Newborn Screening: From Guthrie to Whole Genome Sequencing

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Newborn screening (NBS), a comprehensive system that includes testing, diagnosis, follow-up, treatment, education, and evaluation, was recently named one of the Top 10 Great Public Health Achievements by the Centers for Disease Control and Prevention (CDC).[1] Each year, approximately 10,000 infants are identified with NBS conditions, which frequently go unnoticed at birth.[2] NBS is administered by state public health programs across the country and provides for early identification of newborns with certain congenital, metabolic, endocrine, hematologic, and other genetic conditions. Early identification of these conditions in newborns facilitates timely interventions that result in significant decreases in morbidity, mortality, and disability.[1]

Screening begins by pricking a newborn's heel to get enough blood to fill a few circles on a filter paper card. The specimen, referred to as a dried blood spot, is collected by a health-care provider—typically at the birthing facility—during the first 24–48 hours of life. Some states are required to collect two specimens, in which case the second specimen is collected between seven and 15 days of life. The specimens are then sent to a state-designated NBS laboratory for analysis. When a test result is out of normal range, laboratory or follow-up personnel contact the birthing facility and the newborn's physician to ensure the child receives the appropriate diagnostic work-up and treatment. NBS goes beyond blood-spot screening to include point-of-care testing for hearing and, in some states, critical congenital heart disease. These tests are performed at the hospital shortly after birth, and the state NBS program performs follow-up testing. Although there is some variability in protocols among states, most NBS programs have similar components, including specimen collection, laboratory testing, follow-up, education of providers and the public, verification of a diagnosis, treatment, and ongoing program evaluation.[3]

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THE HISTORY OF NBS

The year 2013 marks an important milestone for NBS: the 50th anniversary of the first legislatively mandated state NBS programs. NBS began in 1963 when Massachusetts, Delaware, Vermont, and Oregon began testing for phenylketonuria (PKU) with Robert Guthrie's bacterial inhibition assay for the quantification of phenylalanine levels in dried blood spots.[4–6] The Health Resources and Services Administration's (HRSA's) Children's Health Bureau funded Guthrie's early efforts. Advocacy groups, including the National Association for Retarded Citizens and the March of Dimes, pressured states to begin NBS for PKU to prevent this cause of mental retardation.[7] The National Academy of Sciences established criteria for population-based screening systems in 1975. Criteria included evidence of substantial public benefit and acceptance; feasibility of screening for the selected disorders; satisfactory laboratory methods; appropriate laboratory facilities and quality control; resources for counseling, treatment, and follow-up; acceptable costs; effective education; and evaluation of program quality.[8] Technological advances helped state NBS programs expand to include conditions such as congenital hypothyroidism, sickle cell disease, congenital adrenal hyperplasia, and galactosemia. NBS was and continues to be organized and administered at the state level.

Historically, budgetary and political restraints, and limited availability of novel multiplex technologies, such as tandem mass spectrometry (MS/MS), resulted in differences in the number and types of NBS tests available among the states. In 2000, the American Academy of Pediatrics' Newborn Screening Task Force published a report indicating that greater uniformity was needed among programs to assist families, professionals, and public health agencies.[3,9] As a result, HRSA's Maternal and Child Health Bureau contracted with the American College of Medical Genetics (ACMG) to outline a process to improve uniformity, which resulted in the creation of a recommended uniform panel of conditions.[10] The ACMG taskforce used data from the National Newborn Screening and Genetics Resource Center to assist with its assessment.[11] Factors in the assessment included the strength of scientific evidence, availability of a screening test, presence of an efficacious treatment, level of understanding of the natural history of the condition, and whether the condition was part of either the differential diagnosis of another condition or the screening test results related to a clinically significant condition. Using these parameters, conditions were categorized as either core or secondary target conditions or deemed not appropriate for NBS. The ACMG taskforce recommended that state NBS programs mandate testing for core conditions and report secondary target conditions that could be identified during screening, including clinically significant conditions and the definitive identification of carrier status.[10]

