60 Years of Newborn Screening in a Nutshell

MAYO CLINIC

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Medical Genetics, and Pediatrics
Biochemical Genetics Laboratory
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1

Learning Objectives

- **Demonstrate a basic understanding of newborn screening (NBS)**
- **Identify some differences between NBS programs**
- **Recall available tools to react appropriately to abnormal NBS results**

2

Newborn Screening

A public health program:

- **Aimed at identification of conditions for which early intervention can prevent**
  - **mortality**
  - **morbidity**
  - **disabilities**
- **Performed by analysis of diagnostic markers in blood spots collected on filter paper on the second day of life**
  (exception: hearing loss and congenital cyanotic heart disease)

3

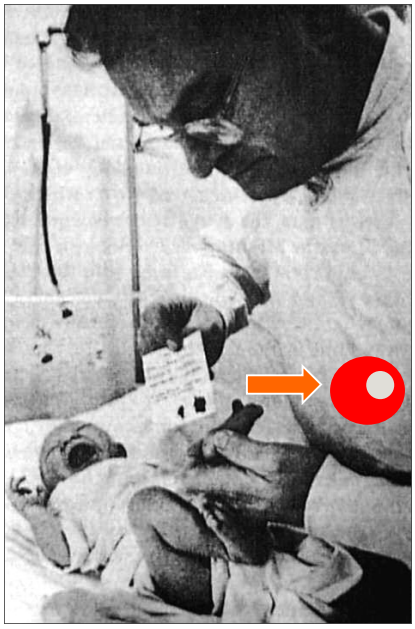
Newborn Screening Pioneers

Horst Bickel
1918-2000

Robert Guthrie
1916-1995

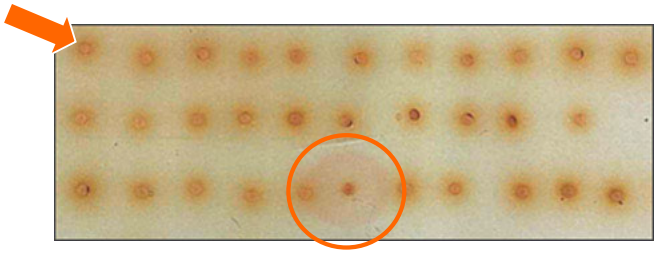
4

Newborn Screening: The Early Years



1958: Bacterial inhibition assay (BIA) for PKU

1961: Newborn screening for PKU



5

History of Newborn Screening

- 1962 JFK promotes a 20-state trial of the "Guthrie test"
- 1963 - Massachusetts mandates NBS for PKU
  - Oregon adds Galactosemia to NBS for PKU

6

The Traditional NBS Model: BIA

- One disease
- One test
- One marker
- One cut-off (N/Abn)

PKU
↓
BIA
↓
Phe
↓
4 mg/dL

7

History of Newborn Screening

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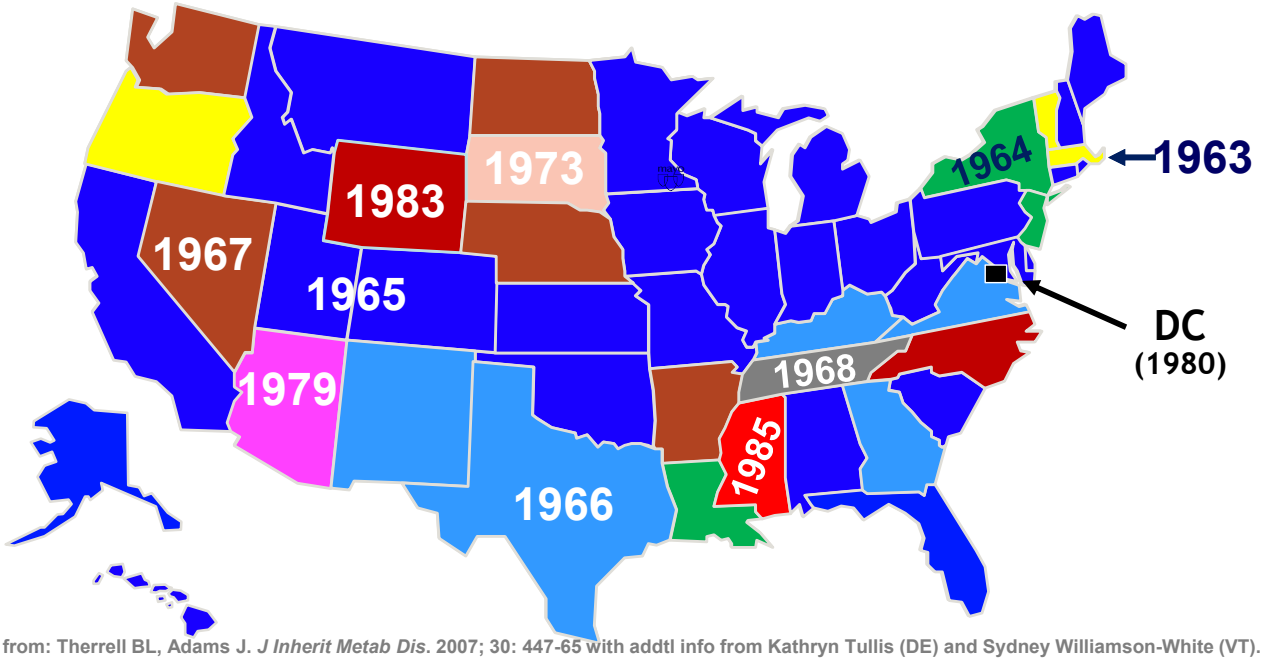
- 1963 MA mandates NBS for PKU; OR adds Galactosemia
- from 1963 NBS extends to all 50 states and includes metabolic and non-metabolic disorders

