The Role of Technology in Newborn Screening

David S. Millington

This commentary traces the expansion of newborn screening for inherited metabolic disorders during the past 55 years, from the first simple test for phenylketonuria to the current panel of over 35 conditions. Emphasis is placed on the role played by technology and the contributions made by researchers in North Carolina.

The Beginnings of Newborn Screening

Screening newborns in the full population for inherited metabolic disorders (IMDs) was the brainchild of Dr. Robert Guthrie, a New England physician and microbiologist with family members afflicted with phenylketonuria (PKU), a defect of phenylalanine metabolism resulting in profound and irreversible intellectual disability if untreated soon after birth. Dr. Guthrie's PKU screening method was based on a simple, inexpensive microbiological test known as the bacterial inhibition assay. It was performed on dried blood spots collected at birthing centers and mailed to the public health laboratory. The test identified at-risk infants based on elevated phenylalanine levels in the dried blood spots [1]. More advanced tests were utilized to ascertain those infants truly affected with PKU, who were promptly treated with a special diet that prevented the onset of intellectual disability. PKU screening began in New England in 1963 and subsequently spread to public health systems in all other states (North Carolina began statewide screening in 1966) and across most of the developed world.

Slow but Steady

The introduction of new dried blood spot tests in the United States for other IMDs required satisfaction of the population screening guidelines established by Wilson and Jungner in 1968 [2]. This required the existence of a suitable screening test, an effective preventive treatment, an early onset form of the disease that would be debilitating if not treated soon after birth, and overall cost-effectiveness, all of which generally took decades to come to fruition. Thus in North Carolina, for example, screening was gradually introduced for congenital hypothyroidism, galactosemia, and congenital adrenal hyperplasia (see Table 1). By the mid-1990s, most newborn screening programs in the United States were still screening for just three to five IMDs, each specific test requiring a separate dried blood spot sample and a laboratory equipped with the means to perform that test. The North Carolina public health laboratory (NCPHL) also began testing for sickle cell disease and sickle trait in 1987, and later expanded the hemoglobinopathy panel to include related conditions, including beta-thalassemia. Although technological improvements needed to screen for most of these conditions were introduced—liquid chromatography replacing the bacterial inhibition assay for PKU, for example—the practice of developing a single test for each IMD screened for was the prevailing paradigm, until the introduction of tandem mass spectrometry (MS/MS) disrupted the status quo.

The Introduction of Tandem Mass Spectrometry – A New Technology to Break the Mold

The MS/MS method for newborn screening was developed during the early 1990s with the help of a grant from the NCPHL to the Duke Biochemical Genetics Laboratory. It was adapted from a clinical diagnostic test for defects of fatty acid oxidation that required an "acylcarnitine profile"—a pattern of signals corresponding to up to 26 endogenous acylcarnitines plus several isotope-labeled internal standards—from which their concentrations in the specimen were measured [3, 4]. By applying the method to dried blood spots and adding simultaneously an amino acid profile, MS/MS became the first "multiplex" newborn screening test, targeting more than 30 IMDs from a single sample. North Carolina was the first state program to adopt this new method in 1997 [5]. Owing to the gradual adoption of MS/MS by newborn screening programs in other states and overseas, the sudden increase in IMDs accessible to newborn screening, many of which did not fit the accepted newborn screening paradigm [2], caused much controversy. Eventually, a committee convened by the American College of Medical Genetics and Genomics determined that MS/MS was capable of recognizing 20 IMDs with high confidence, which were deemed to be primary targets, plus an additional 22 IMDs that MS/MS *might* also detect, but with less confidence, referred to as secondary targets [6, 7] (see Table 2). This committee, which was later

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encompassed by the US Department of Health and Human Services Secretary's Advisory Committee on Heritable Disorders in Newborns and Children [8], also advised that newborn screening programs in the United States adopt a Recommended Uniform Screening Panel of 29 core conditions (the RUSP). Conditions proposed for addition to the RUSP are subject to evidence review by the advisory committee, which has subsequently recommended the inclusion of severe combined immunodeficiencies (SCID), critical congenital heart disease (CCHD), Pompe disease (glycogen storage disease-II), mucopolysaccharidosis type 1 (MPS 1), X-linked adrenoleukodystrophy (X-ALD), and spinal muscular atrophy (SMA), making a total of 35 core conditions at the time of writing. It is noteworthy that several North Carolina scientists and physicians have served on the advisory committee since its inception.

