P-01

Implementation of a Single-Wash, In-Situ, Real-time PCR Screening Procedure for Severe Combined Immunodeficiency in Oklahoma
T. McCallister, R. Thompson and S.T. Dunn, Oklahoma State Department of Health, Oklahoma City, OK

Abstract

Several methods for screening of newborns for Severe Combined Immunodeficiency (SCID) have been documented in the literature, which involve the quantitative assessment of T-cell receptor excision circles (TRECs), as a biomarker of naïve T-cell production. Most laboratories currently screening for SCID perform a DNA extraction from filter paper punches prior to real-time PCR amplification of TRECs; however, recently methods for in-situ amplification of DNA, without prior extraction, have been developed by the Newborn Screening and Molecular Biology Branch of the CDC. In collaboration with the CDC, the Oklahoma Newborn Screening Laboratory is validating a screening method for SCID that employs a single, 15-minute wash of filter paper punches, without DNA extraction, prior to real-time PCR for quantitative measurement of TRECs. Blood spot punches are washed in 96-well plates for 15 minutes with continuous shaking using Generation DNA Elution Solution 2 (Qiagen). The wash solution is then aspirated and PCR master mix is added directly to the wells containing the punches. The samples are amplified by real-time PCR using a Stratagene Mx3005P qPCR system. Analysis includes measurement of TRECs and a control gene (human RNase P protein p30, RPP30). Comparable results were obtained using this method relative to an earlier version of this in-situ real-time PCR procedure, which included two separate washes using Generation DNA Purification Solution 1 (Qiagen) followed by DNA Elution Solution 2. This one-step wash, no DNA-extraction, real-time PCR procedure offers significant benefits for the newborn screening laboratory in terms of time, throughput, and cost-savings as compared to other TREC detection methods and generates highly reproducible and robust results.

Presenter: Tonya McCallister, Oklahoma State Department of Health, Public Health Laboratory, Oklahoma, City OK, Phone: 405.271.5070, Email: tonyaj@health.ok.gov

Summary

Several methods for screening of newborns for Severe Combined Immunodeficiency (SCID) have been documented in the literature, which involve the quantitative assessment of T-cell receptor excision circles (TRECs) as a biomarker of naïve T-cell production. Recently, methods for in-situ amplification of DNA from dried blood spot (DBS) punches without prior extraction have been developed by the Newborn Screening and Molecular Biology Branch of the CDC. The initial method developed by CDC included 2 separate washes of 2 mm punches [using Generation DNA Purification Solution 1 (Qiagen) followed by DNA Elution Solution 2 (Qiagen)] prior to an in-situ quantitative PCR (qPCR) screening method to assess TREC copy number. Subsequently, preliminary observations on qPCR amplification performance using a single-wash step (with Generation DNA Elution Solution 2 only) indicated similar results to the 2-wash approach. Considering the potential for reduced handling, time, and expense by Proceedings of the 2014 APHL Newborn Screening and Genetic Testing Symposium, Anaheim, CA, October 27-30, 2014
using a single-wash approach as opposed to the 2-wash process, we embarked on a formal investigation of the single-wash method as part of an in-situ qPCR screening procedure for SCID. The CDC provided control material for use during validation. Two millimeter punches were obtained from control DBS representing Low, Medium and High levels of TREC, and were subjected to a 2-wash method or a single-wash method followed by in-situ qPCR using a Stratagene Mx3005P. The 3 levels of controls were run over the course of 5 days to obtain 20 values from each control. P values less than 0.05 were considered significant. Comparison of the two methods indicated no significant differences in values for the High and Medium level controls (High Control t = -1.04; p value = 0.30; Medium Control t = -0.38, p value = 0.71). By contrast, a small difference was observed in the values for the Low control between the two methods (t = -2.18, p value = 0.04). To explore PCR efficiency of the single-wash method, 2 mm punches containing one of 7 levels of TREC were run to obtain 20 values for each level. Analysis of these data indicated a qPCR efficiency of 98.9%.

Early in the validation of our SCID assay, we were informed by the manufacturer (Luminex) of the 2 mm puncher that we had been using for the assay that maintenance and repair support for the instrument would be no longer be provided. After consultation with CDC, we decided to explore the option of using a 1.5 mm DBS puncher (PerkinElmer). To investigate the performance of the 1.5 mm DBS as compared to 2 mm punches using the single-wash method, again 3 levels of TREC were subjected to TREC qPCR analysis over the course of 5 days to obtain 20 values for each level. Since the amount of DNA in a 1.5 mm DBS punch would be appreciably reduced from a 2 mm punch, we normalized TREC Cq values relative to those for RNaseP in order to compare the two data sets. Using t-tests to assume equal variances, no differences were observed in the variance of the Low (t = 1.21, p value = 0.23) and Medium (t = 1.59, p value = 0.12) TREC controls between the 2 mm and 1.5 mm punches; however, a significant difference was observed for the High TREC control (t = 2.22, p value = 0.03) where 2 mm punches produced slightly lower TREC and RNaseP Cq values. Efficiency of the qPCR using the 1.5 mm DBS was investigated as previously described, using 7 levels of TREC copy number over the course of several days to obtain 60 values for each level. qPCR efficiency using 1.5 mm punches was reduced (at 93.7%) from that of 2 mm punches but was still within the generally acceptable range of 85% to 115% (CLSI NBSo6-A). Currently, we have analyzed over 5,000 de-identified DBS in our NBS population and have successfully passed a Model Proficiency Evaluation Survey for TREC analysis. In conclusion, use of 1.5 mm DBS processed using a single-wash, in-situ qPCR method as part of TREC analysis is an efficient and cost-effective approach for newborn laboratory screening for SCID.

P-02

Pulse Oximetry Screening for Critical Congenital Heart Disease in Oklahoma: Collaboration between the Newborn Screening Program and the Oklahoma Birth Defects Registry

L. Caton, S. Vaz and D. Christie, Oklahoma State Department of Health, Oklahoma City, OK

Abstract

April 2013, House Bill 1347 was signed into law by Governor Fallin. The Oklahoma Board of Health approved rules to implement HB 1347 on January 14, 2014 requiring all birthing facilities to perform a pulse oximetry screen or an echocardiogram on every newborn prior to discharge to identify Critical Congenital Heart Disease (CCHD). In February 2014, the Oklahoma State Department of Health (OSDH)
notified all birthing facilities and providers of the new required screening and provided recommendations to implement pulse oximetry screening.

The Oklahoma Newborn Screening (NBS) Program and the Oklahoma Birth Defects Registry (OBDR) worked collaboratively to determine screening and follow-up recommendations, provide education to providers and birthing facilities, implement tracking for “failed” results, and monitor compliance related to pulse oximetry screening. Every infant is required to either have a pulse oximetry screen or pediatric echocardiogram performed prior to discharge. Birthing facilities report pulse oximetry screening results to the OSDH by utilizing either the newborn screening filter paper or faxing a Pulse Oximetry Result Form to the NBS program. The OBDR nurses actively review medical records for all infants who “fail” a pulse oximetry screen and who are identified with a heart defect either through screening or diagnostic testing. This collaborative process allows for quality assurance and surveillance of pulse oximetry screening in Oklahoma.

**Presenter:** Lisa Caton, BSN, Oklahoma State Department of Health, Newborn Screening Program, Oklahoma City, OK, Phone: 405.271.6617, Email: lisarc@health.ok.gov

**Summary**

Pulse Oximetry (Pulse Ox) Screening is point of caring testing to identify Critical Congenital Heart Disease (CCHD) in newborns. The screen is performed when the newborn is at least 24 hours old by placing pulse ox probes on the right hand and either foot and measuring the amount of oxygen in the newborn’s blood. A pulse ox is utilized because it is the earliest screen to detect potential structural heart abnormalities. Pulse ox screening targets seven primary and five secondary types of CCHD often associated with hypoxemia in newborns. These defects usually require early medical intervention, including surgery. Pulse ox screening may identify other conditions such as sepsis and pneumonia.

April 2013, House Bill 1347 was signed into law by Governor Fallin. HB 1347 outlined the following components: Every birthing facility is required to administer the pulse ox screening prior to discharge, a birthing facility is defined as an inpatient or ambulatory health care facility licensed by the State Department of Health that provides birthing and newborn care, and the State Board of Health is responsible for the development of rules necessary to care out the purpose of the act. The Oklahoma Board of Health approved rules to implement HB 1347 on January 14, 2014 requiring all birthing facilities to perform a pulse oximetry screen or an echocardiogram on every newborn prior to discharge to identify Critical Congenital Heart Disease (CCHD) and required facilities to report the result on the NBS filter paper or directly to the NBS program.

