

Screening Newborns for Congenital Disorders

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ABSTRACT

The Newborn Screening Laboratory at the Wisconsin State Laboratory of Hygiene (WSLH) tests all newborn babies in the state of Wisconsin for 26 congenital disorders. The screening is mandated by state statute (253.13) and attempts to identify those babies at highest risk for any of the screened-for congenital disorders. The Newborn Screening Laboratory at the WSLH is part of the state's newborn screening program, a cooperative effort between the Wisconsin Department of Health and Family Services, state birthing hospital staff, several public and private specialty clinics, primary care providers, and parents. Screening occurs within the first hours (median 38 hours) of life when a few drops of blood from the baby's heel is collected, applied to a special paper, dried, and sent to the WSLH for analysis. Those babies determined to have at-risk test results get repeat testing to confirm the initial test results and, if warranted, the baby is referred to a specialty clinic for a diagnostic work-up and treatment if necessary. Since its inception in the early 1960s, newborn screening in Wisconsin has saved approximately 1300 children from serious mental or other medical problems.

PUBLIC HEALTH IMPORTANCE

Screening mass populations for congenital disorders has proven to be a very successful and well-accepted public health activity.¹ Most babies born with 1 of the screened-for disorders appear very "normal" at birth and don't show clinical symptoms for a few weeks or months after birth. Even when clinical symptoms are obvious, the diagnosis is often difficult, due to similarities with other childhood medical conditions, and is often too late to reverse the clinical damage that has already occurred. Therefore, for maximum benefit to the infant, detection must occur before clinical symptoms

are evident. Children affected with these disorders, without early detection and treatment, usually depend upon other state-funded programs for long-term care. For example, average lifetime cost of 1 child institutionalized with untreated phenylketonuria (PKU) is approximately \$5 million. Since the early 1960s, about 200 children have been spared this fate, saving approximately \$1 billion in health care costs. This estimate does not include the benefits realized from the fact that these children also become productive members of our society.

ORGANIZATION OF NEWBORN SCREENING (NBS) IN WISCONSIN

Newborn screening is a program consisting of 5 major activities: administration, laboratory testing, patient follow-up, diagnosis/treatment, and program evaluation.² The program administration and evaluation are the responsibility of Wisconsin Department of Health and Family Services (DHFS) and include policy development, treatment services (i.e., dietary supplements), and education for professionals (The Wisconsin Health Care Professionals' Guide to Newborn Screening) and families (parent brochures and disorder fact sheets). The WSLH provides state-of-the-art primary laboratory testing recognized nationally for its quality. The laboratory staff also assists clinicians with the follow-up testing of initial non-normal results to assure that appropriate re-testing is completed. Diagnosis and treatment of affected children are provided by teams of specialists, including expert physicians, nurses, genetic counselors, dietitians, and other support staff at selected public and private metabolic, endocrine, hemoglobinopathy, and cystic fibrosis clinics in the state. Although specialized care programs are created by disorder-specific specialists, routine health care is often the responsibility of the family's primary care provider.

Nationally there are several organizations that are involved in helping or improving newborn screening programs in this country. Through the Association of

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Table 1. History of Newborn Screening at the WSLH

Year	Disorders Added or Deleted
1966	Phenylketonuria (PKU)
1978	Hypothyroidism, Galactosemia, Maple Syrup Urine Disease
1982	Homocystinuria
1988	Hemoglobinopathies (Sickle Cell Disease)
1991	Biotinidase
1992	Discontinued Homocystinuria and Maple Syrup Urine Disease
1993	Congenital Adrenal Hyperplasia (CAH)
1994	Cystic Fibrosis (CF)
2000	Fatty Acid Oxidation (7) and Organic Acidemia (7) disorders
2003	Maple Syrup Urine Disease, Homocystinuria, Tyrosinemia, Citrullinemia, Argininosuccinic Acidemia (ASA)