Expanded Newborn Screening and the Introduction of Screening for Lysosomal Storage Disorders

The adoption of multiplex screening using MS/MS, referred to as "expanded newborn screening," was a revolutionary development, giving impetus to the entire field of newborn screening to consider adding many more conditions and utilizing newer technologies. A significant driving force since the early 2000s has been the development of new therapeutic strategies for conditions that were previously considered untreatable. Thus, the publication of a novel MS/MS assay for the enzyme responsible for Krabbe disease [9], a lysosomal storage disorder (LSD), and successful hematopoietic stem cell transplant (HSCT) treatment for Krabbe [10] led to the introduction of newborn screening for this condition in the state of New York. In this case, the state legislature was lobbied by a special interest group to pass a new act, thus circumventing the consensus system. Lobbying has been an integral part of the history of expansion of newborn screening testing in the United States, including in North Carolina, and continues to influence decisions in several state programs despite the role of the DHHS Advisory Committee on Heritable Disorders in Newborns and Children. Indeed, as MS/MS methods for testing multiple LSDs appeared in the literature [11, 12] and later became available commercially, several state programs including those in Missouri and Illinois were lobbied successfully to screen for as many as five LSDs for which there were new emergent therapies (notably enzyme replacement therapy and hematopoietic stem cell transplantation) available. The MS/MS platform already in use for analysis of amino acids and acylcarnitines cannot simply add on LSD enzyme assays—these tests require additional tandem mass spectrometers and the infrastructure to support them. Most newborn screening programs at that time were not prepared, either financially or managerially, to fulfil these new obligations. In this scenario, another new technology—digital microfluidics (DMF)—became commercially available,

TABLE 1.
Summary of Conditions Screened for in North Carolina

Condition	Current Technology Used	Date of Implementation
Phenylketonuria (PKU)	Was BIA, then HPLC, now MS/MS	1966
Primary hypothyroidism (CH)	Fluorometry	1979
Galactosemia (GALT)	Fluorometry	1988
Congenital adrenal hyperplasia (CAH)	Fluorometry	1988
Sickle cell disease (FS, Hb S/S, others)	Isoelectric Focusing-HPLC	1987; 1994 (expanded)
Amino acid disorders (13)	MS/MS	1997
Organic acid disorders (11)	MS/MS	1997
Fatty acid disorders (9)	MS/MS	1997
Hearing Loss	(POC Test)	2000
Biotinidase (BIOT)	Colorimetry	2004
Cystic fibrosis (CF)	Fluorometry-DNA	2009
Critical congenital heart disease (CCHD)	Pulse oximetry (POC Test)	2014
Severe combined immunodeficiency (SCID)	TREC assay (DNA)	2017
Pompe disease (Pompe) 1	Pending	Pending
Mucopolysaccharidosis type 1 (MPS-I)[1]	Pending	Pending
X-linked adrenoleukodystrophy (X-ALD)[1]	Pending	Pending

Note. For a complete list of conditions, visit https://slph.ncpublichealth.com/newborn/DisordersTested.asp.
[1]Conditions approved for addition to the newborn screening mandate by the NC State legistature in 2018; implementation of these tests is pending the additional technology, infrastructure and personnel requirements. NCPHL is committed to screening for at least all 35 conditions currently on the RUSP including spinal muscular atrophy, and any new conditions added henceforth.

TABLE 2.
Disorders Detectable by Tandem Mass Spectrometry (MS/MS) Newborn Screening Based on the ACMG Recommended Uniform Screening Panel (RUSP)[6][1]