The NBS Program and the OBDR worked with multiple stakeholders including parents of children affected by a CCHD to develop a Pulse Oximetry Education brochure for families. The NBS Program and the OBDR also compiled resources to develop a recommended screening protocol, process of reporting results, documentation of screening refusal, and reasons the pulse oximetry may not be indicated. All birthing facilities and providers were informed of required screening and provided with resources developed.

to pulse oximetry screening. Birthing facilities report pulse oximetry screening results to the OSDH by utilizing either the newborn screening filter paper (pass, fail, or not performed) or faxing a Pulse Oximetry Result Form to the NBS program. The Oklahoma NBS Program and the OBDR worked collaboratively to track “failed” results and monitor compliance related to pulse oximetry screening. The OBDR nurses actively review medical records for all infants who “fail” a pulse oximetry screen and who are identified with a heart defect either through screening or diagnostic testing. This collaborative process allows for quality assurance and surveillance of pulse oximetry screening in Oklahoma.

Current outcomes include the development of the Recommended Pulse Oximetry Screening Protocol and the Pulse Oximetry Screening brochure, notification sent to every birthing hospital and provider to inform them of new required screening, pulse oximetry screen is being performed and reported on the majority of newborns. Issues encountered during implementation include: inappropriate or lack of follow up to the recommended screening protocol, inaccurate interpretation of screening results, and birthing facilities not reporting results if newborn receives the pulse oximetry screen after NBS filter paper was submitted. Future plans include the development of resources to connecting parent with mentors using the partnership of the Oklahoma Family Network (OFN), completion of specific cardiac fact sheets developed in conjunction with parents and stakeholders, online screening education and resources for all stakeholders, and the development of hospital reports.

P-03

Ten Years of MS/MS Screening at the Northwest Regional Newborn Screening Program [NWRNBS]
D. Sesser, S. Willis, S. Dennison, C. Hermerath and M. Skeels, Oregon State Public Health Laboratory, Hillsboro, OR

Abstract

Ten years of testing 1.625 million infants identified 1,174 infants with a disorder detectable by Tandem Mass Spectrometry [MS/MS]. 737 of them were CPT1 Arctic variant cases. 437 other cases were detected for an incidence of 1 case in 3,720 infants (excluding the CPT1A cases).

The NWRNBS has provided Newborn Screening, Follow-up and Medical Consultation for Alaska, Hawaii, Idaho, the Navajo Nation, Nevada, New Mexico, Oregon, Military Bases, Guam, Saipan, and Kwajalein Atoll in the Marshal Islands. Nevada is no longer part of the NWRNBS program.

We test 161,500 infants /year and 125,400 second specimens /year. We began Tandem Mass Spec [MS/MS] testing in October 2002. We are presenting the data on screening 1.625 million infants tested over a ten year period from July 1, 2004 thru June 30, 2014. See table.

Most of the disorders are found on the first specimens. Some disorders have been found only on the first specimens, such as VLCAD. Even in diagnosed cases of VLCAD the second blood spot sample has been normal for C14:1 and C14.

Some disorders we find mostly on the second specimens, such as Tyrosinemia Type 2 and Homocystinuria.
In ten years of testing we have no cases of: IBA Isobutyric Acidemia, MA Malonic Acidemia, GA2 Glutaric Acidemia Type 2, BKT Beta Ketothiolase, and 3MGA 3-Methylglutaconic Acidemia.

**Presenter:** David Sesser, Oregon State Public Health Laboratory, Newborn Screening, Hillsboro, OR
Phone: 503.693.4187, Email: David.e.sesser@state.or.us

**Summary**

The Northwest Newborn Screening Program (NWRNBS) began Newborn Screening in 1963. This year marks our 51st year of providing Newborn Screening service. The NWRNBS program began MS/MS (Tandem Mass Spectrometry) screening in October 2002. We have added several disorders and their analytes to our screening panel since then and now use 49 analytes and ratios to screen for 26 disorders by MS/MS. We will present ten years of data in this poster. From July 1, 2004 thru June 30, 2014 we tested 1,625,529 infants.

The NWRNBS has provided Newborn Screening, Follow-up and Medical Consultation for Alaska, Hawaii, Idaho, the Navajo Nation, Nevada, New Mexico, Oregon, Military bases, Guam, and Saipan. Nevada left the program on July 1, 2014 to start testing their specimens in Nevada.

We began MS/MS testing in October 2002 using the Perkin Elmer derivitized Neonatal Mass Spec Kit and a Sciex mass spectrometer. We changed to Quatro Micro mass spectrometers in January 2008. The derivitized assay was changed to the Perkin Elmer non-derivitized MS/MS kit in June 2010 with SUAC testing added in January 2011. SUAC could not be added to the assay until the assay was changed to a non-derivitized assay. This made detection of Tyrosinemia Type I much easier and prevented many false positives from having diagnostic testing. We changed to the TQD mass spectrometer in December 2013. The TQD Mass Spec requires less maintenance that the Quatro Micro Mass Spec.

Most MS/MS disorders are found on the first specimens. Some disorders have been found only on the first specimens, such as VLCAD. The C14:1 is elevated on the first specimen, but not on the second specimen. A normal C14:1 result on a second specimen does not rule out VLCAD. Diagnostic testing is needed on all infants who have an elevated C14:1 on the first specimen.

Some disorders we find mostly on the second specimens, such as Tyrosinemia Type II and Homocystinuria.

During the ten years in this presentation we found Amino acid disorders occurred one case in 10,983 infants.

Organic Acidemia Disorders occurred one case in 15,335 infants.

Fatty Acid Oxidation Disorders occurred one case in 8,883 infants.

This excludes the many hundreds of CPT1A Arctic variant cases found on Alaska specimens. CPT1A Arctic variant cases were found one case in 2,206 infants and we know we are not finding all of them by MS/MS. Future DNA testing is planned for the Arctic variant on Alaska specimens.
We also detected 9 cases of CPT1A (one case in 180,614 infants) from Pacific Island people living in Hawaii, with a mutation different from the classic CPT1A and the Arctic Variant.

From Hawaiian infants we found 15 cases of VLCAD DNA Mutation 226 for an incidence of one case in 108,369 infants.

We find many NICU infants with elevations due to TPN. Leu, Met, Phe, Tyr, CS, and C3DC_C4OH may be elevated in infants on TPN. We request another blood spot sample, to follow-up on these cases.

1,625,529 infants were tested by MS/MS at the NWRNBS lab in Hillsboro Oregon from July 1, 2004 thru June 30, 2014.

<table>
<thead>
<tr>
<th>DISORDERS</th>
<th>Cases</th>
<th>Incidence</th>
<th>Analytes</th>
<th>Ratios</th>
</tr>
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<tr>
<td>All disorders (except CPT1A)</td>
<td>437</td>
<td>One case in 3,720 infants</td>
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<tr>
<td>CPT1A Variants</td>
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<td>2,173</td>
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<th>AMINO ACID DISORDERS</th>
<th>Cases</th>
<th>Incidence, one case in</th>
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<td>10,983</td>
<td>Amino Acids</td>
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<td>ARG Arginase deficiency</td>
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<tr>
<td>ASA Arginosuccinic Acidemia</td>
<td>13</td>
<td>125,041</td>
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<tr>
<td>CIT Citrullinemia</td>
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<td>Cit</td>
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<tr>
<td>MSUD Maple Syrup Urine Disorder</td>
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<td>HCY Homocystinuria</td>
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<td>MET Hypermethionemia</td>
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<td>108,369</td>
<td>Met</td>
<td></td>
</tr>
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<td>MAT Methionine Adenosyl Transferase</td>
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<td>Met</td>
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<td>PKU Phenylketonuria</td>
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<td>Phe</td>
<td>Phe/Leu</td>
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<td>TYR I Tyrosinemia Type 1</td>
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<td>SUAC, Tyr</td>
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<tr>
<td>TYR II Tyrosinemia Type 2</td>
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<td>Tyr</td>
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<th>Ratios</th>
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<tbody>
<tr>
<td>All cases (except CPT1A Arctic variant cases)</td>
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<td>8,883</td>
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<td>Low C0</td>
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<td>CPT1A Carnitine Palmitoyl Transferase</td>
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<td>CPT1A Arctic variant</td>
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<td>CPT1A Pacific Islander variant</td>
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<td>Disorder</td>
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<td>Incidence, one case in</td>
<td>Analytes</td>
<td>Ratios</td>
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<td>----------------------------------------</td>
<td>-------</td>
<td>------------------------</td>
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<td>MCAD Medium Chain Acyl CoA Def.</td>
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<td>TFP Tri Functional Protein Defect</td>
<td>5</td>
<td>325,106</td>
<td>C14:1, C14, C14O, C16O, C16:1O</td>
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<tr>
<td>LCHAD Long Chain Hydroxyl CoA</td>
<td>3</td>
<td>541,843</td>
<td>C16O, C18O, C18:1O, C16:1O</td>
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<tr>
<td>ORGANIC ACIDEMIAS</td>
<td>Cases</td>
<td>Incidence, one case in</td>
<td>Analytes</td>
<td>Ratios</td>
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<td>All cases</td>
<td>106</td>
<td><strong>15,335</strong></td>
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<td>PA Propionic Acidemia</td>
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<td>C3</td>
<td>C3/C2</td>
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<td>MMA Methylmalonic Acidemia</td>
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<td>C3/C2</td>
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<td>Cbl C Cobalamin C</td>
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<td>C3/C2</td>
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<td>MA Malonic Acidemia</td>
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<td>C3DC_C4OH</td>
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<td>GA II Glutaric Acidemia Type 2</td>
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<td>C4,C5,C6,C8,C10</td>
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<td>3MCC 3-Methylcrotonyl CoA Carboxylase</td>
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<td>MCD Multiple Carboxylase Def.</td>
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<td>HMG 3-Hydroxy-3-methylglutaryl-CoA</td>
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<td>1,625,529</td>
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<td>3MGA 3-Methylglutaconic Acidemia</td>
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<td>na</td>
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<td>2MBCD 2-Methyl-butyryl CoA Def.</td>
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<td>2M3HBA 2-Methyl-3-Hydroxybutiric</td>
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<td>541,843</td>
<td>C5:1, C4DC-C5OH</td>
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<td>BKT Beta Ketothiolase Deficiency</td>
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<td>GA I Glutaric Acidemia Type 1</td>
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<td>C10, C8, C6, C14, C14:1, C16:1</td>
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</tr>
</tbody>
</table>
The Northwest Regional Newborn Screening (NW Regional Newborn Screening) Lab is located at the Oregon State Public Health Laboratories, 3150 NW 229th AV Suite 100, Hillsboro, OR 97124, Phone: 503.693.4173, Fax: 503.693.5601.

