic and congenital conditions including congenital adrenal hyperplasia, congenital hypothyroidism, hemoglobinopathies, biotinidase deficiency, severe combined immune deficiency, cystic fibrosis, galactosemia, organic acidemias, fatty acid oxidation disorders, and amino acidemias. Despite the countless benefits of newborn screening, receiving news about an abnormal newborn screen remains stressful and anxiety-producing for many families. In several cases, there is a gap of time between a family's notification of the abnormal newborn screen result and their appointment with a specialist. The Family Contact Call (FCC) initiative was implemented by the Iowa Newborn Screening Program in September, 2020 as a way to improve family experiences with their child's presumptive positive newborn screen result. The FCC initiative connects families with a genetic counselor who can explain the newborn screening process, the child's abnormal result, answer parent/guardian questions, and review the child's next steps. We will present data from this initiative as well as discuss the challenges and perceived benefits of the FCC initiative. Based on the Iowa experience, families appreciate the timely contact, education, and support during this emotional time. Other newborn screening programs may want to consider implementing a similar technique to improve short-term follow-up for newborns with presumptive positive newborn screen results.

Presenter: Georgianne Younger, University of Iowa Children's Hospital, Email; georgianne-younger@uiowa.edu

Poster #77

KrabbeConnect Patient Journey and Resource Map

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Background: Krabbe disease (KD) is a devastating neurodegenerative genetic disease caused by pathogenic variants in the GALC gene.
A population of parents with children diagnosed with KD through newborn screening are seeking guidance about KD, next steps, monitoring, and (in infantile-onset cases) making rapid informed decisions about hematopoietic stem cell transplantation (HSCT).
The goal of this project was development of a patient-friendly KD roadmap designed to provide information, education, and direction to parents in collaboration with their healthcare providers.
Methods: KrabbeConnect and TOPCAT BIO collaborated to develop a graphic resource utilizing existing literature designed to address informational gaps identified by parents and caregivers of patients living with KD and other community stakeholders including healthcare providers and advocacy representatives.
This resource was then evaluated by a core group of parents, caregivers, and healthcare providers to determine utility and usefulness in addressing the existing informational gaps in KD.
Discussion: The deluge of information provided to parents upon the diagnosis of a genetic disorder such as KD can be overwhelming and make it difficult to prioritize decisions and next steps.
The development of a simple roadmap with easy-to-follow steps supports decision making and closes several of the identified informational gaps.
This resource helps families and the healthcare providers to develop individualized treatment and care plans.
Conclusions: KrabbeConnect has developed a user-friendly, patient journey map to help families and medical professionals understand decision points, next steps, and available resources to support them as they make timely informed decisions that are right for their loved one.

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Workforce Issues

Poster #78

Workflow Consistency in the Kansas Newborn Screening Laboratory during the COVID-19 Pandemic of 2020

M. Mills, Kansas Department of Health and Environment Laboratory, Topeka, KS

Background/Objective: The COVID-19 pandemic ushered in many changes in our lives both outside and inside of the laboratory. The State of Kansas Newborn Screening Laboratory worked diligently to keep the world inside of our lab as similar to pre-COVID-19 as possible. In February 2020, near the beginning of the pandemic, the Newborn Screening Program had started screening for Spinal Muscular Atrophy. In addition, we were at the beginning of our Lysosomal Storage Disorder pilot. Planning, preparing and implementation of the pilot were on the forefront of our minds. The NBS Unit was already wearing masks before the Governor's mandate. We knew if one person contracted COVID-19, it would likely be transmitted to the rest of staff since we are a small, compact lab. Several staff had children and elderly parents which elevated our concern for safety.

Methods/Results: In March 2020, we altered processing to make room for lab staff to be 6 feet apart. We added office cubicles as dividers in processing to separate logging in and labeling of specimens. Screening was moved 6 feet away from its original location. Offices were reduced from 3 analysts to 2 analysts per office. Maintaining consistency when events outside of our control occur was an important part of keeping up morale. We kept the environment within the lab as normal as possible and proceeded with Newborn Screening without too many changes. We discussed implementing our Continuity of Operations Planning (COOP) throughout the thickest of the pandemic and decided to hold firm with minimizing changes. Due to our lab being on the smaller side, with approximately 40,000 specimens per annum, we have always been able to cross-train staff. Having the flexibility of staff knowledgeable on multiple benches kept absences covered, whether due to illness, vacation, or staying home with children. We were also lucky more than half of our staff were experienced analysts and trained on every bench. We were not subject to a hiring freeze as other states were suffering. We were even able to bring in an extra analyst to assist with laboratory operations to keep up our momentum in daily processing and continuing to work towards bringing on new conditions. We not only did not have a shortage, we had an overage which assisted with boosting employee morale. We were impacted by the pandemic, with 3 staff being out multiple weeks with COVID-19.

Conclusions/Implications: The result of our effort was stability for the Kansas Newborn Screening Laboratory through the 2020 COVID-19 pandemic without implementing COOP, being able to pilot new conditions and being able to continue screening babies for genetic conditions. Our conclusion is disrupting workflow to make exception for whatever may be going on outside of the laboratory walls should be minimized, if possible, to keep the workflow consistent, to keep up employee morale, and to continue to move forward with state Newborn Screening program goals.

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