Core Conditions	Secondary Conditions
1. Propionic acidemia (PROP)	1. Methylmalonic acidemia with homocystinuria
2. Methylmalonic acidemia (mutase deficiency)(MUT)	2. Malonic acidemia
3. Methylmalonic acidemia (Cbl A,B)	3. Isobutyrylglycinuria
4. Isovaleric acidaemia (IVA)	4. 2-methylbutyrylglycinuria
5. 3-methylcrotonyl-CoA carboxylase deficiency (3MCC)	5. 3-methylglutaconic aciduria
6. 3-hydroxy 3-methylglutaric aciduria (HMG)	6. 2-methyl-3-hydroxybutyric aciduria
7. Multiple carboxylase deficiency (MCD)	7. Short-chain acyl-CoA dehydrogenase deficiency
8. ß-ketothiolase deficiency (ßKT)	8. Medium/short-chain L-3-hydroxyacl-CoA dehydrogenase deficiency
9. Glutaric aciduria type I (GA1)	9. Glutaric aciduria type II
10. Carnitine uptake defect (CUD)	10. Medium-chain ketoacyl-CoA thiolase deficiency
11. Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)	11. 2,4 mienoyl-CoA reductase deficiency[2]
12. Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	12. Carnitine palmitoyltransferase type I deficiency
13. Long-chain L-3 hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)	13. Carnitine palmitoyltransferase type II deficiency
14. Trifunctional protein deficiency (TFP)	14. Carnitine acylcarnitine translocase deficiency
15. Argininosuccinic acidemia (ASA)	15. Argininemia
16. Citrullinemia type I (CIT)	16. Citrullinemia type II
17. Maple syrup urine disease (MSUD)	17. Hypermethioninemia
18. Homocystinuria (HCY)	18. Benign hyperphenylalaninemia
19. Classic phenylketonuria (PKU)	19. Biopterin biosynthesis disorders
20. Tyrosinemia type I (TYR1)	20. Biopterin regeneration disorders
	21. Tyrosinemia type II
	22. Tyrosinemia type III

[1]The other core conditions that make up the 29 on the original RUSP are congenital hypothyroidism (CH), classic galactosemia (GALT), congenital adrenal hyperplasia (CAH), sickle cell anemia (SS), sickle cell diseases (SB and SC-Thal), biotinidase deficiency (BIOT), cystic fibrosis (CF), hearing loss (HEAR).
[2]This condition is now more correctly referred to as mitochondrial nicotinamide adenine dinucleotide phosphate NADP(H) deficiency (see Houten SM, et al. Hum Mol Genet. 2014;23(18):5009-5016).

with a promise to perform the same LSD enzymatic assays on a simpler platform.

Digital Microfluidics Makes its Mark

DMF refers to a method for manipulating individual droplets on the surface of a printed circuit board by software control using the principle of electrowetting [13]. One of the major developments of this principle emerged from the Duke University School of Engineering and with the help of government grants collaborated with researchers at Duke University Medical Center to develop assays intended for use at the point of care in the neonatal nursery. Further collaboration with the Duke Biochemical Genetics Laboratory and the North Carolina State Newborn Screening Lab resulted in a method based on microfluorometry to screen newborns for up to five LSDs using the extract from a single dried blood spot sample [14]. Advanced Liquid Logic introduced a practical DMF system to screen for four LSDs that was adopted by the Missouri state newborn screening program in January 2013. This system has operated continuously since then and has successfully tested over 350,000 newborns [15]. The DMF platform known as SEEKER™ received approval from the US Food and Drug Administration (FDA) in 2017. Since the recent addition of Pompe and MPS-1 to the RUSP, most US newborn screening programs have opted to screen for at least these two LSDs, with the choice of method roughly divided between MS/MS and DMF. Although the pros and cons of these methods have been hotly debated in the literature, recent results from Illinois, which uses MS/MS [16], and Missouri, using DMF [15], are quite similar. DMF is now well-established as a new technology in newborn screening and could, in the future, be deployed in birthing centers for conditions that are particularly time-critical, such as galactosemia, congenital hypothyroidism, and SCID, thus avoiding delays in their detection and treatment [17].

New Initiatives in North Carolina

Our state's newborn screening program has always prided itself on being progressive and open-minded with regard to adding conditions to its testing panel and embrac-

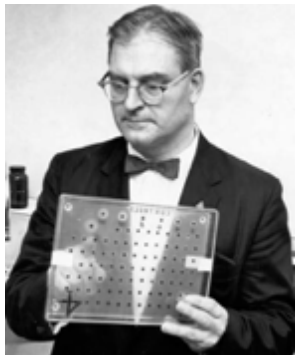
ing new concepts and technology when appropriate. Without their generosity of spirit and cooperation, it might have taken several decades longer for MS/MS to become an established technology in newborn screening. In order to better position North Carolina's response to new challenges, collaborative efforts between RTI International, based in the Research Triangle Park, the University of North Carolina at Chapel Hill, Duke University, and the North Carolina newborn screening Lab have facilitated pilot studies for MPS 1 and X-ALD. A new research initiative from RTI called "Early Check" [18] secured funding to initiate screening for Fragile X syndrome and spinal muscular atrophy on a research basis in North Carolina in the fall of 2018 (see article by Bailey and Gehtland in this issue [19]). Table 1 summarizes the current list of conditions targeted by newborn screening in North Carolina, with the dates of implementation.