P-04

**NW Regional NBS Program - Disorders Detected Over 50 Years of Screening**

D. Sesser, S. Willis, S. Denniston, and C. Hermerath, Oregon State Public Health Laboratory, Hillsboro, OR

**Abstract**

The Oregon State Public Health Laboratory has been performing newborn screening since 1962. In 1975, the Laboratory began providing contracted newborn screening testing and follow-up services to other states and birthing facilities, leading to the formation of the Northwest Regional Newborn Screening Program. Participation in the Regional Program has fluctuated over the years as states, and other birthing centers, have joined or left the Program. Today, the Regional Program includes five states, military, Navajo Nation, and international birthing facilities.

Beginning with screening Oregon infants in 1962, Program participants have included:

- **Alaska**: 1975-1983 and 1987-present
- **Delaware**: 1992-1998
- **Hawaii**: 1997-present
- **Idaho**: 1976-present
- **Montana**: 1975-1985
- **Nevada**: 1978-June 30, 2014
- **New Mexico**: 2007-present
- **Other**: 1991-present

From 1962 through 2013, the Regional Program has screened 4,873,117 infants, detecting 3,094 newborn screening disorders for frequency of 1 in 1,575.

This presentation provides a breakdown and highlights the disorders detected in the Northwest Regional Newborn Screening Program over the last 50 years.

**Presenter**: Cheryl Hermerath, MBA, Oregon State Public Health Laboratory, Newborn Screening, Hillsboro, OR, Phone: 503.693.4172, Email: cheryl.a.hermerath@state.or.us
Colorado’s Strategies to Improve the Timeliness of the Newborn Screening Process Statewide
L. Gillim-Ross and D. Wright, Colorado Department of Public Health and Environment, Denver, CO

Abstract

Background: Timely detection of disorders in newborns allows for babies to be treated early, averting death and preventing or limiting brain damage, disability and a lifetime of costly medical care. The Colorado Department of Public Health and Environment (CDPHE) monitors the performance of health care entities within Colorado in meeting Board of Health (BOH) rules regarding the submission of specimens to the laboratory for newborn screening (NBS). Many facilities within Colorado fail to meet the required BOH performance measures for NBS.

Method: CDPHE and the Colorado Hospital Association (CHA) partnered to convene a work group to develop and implement processes to reduce delays in the transport and processing of NBS blood spot samples within Colorado. The work group consisted of representatives from birthing facilities within Colorado, CDPHE NBS lab, CDPHE NBS follow-up program, and CHA.

Results: The work group mapped the current state of the NBS process within CO from birth to follow up and identified steps in the process where changes and efficiencies could significantly improve the timeliness of NBS. Processes identified and strategies developed to address timeliness of NBS will be discussed.

Implications: Collaborative efforts within Colorado supported the identification of inefficiencies within the NBS system and the development of processes to eliminate delays in the transport and processing of NBS blood spot samples within Colorado. Improvements in the timeliness of the NBS process equate to improved outcomes for the babies of Colorado.

Presenter: Daniel Wright, BS, Colorado Department of Public Health and Environment, Laboratory Services Division, Denver, CO, Phone: 303.692.3673, Email: daniel.wright@state.co.us

Summary

Background:
- Milwaukee Sentinel Journal Article
- CDPHE monitors the performance of health care entities within Colorado in meeting Board of Health rules (5 CCR 1005-2) regarding the submission of specimens to the laboratory for newborn screening (NBS).
- The CDPHE laboratory provides each birthing facility with quarterly reports documenting their performance.
- Many facilities within Colorado are successful at meeting the required Board of Health performance measures for NBS however, some facilities have difficulty

Collaboration Between CDPHE and CHA (Colorado Hospital Association)

GOAL:
To convene a workgroup to develop and implement processes to reduce the time from birth to completion of newborn screening in Colorado.
The work group consists of representatives from birthing facilities within Colorado, the CDPHE laboratory, and CHA.

Newborn Screening Process in Colorado

Improvement Opportunities:

Education Subcommittee:

**GOAL:**

To provide educational materials/opportunities to birthing facilities/HCPs around:
- Proper NBS blood specimen collection and quality
- Proper completion of the NBS laboratory requisition form
- Proper collection and transport time for NBS blood specimens

Transport Subcommittee:

**GOAL:**

To decrease delays in transport of NBS blood spot specimens to the CDPHE laboratory
- Identify method(s) of transport utilized by birthing facilities/HCPs
- Compare method(s) of transport and adherence to turnaround times
- Re-assess current state-wide courier contract and identify needs of system
IT Subcommittee:

**GOAL:**
To decrease delays in reporting of results to HCPs
- Implement electronic reporting
- Availability of NBS test results in HIE

**Plan:**

*Proper collection and transport time for NBS blood specimens*

**CDPHE:**
- Provide courier service

**Pilot Hospitals:**
- Make use of CDPHE courier or overnight mail service
- Train personnel in collection and transport times as put forth in BOH rule

*Proper NBS blood specimen collection and quality*

**CDPHE:**
- Provide DVD “Making a Difference through NBS: Blood Collection on Filter Paper”
- Provide posters
- Provide assistance

**Pilot Hospitals:**
- Provide training to personnel using the DVD and posters
- Implement a NBS blood spot collection competency assessment
- Train personnel to examine specimens prior to transport

**Pilot Project**
- September-December 2014 pilot project initiated in several hospitals
- CDPHE lab monitors quality indicators, turnaround times etc.
- February 2015 collaborative assesses outcomes

**Next Steps:**
- Continue to monitor performance measures around quality of NBS blood spots, completeness of test request forms, and timeliness of collection, transport and reporting.
- Assess outcomes of Pilot Projects.
- Share successes with birthing centers/HCPs throughout CO and develop recommendations to support timeliness of NBS in CO.

From Supportive to Enforcement: Public Health Newborn Screening Strategies to Address Batching & Specimen Delays
J. Luedtke, Nebraska Department of Health and Human Services, Lincoln, NE

Abstract

Managing newborn screening systems to support rapid turn-around times is done in a variety of ways using different models. In Nebraska a historically supportive structure has been expanded to include more intensive oversight and regulation. The supportive elements include health professional training on and distribution of pertinent regulations. These require collection of specimens between 24-48 hours or prior to discharge whichever is first and shipping them within 24 hours of collection. Monetary incentives for hospitals to batch specimens are eliminated by including shipping costs into the lab testing fee. This built in cost for overnight shipping is required by the screening program in the lab contract. By monitoring and reporting on in-lab turn-around times, the laboratory has maximized efficiencies in the lab for specimen processing testing and reporting. Providing regular feedback to hospitals comparing hospital turn-around times to state-wide averages has been another supportive method.

Nonetheless, more timely and meaningful data was needed to be able to accurately identify when specimen handling and delivery delays or batching occurred. We implemented a more specific monitoring feedback reporting procedure to encourage hospital self-evaluation and correction of delays and batching. We realized the supportive infrastructure was not enough to ensure compliance, so we have developed an enforcement procedure for reporting violations of Nebraska’s newborn screening regulations, to the Hospital Licensure unit within the Department. That unit has the authority to administer sanctions and penalties on hospitals when health and safety has been compromised or when there is a risk to health and safety. Specimen batching and late screening due to discharging babies without a screen, clearly risk a delayed diagnosis of a baby with a potentially fatal condition. This poster describes an innovative method for improving quality.