Public Health Laboratories (APHL), newborn screening issues are addressed at regular symposia where personnel from the state programs convene to share and discuss program operational and research experiences. The Centers for Disease Control and Prevention (CDC) address the important issue of testing quality by providing a proficiency testing program for all state, private, or regional newborn screening laboratories. The Maternal and Child Health Bureau of the Health Resources and Service Administration (HRSA) provides resources to state screening programs to help improve testing activities and delivery of appropriate information to primary care providers and/or families. Many resources are provided through the HRSA-supported National Newborn Screening and Genetics Resource Center (NNSGRC). The Wisconsin laboratory staff has been a very active participant in many national newborn screening activities, including membership in the NNSGRC newborn screening advisory committee, participation on site reviews where an individual state program is assessed and improvement recommendations are provided, service on various issues-related committees, and presenting the Wisconsin experience at national meetings as well as organizing and hosting one of the annual national meetings.

HISTORY OF NEWBORN SCREENING IN WISCONSIN

Newborn screening in Wisconsin began in the early 1960s with the introduction of phenylketonuria (PKU) testing using a dried blood specimen. A 1965 statute (Wis 146.02) mandated screening for PKU and other inborn errors of metabolism. Since that time, re-

searchers have developed methods for detecting many other congenital disorders on dried-blood specimens. The first major change in the PKU statute occurred in 1978 when 3 additional disorders (hypothyroidism, galactosemia, and maple syrup urine disease) were added to the screening panel. In addition to mandating these 3 disorders, all testing was centralized to the WSLH. Prior to 1978, the PKU testing was done in many local hospital and clinical laboratories. The rationale for centralizing the testing was based on several perceived advantages. Centralization would minimize the potential for missed cases (liability) because every baby is tested under uniform controlled conditions (analytical methods, quality control/assurance, and experienced staff). The test results are available sooner because every specimen is analyzed on the day of receipt in the centralized NBS laboratory, whereas daily testing is generally not feasible in the hospital laboratory because specimen volume is often not sufficient to efficiently carry out the needed testing and quality assurance protocols. Prompt turnaround of test results is essential because of the life-threatening nature of galactosemia and maple syrup urine disease. Lastly, an effective follow-up system can assure that all affected children statewide are entered into the system, properly diagnosed and treated. A significant program change occurred in 1992 that removed the statutory language mandating specific disorders. It was replaced with statutory language allowing the Wisconsin DHFS to assume, by administrative rule, disorder addition or deletion responsibility. The DHFS has established a Newborn Screening Advisory Group to assist in this activity. The advisory group's first action was to establish criteria to be used making disorder addition or deletion decisions. The criteria include disorder frequency, associated morbidity and mortality, the potential for successful treatment, availability of technology, and screening costs. The chronological addition of disorders to the newborn screening panel is outlined in Table 1. Note that maple syrup urine disease and homocystinuria that were discontinued in 1992 were re-introduced in 2003 due to population demographic changes (more at-risk families moving to the state) and technology improvements allowing more reliable detection of these disorders at lower analyte levels.

SCREENING RESULTS

Table 2 summarizes the screening results from the start of centralized screening, begun in 1978, at the WSLH. During the 25 years of screening newborns at the

WSLH, many improvements have been made to various screening protocols. The following examples of program evolution illustrate how technology and experience have increased sensitivity and specificity of the screening.

Phenylketonuria

The lack of milk feeding prior to testing was recognized to be a cause of false-negatives in the detection of PKU, attributable, in part, to the qualitative technology (the Guthrie bacterial inhibition test [BIA]³) used to measure the amino acid phenylalanine. The Wisconsin laboratory recognized the BIA limitations as early as 1978 and developed a quantitative chemistry test that had much greater sensitivity than the BIA.⁴ The use of this chemistry (fluorometric) test made the detection of PKU less dependent upon the feeding status of the baby prior to blood collection. Testimony to this improved detection ability was the detection of 2 babies with PKU in the 1980s in which the NBS specimens were collected at less than 7 hours of age. In both cases, the phenylalanine level was greater than the established cut-off level.