The development of a core panel, which is now called the Recommended Uniform Screening Panel (RUSP), has been the responsibility of the U.S. Secretary of Health and Human Services' (HHS Secretary's) Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC).[12] SACHDNC has used a nomination and evidence-review process to identify new conditions that should be included on RUSP. The HHS Secretary has traditionally approved or rejected nominations from SACHDNC, and these recommendations serve as guidance for NBS programs to develop state screening panels.[13] In 2010, SACHDNC recommended to the HHS Secretary that severe combined immunodeficiency and critical congenital heart disease be added to the RUSP.[12] Both conditions were approved, bringing the RUSP to 31 conditions. States have been working to adopt the RUSP despite barriers to implementation for some conditions, including lack of funding and other resources.

Subsequent to the Guthrie bacterial inhibition assay for PKU, there have been many technological advances in NBS, including radioimmunoassay, colorimetric and fluorometric immunoassays, isoelectric focusing, high-performance liquid chromatography, MS/MS, and molecular testing (e.g., deoxyribonucleic acid [DNA] tests). In the 1990s, MS/MS allowed the simultaneous testing of an array of metabolic conditions using a single 3 millimeter-sized specimen punched from a dried blood spot.[14] MS/MS was well-suited for the analysis of amino acids and acylcarnitines in dried filter-paper blood specimens.[14,15] It provided a revolutionized means to better use the limited blood specimen and increase screening capabilities through improved sensitivity and specificity.[14] As MS/MS increased in use, NBS programs relied on organizations such as CDC for assistance with quality assurance services. The Newborn Screening Quality Assurance Program assisted state health departments and laboratories in maintaining and enhancing the quality of test results by providing proficiency testing, reference materials, consultation, and training.[16]

Research advances in the late 1980s and early 1990s enabled the extraction of DNA from dried blood spots on filter paper. Subsequently, DNA testing was introduced into NBS, allowing the dual use of the dried blood spot specimen matrices for both biochemical and molecular tests.[17] DNA testing in the context of NBS has, until recently, been primarily used as a second-tier

test for conditions such as cystic fibrosis. It has recently expanded to other uses in programs and as part of the diagnostic work-up as follow-up to the newborn screen.

In NBS, second-tier molecular testing is performed after a primary test using the same specimen. It can improve sensitivity and specificity, increase the speed of diagnosis and treatment, and reduce the number of false-positives that can add significant cost to follow-up.[18] Molecular testing allows for differentiation between specific disorders, such as sickle cell anemia and sickle/beta-thalassemia.[19] Although testing varies by state, second-tier molecular tests are performed for conditions such as hemoglobinopathies, galactosemia, cystic fibrosis, and medium-chain acyl-CoA dehydrogenase deficiency. In 2008, the Wisconsin NBS program began screening for severe combined immunodeficiency (SCID), marking the first time a program used molecular technology as the primary screen.[20,21] The Association of Public Health Laboratories (APHL) has been collaborating with CDC's Newborn Screening and Molecular Biology Branch to address recent trends and developments in molecular testing. This collaboration has led to the development of a data-sharing molecular resources website for NBS programs as well as the molecular assessment program, where NBS laboratories can receive an assessment of their molecular capabilities.[22] Quality improvement for laboratory tests, as well as for the NBS system as a whole, will continue to be a priority in the years to come.

The prospects for advancing NBS are significant in light of new technologies. Microfluidic techniques have been developed to simultaneously perform many of the analytical procedures used in NBS laboratories, including current immunoassays, enzyme assays, and molecular methods.[23] Some of these lab-on-a-chip platforms allow for several or all testing methods to be performed on a single, highly compact chip. Test platforms have become efficient, able to perform high-throughput testing on small volumes of patient samples. They are designed to handle everything from specimen preparation to specimen processing, mixing, incubation, and detection.[23] It is possible that these platforms will be used at the bedside, but they will more likely be used in the hospital's clinical laboratory. In theory, the newborn's test results would be known before discharge, allowing for faster time to diagnosis and treatment initiation. This type of testing would also reduce the number of missed diagnoses due to untestable specimens or the inability to locate infants with out-of-range results after discharge. Additionally, DNA sequencing technologies are entering clinical laboratory practice and, in some circumstances, may augment current NBS strategies. Further work is needed to determine if DNA sequencing has utility in this setting and whether the benefit of using this technology outweighs the cost.