Presenter: Julie Luedtke, BS, Nebraska Department of Health and Human Services, Newborn Screening & Genetics Program, Lincoln, NE, Phone: 402.471.6733, Email: julie.luedtke@nebraska.gov

Summary

Managing newborn screening systems to support rapid turn-around times is done in a variety of ways using different models. In Nebraska a historically supportive structure is being expanded to include more intensive oversight and regulation. The supportive elements are multifaceted. One is providing health professional training on pertinent regulations, standards and guidelines and distribution of these to the hospitals. Another is the regulations require collection of specimens between 24-48 hours or prior to discharge whichever is first and shipping them within 24 hours of collection. Thirdly, monetary incentives for hospitals to batch specimens are eliminated by including shipping costs into the lab testing fee. This built in cost for overnight shipping is required by the screening program in the lab contract. A fourth mechanism is by monitoring and reporting on in-lab turn-around times, the laboratory has maximized efficiencies in the lab for specimen processing testing and reporting. Finally,
providing regular feedback to hospitals comparing hospital turn-around times to state-wide averages has been another supportive method. Nonetheless, more timely and meaningful data was needed to be able to accurately identify when specimen handling and delivery delays or batching occurred.

We implemented a more specific weekly reporting procedure to provide hospital specific data and encourage hospital self-evaluation and correction of delays and batching. The weekly data reports on all specimens received on any date in which any specimen is greater than 3 days old since collection. It provides them with enough specific demographics from the filter paper to facilitate their evaluation. This has been in place for a year, and the frequency of incidents has decreased. In addition we implemented a specific weekly report providing hospital’s data on any specimens that were collected at greater than 48 hours. It was satisfying to see the very low frequency of this data. Hospital investigations have found a variety of reasons for late specimen collection, but most frequently it is due to a recording error of dates/times on filter paper. This affords an opportunity to refocus training for specimen collectors at the hospital.

We realized the supportive infrastructure was not enough to ensure compliance, so we have developed an enforcement procedure for reporting violations of Nebraska’s newborn screening regulations, to the Hospital Licensure unit within the Department. That unit has the authority to administer sanctions and penalties on hospitals when health and safety has been compromised or when there is a risk to health and safety. As with all major procedural revisions, we have utilized the expertise of our Advisory Committee to help us refine aspects of this procedure. Specimen batching or shipping delays, unsatisfactory specimens, late specimen collection and discharging babies without a screen, clearly risk a delayed diagnosis of a baby with a potentially fatal condition.

Implementing an enforcement procedure alone, threatened to damage the collaborative relationship the newborn screening program had built with hospitals across the state. Therefore the program is developing a system for providing meaningful positive feedback to hospitals that they could choose to use however they liked (e.g. promotional marketing information). We proposed a list of potential benchmarks and the concept of levels of achievement that hospitals could attain. For example meeting the 5 benchmarks throughout a year could attain a platinum rating among hospitals providing newborn screening services in Nebraska. Four of five could achieve Gold status, and so forth.

To ensure the new benchmarks were realistic, met the goals of the program, and had buy-in from the hospitals we invited review, input and participation in development of the proposed plan. All of the individuals from every birthing facility identified by their administrator or CEO as those involved with newborn screening quality assurance monitoring at their hospital were provided both the “Proposed QA/QI Plan for Nebraska’s Newborn Screening”, and the proposed “Procedure for Reporting Violations” to the Licensure Unit in the Department of Health and Human Services. This involved laboratory directors, mother/baby unit coordinators, and QA officers. A good cross section of representatives have been participating in review of the documents and providing feedback on the measures and reports.

This participation has proven invaluable in making sure the benchmarks are realistic and achievable but result in quality improvement, especially in consideration of the vast differences between facilities. There is considerable disparity in number of births in the 56 Nebraska birthing facilities (from < 10 per year to > 4000 per year). Some communities are fairly close to metropolitan areas which facilitates transport of specimens, while some are more than 4 hours from the closest airport served by the commercial courier. There is a wide variety in geography and services available to communities.

Despite these challenges, the laboratory with which Nebraska contracts for services, PerkinElmer Genetics, has worked diligently to make specimen pick up available to all facilities on Saturdays over the last year. (Previously only Monday through Friday pickup, and Monday through Saturday delivery were available). Only one small, fairly remote community hospital is not served on Saturday by any commercial courier.

The plan continues to evolve at this time. Data and data reporting capacity produced by PerkinElmer Genetics Inc. has also been invaluable in helping with this project. In the meantime the increased awareness and attention by hospital personnel on newborn screening metrics of: time of collection, collection to receipt, specimen quality, and ensuring no baby is discharged without a screen is at levels not seen before. Hospital personnel’s participation in development of the QA/QI plan along with the expectation for them to investigate and identify ways to prevent recurrence of reported errors (or risk getting reported to licensure) have been an important part of this increased attention.

P-07

Maryland’s Experience of Implementing Quality Assurance Initiative

F. Gulamali-Majid and M.D. Celiz, Maryland Laboratory Administration, Baltimore, MD

Abstract

The Discretionary Advisory Committee on Heritable Disorders in Newborns and Children, whose mission is to reduce morbidity and mortality in newborns and children who have, or are at risk for heritable disorders, has recommended timeframes related to newborn screening. One key recommendation is newborn screening specimens should be received at the Laboratory within 24 hours of collection. Thus, Maryland Newborn Screening laboratory (NBS) has launched a Quality Assurance (QA) initiative aimed at improving the number of samples received in a timely manner from hospitals. The goal is to obtain at least 90% specimens from hospitals within 48 hours or 72 hours.

Our study evaluated 32 birthing hospitals in Maryland. The number of specimens received within 48 hours and 72 hours, from blood collection to receipt in the laboratory, were gathered from January 1, 2013 to June 30, 2013. This initial data showed an average of 60% of specimens as received within 72 hours, with two hospitals achieving the set goal of 90%. However, an average of 36% of samples was received within 48 hours, with the highest at 75%.

Each hospital was asked to submit a plan that would effectively meet the objective.

The plans submitted included
a) an improvement of their internal processes of transporting the specimens within 12 hours of collection
b) Using a private couriers or FedEx to transport specimens daily
c) Transporting specimens on Saturdays and holidays to NBS.

After implementation of these plans by the hospitals and changes in workflow within NBS to accommodate the plans, an improvement was observed. For three months starting on October 1, 2013, the average number of specimens received within 72 hours rose to 86%, with 18 hospitals reaching the
goal. During the same period, 70% of samples were received within 48 hours and 6 of 32 hospitals achieved the 90% goal. During January-March 2014 an 85% average of specimens were received within 72 hours, with 15 hospitals achieving the 90% goal and an averages of 67% of samples were received within 48 hours with 5 hospitals achieving the 90% goal.

This study indicates that upon addressing the barriers or gaps in the work flow and procedures employed by hospitals, laboratories, and other entities, timely reporting of results can be achieved.

**Presenter:** Fizza Gulamali-Majid, PhD, Maryland Laboratories Administration, Baltimore, MD Phone: 410.767.6099, Email: Fizza.Majid@maryland.gov

**Summary**

**Background:** One of the recommendations by The Discretionary Advisory Committee on Heritable Disorders in Newborns and Children (DACHDNC), is that newborn screening specimens should be received at the Laboratory within 24 hours of collection. Thus, Maryland Newborn Screening laboratory (NBS) launched a Quality Assurance (QA) initiative aimed at improving the number of samples received in a timely manner from the birthing hospitals.

**Objective:** To receive at least 90% specimens from hospitals within 72 hours in laboratory.

**Method:** Baseline data from the first six months in 2013 indicated 6% of the 32 birthing hospitals delivered 90% of their specimens within 72 hours of specimen collection to the NBS Laboratory and 59.66% of all specimens met the transit time of 72 hours. No birthing hospitals delivered 90% of their specimens within 48 hours to the NBS Laboratory and 35.52% of all specimens met the transit time of 48 hours. (Figure 1)
In September, 2014 a letter was sent to the Chief Executive Officer/President, Laboratory Director and Nurse Manager of each birthing hospital requesting a plan that would effectively improve the timeliness of specimens submitted to the NBS laboratory. Each hospital was also provided with a chart indicating the percentage of specimens delivered to NBS laboratory within 72 hours of collection during the first six months of 2013.

Improvement plans from all hospitals were received by end of September 2013. The plans to improve the timeliness of specimen delivery included using a private couriers or FedEx to transport specimens daily; transporting specimens on Saturdays and holidays to NBS and changing the internal processes of transporting the specimens within 72 hours of collection.