Galactosemia

In this disorder, the lactose metabolites (galactose and galactose-1-phosphate) may not be elevated in babies who have received little or no lactose (milk) feeding prior to specimen collection. Recognizing this fact as early as 1978, the laboratory developed a 2-tiered testing process that included the addition of enzyme (galactose-1-phosphate uridyl transferase) testing for all babies falling into the highest 5% of the daily metabolite results. This 2-tiered testing process was successful in detecting 4 babies with galactosemia that had little or no lactose intake prior to specimen collection. In 2 of these cases, because of family history, amniocentesis had diagnosed galactosemia prior to birth. The mother's lactose intake was restricted during pregnancy. Specimens were collected 24 hours after birth in these 2 cases before any feeding and the screening laboratory was successful in detecting galactosemia in these babies.

The PKU and galactosemia testing processes described above were extremely beneficial in the early 1990s when babies and their mothers were being discharged from the hospital in 24 hours or less. Since most of the newborn screening laboratories in this country were still using BIA for the measurement of phenylalanine, there was great concern about the ability to detect these disorders in early-discharge specimens.⁵ This concern was considerably less in Wisconsin

Table 2. Confirmed Cases and Prevalence of Disorder in Wisconsin Population since 1978's Centralized Screening

Disorder	Number Screened	Confirmed Cases	Prevalence
Phenylketonuria	1.7 million	162	1:15,880
Hypothyroidism	1.7 million	498	1:3,464
Galactosemia	1.7 million	30	1:57,507
Maple Syrup Urine Disease	1.1 million	0	—
Homocystinuria	750,000	0	—
Hemoglobinopathies	1.0 million	289	1:3,460; 1:242*
Biotinidase	850,000	8	1:100,000
Congenital Adrenal Hyperplasia	720,000	50	1:13,189
Cystic Fibrosis	600,000	136	1:4,338
Fatty Acid Oxidation Disorders	185,000	24	1:7,754
Organic Acidemia Disorders	185,000	22	1:8,459
Tyrosinemia	20,000	0	—
Citrullinemia	20,000	0	—
Argininosuccinic Acidemia	20,000	0	—

* African Americans

due to the increased sensitivity and specificity of the testing processes used. Although the disorder-detection sensitivity in early-discharged babies is very high, specimens collected prior to 24 hours of age should be repeated, as recommended by the American Academy of Pediatrics.⁶

Hypothyroidism

This devastating disorder affects approximately 1 in every 3400 babies born in Wisconsin, or about 20 per year. With the early hospital discharge situation in the 1990s, there was concern that hypothyroidism in a baby might go undetected because the thyroxine (T4) level, the primary test used at the time, would not be low enough to separate babies with the disorder from those without it. In fact, several Wisconsin babies with hypothyroidism had initial T4 levels very close to the cutoff level. The thyroid stimulating hormone (TSH) levels—the second-tier test—on these babies were clearly elevated. This fact resulted in a decision to replace the T4 testing assay with a TSH assay in 1996. The identification of hypothyroidism in premature babies, however, has been less than ideal. In at least 3 premature babies, the initial thyroid-stimulating hormone (TSH) levels were normal when the specimen was collected in the first day or two of life and extremely elevated when repeat specimens were collected upon hos-

pital discharge (3 to 4 months of age). A hypothyroidism diagnosis was made in each case. Early in 2002, in an effort to decrease the time at which diagnosis is made in premature babies, re-testing guidelines were issued that requested an initial specimen by the sixth day of life, a second specimen at 2 weeks, a third at a month, and re-testing monthly thereafter until discharge. These guidelines were responsible for the identification of hypothyroidism in 2 premature babies in 2002, possibly several weeks earlier than if a second specimen had been taken only at hospital discharge.