Advances in our understanding of the human genome and associated technologies are anticipated to provide important tools for evolving NBS services. The map and sequence of the human genome were completed in 2003, thus enhancing the study of genetic disease and predisposition. In the future, entire human genomes may be sequenced at birth, allowing individuals to have the option of receiving information about later-onset diseases for which effective interventions may be available. In 2012, The National Human Genome Research Institute and the Eunice Kennedy Shriver National Institute of Child Health and Human Development initiated a $25 million grant program to fund studies that explore how genome sequencing may be used in NBS. The intent of this program was to encourage research associated with the challenges and opportunities of applying whole genome sequencing or whole genome data into newborn care.[24] As research in this area continues, there will be debate regarding the risks and benefits of disclosing and using genetic information in the NBS realm.[25] Genome sequencing provides the means to report health-related information that goes beyond immediate risks to the newborn. The extent to which NBS programs will evolve in this direction requires significant consideration.

CHALLENGES FOR NBS PROGRAMS

Logistical, ethical, and legal challenges have always existed for NBS programs. Most recently, some of the most prominent issues have involved informed consent, justification for the storage and use of residual dried blood spots, addressing additional information that results from genetic testing, and dealing with practical issues that affect NBS programs, such as increased costs.

Most state NBS programs have an opt-out policy, requiring testing for all newborns unless parents or guardians decline testing due to religious or other reasons. Some groups have opposed these opt-out policies, arguing that parents and guardians should submit consent documentation for testing.[26] Screening programs have typically relied on the opt-out policy with the premise that the best interest of the child is priority and should override the family's decision-making rights, as many NBS conditions have severe and rapid consequences when undetected and left untreated.[27] NBS programs continue to work with organizations such as APHL and Genetic Alliance to encourage NBS by educating parents and providers on the benefits of screening babies.[28,29] Additionally, screening programs

ensure that measures are in place to protect privacy and confidentiality throughout the process.

Residual sample present on dried blood spots has proven absolutely essential as quality control material, for the improvement of current methods, and to add new conditions to the RUSP. After screening, a small amount of dried blood remains on the filter paper card.[30] This residual blood is often stored for varying durations for use by laboratories in accordance with state statutes and/or policies and may not require parental consent.[30–32] Justifying the importance of using and storing residual dried blood spots when NBS has been completed is a sensitive issue for NBS programs and parents.[27] Residual dried blood spots are primarily used for internal laboratory quality control and quality assurance purposes, including confirmation of original results, method validation, assay quality control, and lot-to-lot reagent validations, which are required of clinical laboratories. Residual dried blood spots are also used for quality improvement initiatives, such as refinement of current methodologies and new method development.[32] These activities are performed by the testing laboratories and support the public health mission to improve the NBS system.

Some state NBS programs allow controlled access to residual dried blood spots for purposes other than NBS, including requests for additional screening as well as for academic, public health, or medical research.[27] The Michigan Newborn Screening Program stores residual dried blood spots indefinitely in the Michigan BioTrust for Health, where specimens may be used for research purposes such as studying birth defects, genetic and chronic diseases, and exposures to toxic substances.[33] Additionally, the Newborn Screening Translational Research Network has developed a Virtual Repository of Residual Dried Blood Spots, where researchers can have access to de-identified information from more than two million dried blood spots.[34] Although the use of residual dried blood spots is proving beneficial in many arenas, it has not been without challenges.