**Results:** After implementation of these plans by the birthing hospitals and changes in workflow within NBS laboratory to accommodate the plans, an improvement was observed. For Q4 2013, the average number of specimens received within 72 hours rose to 86%, with 56% of hospitals reaching the 90% goal. During the same period, 70% of specimens were received within 48 hours and 19% hospitals achieved the 90% goal (Figure 2).

During Q2 in 2014, a 91% of specimens were received within 72 hours, with 66% hospitals achieving the 90% goal and an average of 75% of samples were received within 48 hours with 25% hospitals achieving the 90% goal. (Figure 3)
Conclusion: With a multipronged approach it is possible to meet the recommendation made by DACHDNC. This study indicates that upon addressing the barriers or gaps in the workflow and procedures employed by hospitals, laboratories, and other entities, timely reporting of results can be achieved.

P-08

Achieving Dramatic Improvements in Sample Transit Time – The Arizona Model

C. Nabor and S. Aponte, Arizona Department of Health Services, Phoenix, AZ

Abstract

A nationwide story reported by the Milwaukee Journal Sentinel identified nationwide delays in transit time for newborn screening samples from hospitals to NBS laboratories. Arizona hospitals were singled out as being among the worst in the nation. Every day of unnecessary delay has the potential to delay identification and critical treatment of affected newborns.

Arizona chose a multi-pronged approach to address this problem. First, ADHS Director Will Humble publically set an aggressive and challenging goal that, by July 1, 2014, 95% of first screen samples would be received at the state laboratory within 3 days of sample collection. Then, ADHS leadership assigned and funded a dedicated team to review data and develop strategies to reach that goal. The strategies included partnering with the state’s Hospital and Healthcare Association to leverage limited resources, hospital site visits, and a commitment to transparent regular disclosure of individual hospital performance toward the goal. Targeted outreach to lagging performers and public recognition of top performers were also helpful. Finally, supplemental funding was made available to establish a contract with an Arizona courier service for daily pickups from each hospital.
Baseline data from 2013 indicated only 67% of hospitals met the three day transit time goal. As of April (courier service began in mid-April), 89% of hospitals met the goal. We are confident that the 95% goal is within reach by July 1st.

Leadership involvement is essential to achieving an effective solution. Setting an aggressive goal and making the necessary resources available sends an unmistakable message about commitment. Our decision to work collaboratively with our partners to remove barriers, while maintaining fully transparency about project progress, led to the realization of dramatic improvements in a short period of time.

**Presenter:** Celia Nabor, MPA, Arizona Department of Health Services, Office of Newborn Screening, Phoenix, AZ, Phone: 602.364.2579, Email: celia.nabor@azdhs.gov

**P-09**

**Improving Newborn Screening Sample Delivery Time in Arkansas**

P. Scott and L. Himstedt, Arkansas Department of Health, Little Rock, AR

**Abstract**

**Problem:** In 2012, only 10.6% of newborn screening samples in Arkansas arrived to the Public Health Laboratory (PHL) within the recommended 48 hour time frame from sample collection.

**Methodology:** In 2012, we assessed gaps in our service, education and technical assistance to hospitals that could be contributing to delayed sample delivery. In addition to annual hospital site visits, we implemented the free, daily ADH-PHL-local health unit courier that was in place, but not being used for NBS samples.

In 2013, there was some, but not substantial improvement after implementing the courier.

We initiated a QI project that was focused on educating nursery nurses. This was founded on principals of community-based participation and included:

- Informal brainstorming with nursery nurses about their learning needs.
- Nursery manager survey.
- Development of an educational program informed by the brainstorming session and survey results.

The education program included a video available on our website and a DVD delivered to every hospital; an online training program providing nursing education units; a tool-kit; and continued annual site visits plus as-needed contacts.

Also in 2013, we implemented recommendations made by our NBS Advisory Committee:

- Quarterly newsletters to hospitals and stakeholders.
- Quarterly certificates of improvement to the hospital with most improved sample delivery time.
- Email blasts hospitals prior to holidays reminding them to use same day or overnight delivery.
- Quarterly trend reports of sample delivery times to birthing hospital administrators and lab directors, and annual reports with hospital comparisons

• A letter from our Director of Health to each hospital administrator stressing the importance of prompt sample delivery.

Results: In 2012, 10.6% of samples arrived to the PHL within 48 hours. In 2013, 15% of samples arrived to the PHL within 48 hours. In the first quarter of 2014, 22.7% of samples arrived to the PHL within 48 hours.

Conclusions: It takes a multi-focal approach with stakeholder input to improve sample delivery times. The approach that is working for Arkansas includes education; regular and varied communication; provision of sample delivery data; recognition of improvement; courier service; and involvement of high-level administrators.

Presenter: Leslie Himstedt, BS, MT(ASCP), Arkansas Public Health Laboratory, Little Rock, AR, Phone: 501.661.2445, Email: leslie.himstedt@arkansas.gov

Summary

Problem: In 2012, only 10.6% of newborn screening samples in Arkansas arrived to the Public Health Laboratory (PHL) within the recommended 48 hour time frame from sample collection.

Methodology: In 2012, we assessed gaps in our service, education and technical assistance to hospitals that could be contributing to delayed sample delivery. In addition to annual hospital site visits, we implemented the free, daily ADH-PHL-local health unit courier that was in place, but not being used for NBS samples.

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• Informal brainstorming with nursery nurses about their learning needs.
• A nursery manager survey.
• Development of an education program informed by the brainstorming session and survey results.

The education program included a video available on our website and a DVD delivered to every hospital, an online training program providing nursing education units, a tool-kit, and continued annual site visits plus as-needed contacts.

Beginning in 2013, we implemented recommendations made by our NBS Advisory Committee and sent:
• Quarterly newsletters to hospitals and stakeholders.
• Quarterly certificates of improvement to the hospital with most improved sample delivery times.
• Email blasts to hospitals prior to holidays reminding them to use same-day or overnight delivery.
• Facebook messages and news about NBS.
• Quarterly trend reports of sample delivery times to birthing hospital administrators and lab directors, and an annual report with hospital comparisons.
• Director of Health letters to each hospital administrator stressing the importance of prompt sample delivery.

Results: In 2012, 10.6% of samples arrived to the PHL within 48 hours. In 2013, 15% of samples arrived to the PHL within 48 hours. In the first quarter of 2014, 32% and by the second quarter, 50% of samples arrived to the PHL within 48 hours.

Conclusions: It takes a multi-focal approach with stakeholder input to improve sample delivery times. The approach that is working for Arkansas includes education, regular and varied communication, provision of sample delivery data, recognition of improvement, courier service, and involvement of high-level administrators.

P-10

The Discrepancy between the Concentration of Phenylalanine Measured in Dried Blood Spots Compared to Plasma in Individuals with Phenylketonuria

S. Van Calcar, M. Clayton, B. Stroup and M. Baker, University of Wisconsin School of Medicine and Public Health, Madison, WI

Abstract

Introduction: Metabolic control of phenylketonuria (PKU) and compliance with the low-phenylalanine (phe) diet are frequently assessed by blood phe concentrations analyzed from dried blood spots. A previous report found a mean blood phe concentration that was 19% lower compared to the mean plasma phe concentration when venous blood was collected and spotted on filter paper by trained personnel (Genet Med 2007:9(11): 761-765).
**Objective:** To investigate the difference in blood phe concentrations collected by patients and analyzed using tandem mass spectrometry (MS/MS) compared to plasma phe concentrations analyzed using an Amino Acid (AA) Analyzer.

**Methods:** Three to four fasting blood samples were obtained from each of 18 subjects with PKU, ages 15-37 years. Capillary blood was spotted on filter paper by each subject and analyzed using MS/MS. Venous blood was collected into EDTA tubes; plasma was isolated and analyzed using an AA Analyzer. Both blood spot and venipuncture were collected simultaneously.

**Results:** When the patients spot the filter paper, blood phe concentrations are 29% ± 14% (mean ± SD, n=68) lower than plasma phe concentrations. The discrepancy between the blood and plasma phe concentrations exceeds a previous report, where trained personnel spot the filter paper with venous blood.

**Discussion:** Measuring blood phe concentrations from dried blood spots by MS/MS was not designed for metabolic monitoring. However, it is widely used for patient management. The lower phe concentration measured from blood spots compared with plasma needs to be considered in the implementation of the 2014 management guidelines that recommend target phe concentrations of 120 to 360 umol/L (2 to 6 mg/dl) for individuals with PKU of all ages.