Congenital Adrenal Hyperplasia (CAH)

Screening for the 21-hydroxylase type of CAH by measuring 17-hydroxyprogesterone (17-OHP) has been very successful in preventing early death in male babies with the salt-losing condition. Although some females have also been detected with salt-losing CAH, there have been female babies with variant types of CAH that have escaped detection on the newborn screen. In these cases, the initial 17-OHP levels have been just below the cutoff but continued to increase in the weeks after birth. In 1 female infant, the initial 17-OHP was borderline (56 ng/mL) at 2 days of age, rose slightly (76 ng/mL) by a week of age, and reached a panic level (113 ng/mL) at 1 month of age. Since the current testing process does not routinely capture these cases, the NBS laboratory is investigating a 2-tiered testing process with an initial screen for 17-OHP levels followed by DNA analysis for several common CAH mutant alleles.

Another example of a NBS testing dilemma involved a male baby whose specimen was delayed 7 days in the US Postal Service. When testing was performed, the 17-OHP level was well above the panic level. When the physician was notified, the baby had already been admitted to a neonatal intensive care unit and was not expected to survive, but 4 days after treatment for CAH was started, the baby was well enough to go home. The issue of specimen delay has been minimized by the NBS laboratory providing, free-of-charge to all Wisconsin hospitals, next-day courier service by United Parcel Service.

Cystic Fibrosis (CF)

In 1985, the NBS laboratory collaborated with the University of Wisconsin Cystic Fibrosis Clinic, under the direction of Philip Farrell, MD, PhD, to investigate the benefits of early detection of cystic fibrosis.⁷ With funding provided by the National Institute of Health, Wisconsin conducted one of the largest prospective human health studies ever attempted involving over

600,000 babies born in Wisconsin between the years 1985-1994. The NBS laboratory developed a testing process that involved randomizing the baby specimens into 2 groups. One group was tested immediately for CF and treatment for affected babies begun within the first few weeks of life. Cystic fibrosis-affected children in the other group were detected clinically. The health status of affected children in the 2 groups was compared to determine the benefits of early detection. The NBS laboratory also developed an analytical testing process based upon a 2-tiered testing protocol that includes an initial test for the pancreatic enzyme, immunoreactive trypsinogen, followed by DNA analysis for the Δ F508 mutant allele (the most common and severe CF mutation). In 2002, the number of mutant CF alleles routinely screened for was expanded to 25. This 2-tiered testing process became the national model used by several other state newborn screening programs that have implemented CF screening in recent years.

FATTY ACID OXIDATION, ORGANIC ACIDEMIA, AMINOACIDOPATHIES

In the late 1990s, the introduction of newborn screening testing based upon tandem mass spectrometry technology allowed for the routine testing for 3 classes of metabolic disorders: fatty acid oxidation, organic acidemia, and aminoacidopathies. In 2000, the NBS laboratory was one of the first state public health newborn screening laboratories in the nation to implement tandem mass spectrometry technology.⁸ This technology introduced a new testing paradigm, changing operation from a "1 analytical method" per disorder approach to a laboratory operation where 1 analytical method is capable of detecting multiple disorders. With the use of tandem mass spectrometry, 20 different disorders are simultaneously identified from a single 1/8" dried-blood spot. Table 3 lists each of the current 26 disorders screened for, the analyte measured, and type of technology used.

REPORTING POLICIES AND PRACTICES

Since newborn screening attempts to identify those babies at highest risk for specific congenital disorders, reporting protocols are designed to detect all potentially affected babies. By emphasizing high sensitivity, screening a large population will necessarily result in a number of false-positives. The WSLH has tried to minimize the number of false-positives by establishing, through its advisory committees, highly refined cut-offs based upon such parameters as birth weight or