Will New DNA Technology Create Another Revolution in Newborn Screening?

There has been a major revolution in DNA sequencing technology during recent years, and few can doubt its impact on the current and future practice of medicine [20]. DNA tests are already widely utilized in newborn screening, primarily as second-tier methods to improve the diagnostic specificity of primary screening tests, such as cystic fibrosis, for example. The primary newborn screening test for SCID uses a DNA-based assay, albeit for a unique biomarker rather than for identifying gene pathogenic variants [21].

FIGURE 1.
Technology Developments in the Newborn Screening Laboratory

From the bacterial inhibition assay (top left), to the benchtop microfluorometer (top right), isoelectric focusing gel electrophoresis (center left) high performance liquid chromatography (center right), tandem mass spectrometry (bottom left) and digital microfluidics (bottom right)



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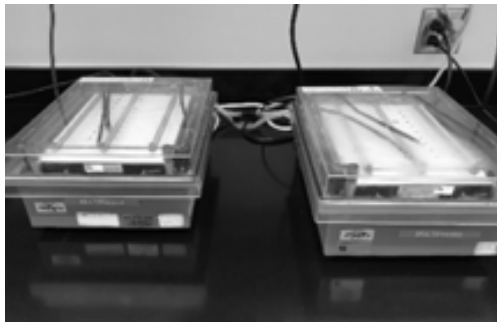

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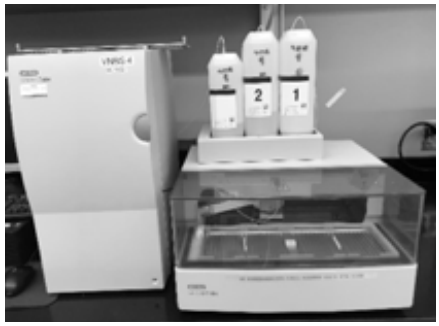

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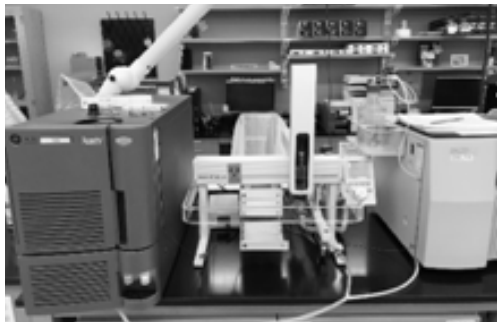

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Newborn screening for spinal muscular atrophy requires a targeted DNA test for deletion of exon 7 in the SMN1 gene because there is no suitable biomarker. A new research study to screen for Fragile X syndrome will also depend on novel DNA technology. But the big question remains: Is there a case for whole genome or exome sequencing as a primary newborn screening test for all newborns, rather than for a select group of neonatal intensive care unit (NICU) babies with undiagnosed clinical problems? Many believe so, leading to a sense of inevitability that we might expect to see this occur in at least one newborn screening program, possibly in Europe. Counter-arguments center mostly on cost, ethical/legal issues, and on lack of resources to follow-up on results from the initial testing, many of which will be uncertain or uninterpretable [22] and likely will prevent or delay genome sequencing in the United States. Approximately five years ago, the National Institute of Child Health and Human Development awarded grants worth $25 million to four centers in the United States, including the University of North Carolina, to study various aspects of next-generation DNA testing in newborns. The North Carolina project (NC-NEXUS) includes a research study to explore the utility of next-generation sequencing in newborn screening and parental decision-making [23]. The results of these studies will enable a deeper understanding of the issues surrounding DNA screening in newborns [20]. The most likely use of novel DNA sequencing technology in newborn screening is a comprehensive panel targeting conditions currently on the RUSP. This could provide valuable information, when combined with existing biochemical testing methods, to indicate or refute a potential diagnosis.

In summary, technology is a vital and principal force that moves newborn screening forward (see Figure 1). The North Carolina newborn screening program, through its collaboration with neighboring institutions, has achieved a unique record of success in the promotion and adoption of new technologies, with significant and ongoing benefit to the newborns in North Carolina affected by IMDs. NCMJ

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