**Presenter:** Sandra Van Calcar, RD, PhD, University of Wisconsin School of Medicine and Public Health, Madison, WI, Phone: 608.263.5981, Email: vancalcar@pediatrics.wisc.edu

**P-11**

**VLCADD Screening: Wisconsin’s Experience**

J. Scott Schwoerer, S. Van Calcar, M. Baker and G. Rice, University of Wisconsin School of Medicine and Public Health, Madison, WI

**Abstract**

Very Long Chain Acyl CoA Dehydrogenase Deficiency (VLCADD) is an inborn error in long chain fatty acid oxidation that can present clinically in the neonatal period with severe cardiomyopathy and hypoglycemia. This disorder can also present in early childhood with hypoketotic hypoglycemia upon fasting or illness or, in the late-onset form, with rhabdomyolysis, muscle cramps or pain, and exercise intolerance. Introduction of tandem mass spectrometry to newborn screening has enabled detection of VLCADD by a characteristic acylcarnitine profile, often prior to onset of symptoms. As individuals with VLCADD have been identified by newborn screening, greater variability in the clinical phenotype has been recognized.

This study reviews screening results from the Wisconsin Newborn Screening Laboratory for more than 50 infants identified from 2000 to 2014 with an abnormal newborn screen suggesting a possible diagnosis of VLCADD. Status of VLCADD diagnosis was evaluated by a combination of biochemical, enzymatic and/or molecular testing. Over time, the screening algorithm has been modified to improve the positive predictive value for this disorder.

In review of the data, the following acylcarnitine species and ratios (C14:1, C14, C14:2, and C14:1/C16) were found to be helpful in predicting an infant’s likely hood to have VLCADD. Affected infants typically had a C14:1 value greater than 2 umol/L, although the significantly elevated concentration did not

necessarily predict the initial clinical status. Infants with asymptomatic forms of VLCADD or carriers of the disease had C14:1 concentrations ranging from 1-2 umol/L. False positives also had C14:1 concentration as high as 2 umol/L, although several of these infants were found to have other metabolic disorders. In summary, VLCADD remains a difficult disease to screen for, given the overlap in analyte concentrations between affected infants, carriers, and false-positives.

**Presenter:** Jessica Scott Schwoerer, MD, University of Wisconsin School of Medicine and Public Health, Pediatrics, Madison, WI, Phone: 608.263.5993, Email: jscottschwoerer@pediatrics.wisc.edu

**P-12**

**Wisconsin’s Approach to Addressing Quality Assurance within the Pre-Analytical Testing Phase of Newborn Screening**

P. Held, S. Dawe, M. Anderson, M. Baker and C. Brokopp, Wisconsin State Laboratory of Hygiene, Madison, WI

The mission of newborn screening laboratories is to identify infants affected with certain heritable disorders, so that treatment can be initiated prior to the onset of symptoms. Within laboratory testing, there are three defined stages: pre-analytical, analytical, and post-analytical. Historically, significant efforts have been focused on improving the sensitivity and specificity of analytical tests. However, as recent news articles have highlighted, the pre-analytical phase of testing, specifically the timing from specimen collection to receipt in the laboratory, is critical to the success of a newborn screening program. In addition, Centers for Medicare & Medicaid Services (CMS) regulations state that laboratories must establish and follow written policies and procedures for an ongoing mechanism to monitor, assess, and when indicated, correct problems identified in the pre-analytical systems. As a quality assurance project, Wisconsin sought to not only improve transit times, but also address other pre-analytical quality indicators related to proper collection of the newborn screening specimen and essential demographic information. Wisconsin developed several strategies aimed at improving the pre-analytical phase of testing, such as implementation of monthly submitter quality assurance reports and rapid notification of specimens failing to meet established standards. Additionally, the laboratory provided consultation and resources to submitters failing to meet the recommended standards. Initial results of these efforts demonstrated dramatic improvement in transit times. The long term effects of these strategies on the improvement of pre-analytical quality indicators will be reviewed and suggestions will be provided based upon the Wisconsin experience.

**Presenter:** Patrice Held, PhD, Wisconsin State Laboratory of Hygiene, Madison, WI, Phone: 608.265.5968, Email: heldpk@slh.wisc.edu
Improving Newborn Screening Rates within the Michigan Homebirth Community
L. Turbett, M. Kleyn, K. Urquhart and J. Bach, Michigan Department of Community Health, Lansing, MI

Abstract

Background: Midwife-attended homebirths were less likely to receive a newborn screen (65%) than the general population (99.6%) in 2011. In January 2013, Michigan’s Newborn Screening (NBS) Program received a Baby’s First Test Challenge Award to identify ways to increase the number of infants born at home who receive NBS. The project’s goals were to: 1) increase understanding of barriers, experienced by midwives and parents, to obtaining NBS for infants born at home; 2) increase NBS education provided to midwives and interested community members; and 3) increase partnership relationships with midwives and local health departments to promote NBS for homebirths.

Methods: Staff from multiple programs including NBS, vital records, and EHDI collaborated to develop a survey sent to all midwives who attended three or more planned homebirths in Michigan in 2011.

Results: Thirty-four midwives returned the survey out of 51 (67%). The majority (85%) of respondents reported they discuss NBS with every client and all felt confident in their ability to explain the purpose and benefits of NBS. The most common reasons for parents refusing screening were: personal reasons (N=24), client does not want state to store infant’s blood (N=24), and religious reasons (N=18). The most common barriers midwives experienced for collecting the screen were: distance to a client’s home (N=10), family plans to obtain NBS elsewhere (N=6), and midwife is too busy (N=5).

Conclusions: Four NBS trainings were provided to address barriers identified by the survey, with 2 targeted to Amish communities. Forty-six midwives and community members attended these trainings. Using feedback from the survey and trainings, a NBS guide for homebirths was developed. Multiple approaches were used to improve communication and foster understanding between the state NBS Program and midwife community. Monitoring of screening rates among midwife-attended homebirths will be used to assess the effectiveness of these efforts.

Presenter: Lois Turbett, MSN, Michigan Department of Community Health, Lansing, MI, Phone: 517.335.1966, Email: turbettl@michigan.gov

Summary

Background: Michigan law requires all birth attendants to offer newborn screening (NBS), though parents have the option to decline. By linking vital records with the NBS database, it was determined that midwife-attended homebirths were less likely to receive a newborn screen (65%) than the general population (99.6%) in 2011. The completed screens were more likely to be collected after 36 hours and were slow to arrive to the State NBS Laboratory.

In January 2013, Michigan’s Newborn Screening Program received a Baby’s First Test Challenge Award to identify ways to increase the number of infants born at home who receive NBS. The project’s goals were to: 1) increase understanding of barriers, experienced by midwives and parents, to obtaining NBS for infants born at home; 2) increase NBS education provided to midwives and interested community members; and 3) increase partnership relationships with midwives and local health departments to promote NBS for homebirths.

**Methods:** Staff from multiple programs including NBS, vital records, and Early Hearing Detection and Intervention (EHDI) collaborated to develop a survey sent to all midwives who attended three or more planned homebirths in Michigan in 2011. The survey modules included preparing for NBS, collecting and transporting the newborn screen, Michigan BioTrust for Health, pulse oximetry screening, newborn hearing screening, birth certificates, and midwife and client characteristics.

Surveys were mailed in Spring, 2013. A second survey was sent two weeks later to midwives who had not returned the first survey. Included with the survey were the Michigan Department of Community Health (MDCH) NBS Guide, a NBS brochure, an EHDI brochure, the BioTrust for Health consent booklet, a critical congenital heart disease (CCHD) brochure and the Spring, 2013 NBS newsletter. A $10 gift card to a local store and five NBS Program-approved Microtainer Quikheel lancets were offered as an incentive to complete the survey. Participants were told in advance that the surveys would be de-identified and that the contact information was requested just so that the incentives could be sent.

**Results:** Thirty-four midwives returned the survey out of 51 (67%). The majority (85%) of respondents reported they discuss NBS with every client and all felt confident in their ability to explain the purpose and benefits of NBS. The most common reasons for parents refusing screening were: personal reasons (N=24), client does not want state to store infant’s blood (N=24), and religious reasons (N=18). The most common barriers midwives experienced for collecting the screen were: distance to a client’s home (N=10), family plans to obtain NBS elsewhere (N=6), and midwife is too busy (N=5).

The majority of the respondents had been practicing for more than 10 years (76%), attended 10 or more births per year (85%), practiced independently (74%) and belonged to at least one professional organization (85%). A large percent reported having at least one client whose newborn was diagnosed with a disorder through NBS (35%).

There was a diversity in geographic, age and education distribution among the clients served though a large number of midwives (65%) reported serving primarily Caucasian women. Half of the midwives reported working with Amish or Mennonite clients. A majority of the respondents (73%) reported that more than 75% of their clients allow NBS. Collections were more likely to be made during the recommended timeframe of 24-36 hours of age (67%) though some were collected at 36-48 hours (24%) or more than 48 hours (9%).

Some midwives (27%) had already implemented pulse oximetry screening as part of their practice. If added to the Michigan NBS panel, approximately half (56%) said they would do the screen themselves, six (18%) would refer to a provider and two (6%) would either not perform the screen or would refer.