In recent years, Texas and Minnesota courts handled lawsuits pertaining to issues of storage and use of residual dried blood spots. In Texas, privacy concerns brought up in the case of *Beleno et al. v. Texas Department of State Health Services et al.* (2009)[35] led to changes in legislation that required a parental option to request the destruction of residual dried blood spots after completion of NBS. As a result of the case of *Higgins et al. v. Texas Department of State Health Services* (2012), further statutory changes became effective requiring parental consent for the use of residual dried blood spots for public health research outside the state public health agency and storage for more than two years.[36] The Minnesota Supreme Court ruled against the Minnesota Department of Health in the case of *Bearder et al. v. State of Minnesota* (2011).[37] Written consent is now required for the long-term storage and use of residual dried blood specimens and test data. Legislation was passed in 2012 specifying timelines by which blood specimens and test data must now be destroyed. Specimens with negative results may be retained for 71 days, presumptive positive specimens for two years, and data for two years unless authorized by written consent.[38]

NBS programs are making it a priority to ensure the transparency of state policies and create an atmosphere of open dialogue with the public when it comes to issues related to the storage, use, and destruction of residual dried blood spots. Education about the benefits of using residual dried blood spots to support NBS and the public health mission may help alleviate public misperceptions.[28] Residual dried blood spots are a unique matrix and an invaluable resource for quality control and improvements in NBS. Evidence exists that these residual dried blood spots can be used anonymously, responsibly, and without privacy risk to the infant from whom the blood was collected. Therefore, continued efforts are important to protect this valuable resource.

One of the byproducts or results of NBS is that occasionally, clinical and family information is revealed from the screen, including carrier status. For example, newborns heterozygous for hemoglobinopathies, cystic fibrosis mutations, and other conditions may be detected by screening. State programs differ with reporting and follow-up services for detected carriers. Additionally, DNA sequence analysis performed as part of screening protocols can identify variants of unknown clinical or functional significance, making it difficult to interpret their impact on infant health. Although some variants may be benign, there are inadequate data to assess whether they cause disease. Information about paternity or about a mother's genetic risk may be identified through molecular testing of family members during clinical follow-up. Biochemical testing can also elucidate a mother's genetic status. State NBS programs are typically restricted to reporting results solely from tests on their approved panels. However, when programs report only the mandated information when more is available, it may appear that important medical information was withheld.[39] For example, there are several conditions that can be detected through NBS that do not fit the description of classic SCID, and these conditions may or may not be reported.[40] The need for consensus recommendations for reporting and follow-up of this additional clinical information obtained as a result of NBS exists for the public health community.

CONCLUSIONS

As advancing technologies allow for the detection of biomarkers for more conditions and public advocacy increases, there will be continued pressure for state NBS programs to expand their screening panels. However, many programs are experiencing stagnant or reduced funding levels, making this goal difficult to attain. Implementing new conditions for screening requires significant effort, not only in developing and implementing the laboratory test, but also in quality management, follow-up, diagnosis, and the education of parents and the medical community. Test development requires expenditures for instrumentation and equipment, personnel, supplies/reagents for validation studies, the availability of residual blood specimens from infants with the condition, and regulatory inspections. These considerations, along with the limited amount of blood on a specimen collection form, have driven laboratories to employ a strategic process of adding new disorders to their NBS testing panels. Fortunately, improvements in the sensitivity and specificity of screening technologies, as well as advances in multiplexing capability, or the screening of multiple disorders at the same time, will make future program expansions more practical and help keep costs more manageable. NBS programs continue to collaborate with federal agencies, parent advocates, and public health organizations to find solutions to these challenges as part of their mission to provide high-quality services.

Since 1963, NBS programs have worked to establish a comprehensive system that identifies, saves, and improves the lives of infants affected with a variety of genetically based conditions. The lessons learned over time and the advent of molecular testing have led NBS programs to incorporate new ideas, technologies, and processes into their systems. Continued emphasis on applying technologies to screen for and detect genetic disorders is critical for advancement and quality improvement. NBS programs will be critical in determining which technologies will lead to improvements in the overall health of newborns and can appropriately be integrated into the NBS setting.

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