Table 3. Newborn Screening Technology

Disorder	Analyte Measured	Technology
Phenylketonuria (PKU)	Phenylalanine	Tandem Mass Spectrometry
Hypothyroidism	Thyroid Stimulating Hormone	Immunochemistry
Galactosemia	Galactose	Chemistry (quantitative)
	Galactose-1-Phosphate	
	Galactose-1-Phosphate Uridyl Transferase	
Maple Syrup Urine Disease	Leucine, Isoleucine and Valine	Tandem Mass Spectrometry
Homocystinuria	Methionine	Tandem Mass Spectrometry
Hemoglobinopathies	Hemoglobin Fractions	Electrophoresis (qualitative)
		Liquid Chromatography (quantitative)
Biotinidase	Enzyme	Chemistry (qualitative)
Congenital Adrenal Hyperplasia (CAH)	17-hydroxprogesterone	Immunochemistry
Cystic Fibrosis (CF)	Immunoreactive Trypsinogen	Immunochemistry
	mutation analysis (25 mutations)	Polymerase Chain Reaction
Fatty Acid Oxidation (7 disorders)	Acylcarnitines	Tandem Mass Spectrometry
Organic Acidemia (7 disorders)	Acylcarnitines	Tandem Mass Spectrometry
Tyrosinemia	Tyrosine	Tandem Mass Spectrometry
Citrullinemia	Citrulline	Tandem Mass Spectrometry
Argininosuccinic Acidemia (ASA)	Citrulline	Tandem Mass Spectrometry

specimen collection age. False-positives are further reduced by second-tier testing processes as previously described for galactosemia and cystic fibrosis. In addition to these modifications, non-normal test results are placed into 1 of 2 classifications. Those non-normal test results that are highly indicative of a disorder are telephoned to the primary-care provider and followed by a written "Definite" report. The recommendation is that the primary-care providers have confirmatory testing completed immediately, usually in consultation with a volunteer physician (specialist in the particular disorder). Those non-normal test results that are not considered urgent are reported as a "Possible" abnormal, with a recommendation to repeat the newborn screen. As a general rule, the primary-care provider is notified of "Possible" abnormal results by a written report only.

The newborn screening laboratory activities also include follow-up of all non-normal test results. The goal is to make sure that proper re-testing of the at-risk baby has been completed in a timely manner. With every non-normal result issued, laboratory staff contacts the primary-care provider regarding the re-testing progress, and for those babies with confirmed disorders, continues to monitor the child until treatment has begun (i.e., the baby has properly entered the health care system). This follow-up has prevented a number of affected babies from becoming lost to the health care system and possibly denying them essential timely treatment. One such case was a baby with an initial phenylalanine level that was non-normal, which prompted a call to the primary care provider requesting

a second NBS specimen. After several days, the newborn screening laboratory had not received the repeat specimen, which prompted a return call to the primary-care provider. The primary-care provider had sent an order to the clinic laboratory staff for a "neonatal" screen, which was interpreted as a "bilirubin" test. The bilirubin level was normal and the family was sent home. A second specimen sent to the NBS laboratory at about 10 days of age had an extremely elevated phenylalanine level that was clearly indicative of PKU.

FUTURE DIRECTIONS

As knowledge, treatment models, and technology advance, the list of disorders screened for at birth will continue to expand. Ongoing research suggests a number of disorders that might be included in future NBS programs: muscular dystrophy, type I diabetes, cerebral palsy, autism, hemochromatosis, and others not yet considered as positive interventions become available. The completion of the human genome project could potentially expand the number of genetic conditions tested for—especially as DNA molecular-based methods are developed. Further, screening is not limited to genetic abnormalities, but may also include infectious diseases such as human immunodeficiency virus (HIV) and cytomegalovirus. A 1990s national, CDC-funded, program demonstrated the ability of NBS programs to detect maternal HIV antibody. This program was done on blinded specimens.

Regardless of the make-up of future testing panels and advances in methodologies and therapy, newborn screening will continue to play an important role in the

detection and prevention of serious medical complications in Wisconsin's population. As with all public health, the benefit is in costs not encountered and expenditures not made. Wisconsin's newborn screening program is 100% fee-funded (no tax dollars). The laboratory cost, in 2003, is \$35.50 per baby or \$2.4 million per year. The lifetime institutional care for 1 PKU or hypothyroid child is approximately \$5 million. In a typical year, approximately 24 (4 PKU and 20 hypothyroid) children are spared the fate of severe mental retardation and possible institutionalization—resulting in a cost-benefit ratio of 1:50. The benefit to the child and family is incalculable; the benefits to society are immeasurable. The Wisconsin State Laboratory of Hygiene is proud of its role in this very important public health program.

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