This survey was the first time Michigan has assessed NBS practices and training needs of homebirth attendants. Based on the results, four NBS trainings were provided to address identified barriers, with two targeted to Amish communities. Forty-six midwives and community members attended these trainings. Participants were taught by a professional phlebotomist how to collect the NBS bloodspot. The EHDI consultant reviewed how to perform the hearing screen using otoacoustic emissions (OAE) equipment. Because screening for CCHD was expected to become mandatory in April, 2014, the NBS CCHD Nurse Educator instructed participants in how to use a pulse oximeter to screen for CCHD. She also reviewed the two pulse oximeters that are currently approved by the U.S. Food and Drug Administration for use with neonates.

Three public health nurses from a district health department (DHD) attended one of the trainings. Their three counties have a large number of Amish communities whose members usually choose homebirth. It is estimated that one-third of their infants born at home do not have a newborn screen. This DHD was awarded a 2014 Baby’s First Test Challenge Award to promote perinatal health and create demand for newborn screening among Amish mothers residing in their counties.

**Conclusions:** Using feedback from the survey and trainings, a NBS guide for homebirths was developed and is posted to the NBS website, www.michigan.gov/newbornscreening. Multiple approaches were used to improve communication and foster understanding between the state NBS Program and midwife community. A comprehensive contact list of midwives, doulas, and other persons interested in receiving ongoing communication about NBS was developed and has been shared with vital records, EHDI and the Perinatal Hepatitis B Prevention Program. Persons on the list receive quarterly information, either electronically or by postal mail, about the NBS Program and updates.

Lines of communication between the NBS Program and the homebirth community have been greatly improved. The Michigan Midwives Association, which has historically promoted NBS, invited the EHDI Program Consultant, NBS CCHD Nurse Educator and the NBS Nurse Consultant to speak at their fall, 2013 conference. Homebirth midwives call the program more frequently for guidance on specific matters, including picture feedback on unsatisfactory bloodspot specimens. The EHDI Program has been able to place OAE equipment at sites throughout the state for loan to homebirth midwives who have been trained in its use. The NBS Program is in the process of developing a similar loan program for homebirth midwives who would like to have access to pulse oximeters.

Monitoring of screening rates among midwife-attended homebirths will be used to assess the effectiveness of these efforts. The NBS epidemiologist will do this by periodically linking vital records to the NBS database.

_Acknowledgement:_ The midwife survey and trainings were supported by a 2013 Baby’s First Test Challenge Award from Genetic Alliance.

**P-14**

**A DNA Stability Study for the T-cell Receptor Excision Circle (TREC) Real-time PCR Assay Screen for Newborn Immunodeficiency**

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**Abstract**

**Objective:** To determine the stability of the T cell receptor excision circle (TREC) and β actin levels by conducting a controlled stability study over two months with normal cord blood dried blood spots (DBS) kept in storage temperatures ranging from -20 oC to +37 oC. MI newborn screening DBS samples, stored in varying conditions, from 1984 to 2011 were also evaluated.

**Methodology:** A normal cord blood DBS is used as one of the daily controls for the TREC assay. The DBS controls are stored at -20 oC with desiccant for long-term storage. The testing conditions included temperatures from -20 oC to +37 oC with and without desiccant. The stability samples were added in
duplicate to the daily run of the TREC assay for 2 months. The MI method includes an automated DNA extraction from a 3 mm dried blood spot (DBS) followed by a duplex real time PCR assay for TREC and β actin. Stability results of the normal cord blood DBS and MI newborn screening samples from 1984 to 2011 will be presented.

**Results:** All of the normal cord blood controls used in this study remained stable throughout the duration of the testing period. All patient samples tested from 1984 to 2011 had normal TREC and β actin Ct values with the exception of one patient sample from 1990. This sample was inconclusive as both the TREC and β actin Ct values were outside the acceptable range.

**Conclusions:** This study demonstrates the TREC and β actin targets used in the TREC assay are very stable. Considering the TREC and β actin amplicons are small, 88 bps and 350 bps respectively this is not surprising. A larger study with the older patient samples from the Michigan Neonatal Biobank is being considered. Additional studies may also be conducted to evaluate the stability of the DBS in high humidity conditions.

*Partially funded by Grant Number 1U01EH000936 from CDC/NCEH*

**Presenter:** Heather Wood, MS, Newborn Screening Program, Michigan Department of Community Health, Lansing, MI, Phone: 517.335.9381, Email: WoodH@michigan.gov

**Summary**

A stability study to analyze T cell receptor excision circles (TREC) and β actin levels using normal cord blood dried blood spots (DBS) was performed from August 1, 2013 to September 30, 2013. This is the first stability study to date to test the stability of the targets of the TREC assay.

TRECs are small circular pieces of DNA that are formed during the differentiation of T-cells in the thymus as a result of rearrangement of the T cell receptor genes. It has been demonstrated that the quantification of T cell receptor excision circles using quantitative real time PCR (qPCR) will estimate naïve T cells that recently emigrated from the thymus (1,5,6). This technique can be used to determine a reduction in naïve T cells regardless of the underlying defect. Therefore any primary immune disorders due to the low T cell concentration can be identified.

This method made it feasible to include severe combined immunodeficiency (SCID) and other primary immune deficiency syndromes (PIDs) in the newborn screening panel. Infants with SCID have been shown to have TREC values near zero or undetectable. The published methods were further streamlined by the MI NBS to include a single reagent extraction with partial automation of the DNA extraction from the dried blood spot (DBS) followed by simultaneous analysis of TREC and β-Actin by qPCR (4). Modifications of the procedure included streamlining the DNA extraction method, duplexing TREC and β-Actin, and utilizing the assay as a qualitative assay rather than a quantitative assay (4,7).

Quantitative real time PCR measures the cycle threshold (Ct), an instrument value used to determine positive, negative and inconclusive results. The Ct is the number of DNA amplification cycles at which the amplification passes a fixed threshold and is inversely proportional to concentration of the target gene. In other words, the lower the Ct value the larger the amount of target DNA is present. For the MI newborn population a TREC Ct value greater than 36.3 is considered a positive result if there is a normal level of β-Actin. β-Actin is a reference gene included to determine if there is adequate amount of DNA present to amplify. This is especially important because we are looking for the absence of TRECs in a Proceedings of the 2014 APHL Newborn Screening and Genetic Testing Symposium, Anaheim, CA, October 27-30, 2014
sample. If a sample has a low amount of both TRECs and β-Actin then it is considered inconclusive. MI NBS began testing for SCID and other PIDs using the modified TREC assay on 10/1/2011 since then over 320,000 newborns have been screened. A total of 43 primary and secondary primary immunodeficiency cases have been identified. Of these cases there have been 5 SCIDs.

A normal cord blood control is used as one of the daily controls for the TREC assay. The DBS controls for the TREC assay are produced in the MI NBS lab. For a normal cord blood control a single source sample is spotted onto several filter cards. The controls are then stored at -20 oC with desiccant for long term storage. The normal cord blood DBS samples used for the study were prepared 12/12/12 and have been stored at -20oC with desiccant. Twenty initial data points were obtained to establish a reference point at time zero. The normal DBS cards were then separated into different bags for each testing condition. The testing conditions included varying temperature with and without desiccant (See Table 1 for full descriptions of the testing conditions). The stability samples were added in duplicate to the daily run of the TREC assay during the study period. These data points (TREC Ct and β actin Ct values) were plotted and the averages, standard deviations, and percent of coefficient of variation (%CV) were calculated. It is expected to see variability from day to day for a number of reasons including the lack of homogenous nature of the cellular distribution in the dried blood spot. This is especially true with patient samples. Therefore there is an accepted amount of variability for the assay. It is also important to remember this is a screening assay for PIDs and we are looking for the absence or low levels of TREC which would result in either a Ct value of greater than 36.3. Although real time PCR is typically used to quantitate a target of interest in the case of the Michigan’s version of the TREC assay it is used as a qualitative test.

All of the normal controls used in this study remained stable throughout the duration of the testing period. Performing a stability study with normal cord blood samples allowed for a controlled stability study however, the most interesting question is: what about the stability of true newborn blood spot samples? Patient specimens ranging from 2 to 29 years ago were also tested for this study. Three samples from each of the following years were requested from the record center and the MI neonatal biobank: 1984, 1987, 1990, 1993, 1996, 1999, 2002, 2005 and 2011. Specimens from 1984 to 2008 were stored in uncontrolled conditions. Specimens from 2011 to current were stored in -20oC with desiccant. Each of the patient samples were tested in triplicate. The TREC and β actin Ct averages were calculated. All patient samples tested from 1984 to 2011 had normal TREC and β actin Ct values with the exception of one patient sample from 1990. This sample would be considered inconclusive as both the TREC and β actin Ct values were outside the acceptable range. This could be due to other factors rather than stability such as the presence of PCR inhibitors.

This study demonstrates the TREC and β actin targets used in the TREC assay are very stable. Considering the TREC and β actin amplicons are small, 88 bps and 350 bps respectively this is not surprising. A larger study with the older patient samples from the Michigan Neonatal Biobank is being considered. More information can be found at the following link (www.mnbb.org). Additional studies may also be conducted to evaluate the stability of the DBS in high humidity conditions.

*Partially funded by Grant Number 1U01EH000936 from CDC/NCEH

References:
4. Wood et al 2013, “An improved method for DNA extraction method from Dried Blood Spots for T receptor excision circle (TREC) analysis and other Newborn Screening assays” Newborn Screening & Genetic Testing Symposium, May 5-10, Atlanta, GA.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Description of condition</th>
<th>TREC Ct Average</th>
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<th>β actin Average</th>
<th>β actin Ct Stdev</th>
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**P-15**

Development and Implementation of an Automated DNA Extraction Protocol with Selective Sample Transfer, or ‘Cherry Picking’ Capabilities for Cystic Fibrosis (CF) Screening

**Abstract**

**Background:** The Michigan Newborn Screening (MI NBS) program recently introduced a method for the analysis of T cell receptor excision circle (TRECs) that includes a DNA extraction. A small volume of DNA is required for the analysis of TRECs, thus creating a surplus of DNA arrayed in 96-well plates that is available for additional molecular tests.

**Objective:** To implement an automated mechanism for selecting DNA for 2nd tier CF analysis from 96-well plates extracted for TREC analysis, thereby using one dried blood spot (DBS) as the starting point for both molecular methods.
Methodology: DBS were punched into 96-well plates that were loaded onto an Eppendorf epMotion 5075 liquid handler for DNA extraction with Qiagen Generation DNA Elution Solution. The DNA was then used for TREC analysis. A subset of the surplus DNA was cherry picked by the epMotion 5075 from the source TREC plates via a novel application developed by Perkin Elmer (PE) specifically for this endeavor. The application converts a worklist into a CSV file recognized by epMotion 5075 epBlue software. The CSV file contains instructions that enable the epMotion to cherry pick DNA from source TREC plates and transfer the DNA into a receiver plate. The cherry picked samples then underwent targeted mutational analysis for CF screening with the Hologic CFTR InPlex 40+4 assay.

Results: 100% of the DNA was accurately transferred with the cherry picking application from source TREC plates into a receiver plate for CF analysis. The transferred DNA was of sufficient quality and quantity for CF analysis.

Conclusions: By utilizing the application developed by PE, in conjunction with the epMotion 5075 liquid handler, our laboratory was able to implement a protocol for selectively transferring DNA from TREC plates into a receiver plate for CF analysis. This cherry picking protocol demonstrates that multiple molecular analyses can stem from one DBS extraction while maintaining positive patient identification. This flexible, automated cherry picking protocol may facilitate the addition of expanded molecular screening in the NBS laboratory in that it utilizes preexisting DNA extractions, thus eliminating the need for an additional DBS and sample preparation.

Partially funded by Grant Number 1U01EH000936 from CDC/NCEH

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Summary

Background: The increasing appearance of molecular methods and PCR-based tests in the newborn screening (NBS) laboratory highlights the expanding potential that these methods bring to the field of newborn screening. As the role of DNA-based molecular methods continues to advance, the capability to utilize DNA extracted from one dried blood spot (DBS) for multiple analyses in an automated fashion is advantageous.

Like many NBS programs, the first molecular method introduced in the Michigan (MI) NBS program was a second-tier test for the screening of cystic fibrosis (CF). Only a subset of samples undergo this molecular screening on a daily basis. The MI CF screening program utilizes a primary screen for all DBS samples to determine the concentration of immunoreactive trypsinogen (IRT) present in the sample. The samples with the highest IRT concentrations are reflexed to a secondary test to determine if CF-causing mutations are present in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The molecular test for CF begins with a DNA extraction, and is followed by targeted mutational analysis using the Hologic 40+4 InPlex assay. Approximately 4% of the daily samples undergo the CF molecular test.

In October 2011, the MI NBS program introduced its first molecular primary screening method. This method was developed for the analysis of T cell receptor excision circles (TRECs) for the detection of primary immune deficiency syndromes (PIDS). All babies born within the state of Michigan receive this screening. The implementation of primary molecular screening with the TREC assay necessitated a DNA
extraction for all samples tested within the lab. Automation was incorporated to accommodate the daily high-throughput of samples in an otherwise labor-intensive method.

Both the CF and TREC protocols begin with a 3mm dried blood spot ‘punch’ for analysis, and have a similar DNA extraction protocol. Only a small volume of DNA (5-8 µl) is required for each analysis, thus creating a surplus of extracted DNA arrayed in 96-well plates that is available for additional molecular testing. Rather than regarding the surplus DNA extracted for the TREC protocol as waste, our laboratory explored the possibility of using an automated liquid handler to utilize this DNA for the second-tier CFTR analysis for CF. Consequently, both molecular methods within our laboratory could share a DNA extraction originating from the same 3mm DBS ‘punch.’

**Objectives:**
1) To determine if DNA extracted for the TREC assay is of sufficient quality and quantity for CFTR analysis.
2) To replace the laborious, manual, multi-wash CF DNA extraction with an automated extraction utilizing instruments already in-house for TREC analysis.
3) To develop an automated mechanism for selecting, or ‘cherry-picking,’ DNA for CF analysis from 96-well microplates already extracted for TREC analysis, thereby using a single “punch” and sample preparation as the starting point for both molecular screening assays used within our laboratory.
4) To implement the automated cherry-picking transfer of DNA while maintaining positive patient identification, thus replacing the manual DNA transfer performed by a technician.

**Methodology:** The study occurred in two stages. The first stage was a comparison between the manual DNA extraction currently in use for the CF assay, and the automated DNA extraction used for the TREC assay to determine if the automated method yielded DNA of sufficient quality and quantity for CFTR analysis. The manual DNA extraction requires the use of a handheld multichannel pipette to wash the DBS multiple times with two different reagents manufactured by Qiagen: Generation Purification Solution 1 and Generation Elution Solution 2. After the last wash, a thermal cycler is used to apply heat. In contrast, the automated extraction utilizes the epMotion 5075 liquid handler (Eppendorf) to extract DNA from the DBS through the addition of only Qiagen Generation Elution Solution 2, followed by the application of heat and shaking. A Mastercycler (Eppendorf) is then used to apply heat.

Samples used for the extraction comparison and subsequent validation were obtained through the Michigan BioTrust for Health, which is a program that oversees the state’s stored blood spots. Only blood spots from infants whose parents consented to research were used in this study.

DNA extractions from both methods underwent targeted mutational analysis for CFTR mutations with the CF Hologic InPlex 40+4 screening assay. EpEvaluator software was used to statistical compare data, and to assess metrics such as accuracy and precision.

The second stage of the study involved developing and implementing an application that selects, or ‘cherry-picks’, DNA from individual wells of a 96-well plate and transfers it to another reaction plate for amplification and further analysis. To accomplish this, epBlue software (Eppendorf) was utilized on the epMotion 5075, in conjunction with a novel application developed by Perkin Elmer (PE) specifically for this endeavor. The application developed by PE converts an existing CF worklist into a CSV file recognized by the epMotion. The CSV file contains instructions that enable the epMotion to ‘cherry-pick’ DNA from source TREC plates and to then transfer the DNA into a receiver plate for further CFTR analysis. The PE application also creates a worklist that is compatible with the Hologic platform for the final results readout.
**Results:** The automated extraction used for TREC analysis yields DNA of sufficient quality and quantity for CFTR analysis. There were no sample failures with the automated extraction. 100% of the DNA was accurately transferred with the ‘cherry-picking’ application from source TREC plates into a reaction plate for CFTR analysis.

**Conclusions:** A fast, inexpensive, automated one-wash DNA extraction is now validated for use with TREC and CF assays in the MI NBS laboratory. Depending upon the operational needs of the laboratory, the DNA extraction for each method can be performed separately, or DNA can be extracted once from a DBS and used for both methods. The later of the two options is made possible by an application developed by PE, in conjunction with the epMotion 5075 liquid handler with epBlue software. These two components enable our laboratory to develop a ‘cherry-picking’ protocol by which DNA from both samples and quality control material is selectively transferred from TREC 96-well plates to another 96-well plate for CFTR analysis. This ‘cherry-picking’ protocol demonstrates that multiple molecular analyses can stem from one DBS extraction while maintaining positive patient identification. This flexible, automated ‘cherry-picking’ protocol may make the addition of new molecular methods easier for a NBS lab, since it allows for the use of already existing DNA extractions and eliminates the need for an additional DBS punch. This ‘leftover’ DNA can be utilized for additional second-tier screening, or future primary screening methods. Additional benefits to automating the CF extraction and transfer include: a. decreasing technical hands-on time, which frees the operator for other tasks, and b. eliminating transfer errors that may occur when a sample is transferred manually from a source DNA extraction plate to a receiver plate for further testing.