8

Newborn Screening Implementation in the USA



adapted from: Therrell BL, Adams J. *J Inherit Metab Dis*. 2007; 30: 447-65 with addtl info from Kathryn Tullis (DE) and Sydney Williamson-White (VT).

9

History of Newborn Screening

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10

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- **1989 McCabe* et al describe molecular genetic analysis as a 2nd tier test for sickle cell disease screening**

*Baylor College of Medicine, Houston, TX

11

MCAD Deficiency

First described by Kolvraa et al in 1982

Incidence: ~1 : 10,000 live births

Gene: ACADM (1p31) (common mutation 985A→ G)

Symptoms: - Hypoketotic hypoglycemia
- Reye-like syndrome
- Sudden unexpected death

Treatment: Avoidance of fasting, IV glucose during stress

Prognosis: - Excellent when treated before onset of symptoms
- 30-50% of mortality during first acute episode

Diagnosis: Acylcarnitine profile by tandem mass spectrometry (MS/MS)

12

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- **1993 Chace et al describe NBS for PKU using MS/MS**

13

Tandem Mass Spectrometry Systems (MS/MS)

14

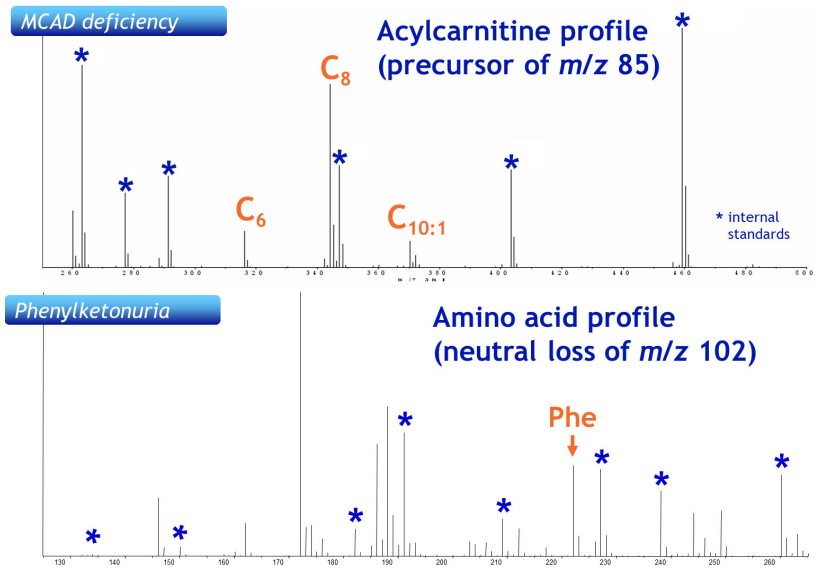
Application of MS/MS to NBS

- **Primary screening (multiplex platform)**
  - **Acylcarnitines, amino acids, succinylacetone, creatine*, creatinine*, guanidinoacetate***
  - **Lysosomal enzyme activities, lysophophatidylcholines^**
  - **(Bile acohols for Cerebrotendinous xantomathosis, bile acids for Niemann-Pick C disease, hemoglobin for Hemoglobinopathies)**

*for creatine deficiency disorders
^for X-adrenoleukodystrophy (ALD)

15

NBS by MS/MS



16

A New NBS Model: MS/MS

- Many conditions
- One test 
- Many markers
- Many cut offs

$(IEM)_n$

↓

MS/MS

↓

$(AA, AC)_n$

↓

0.1-1,000 µM

17

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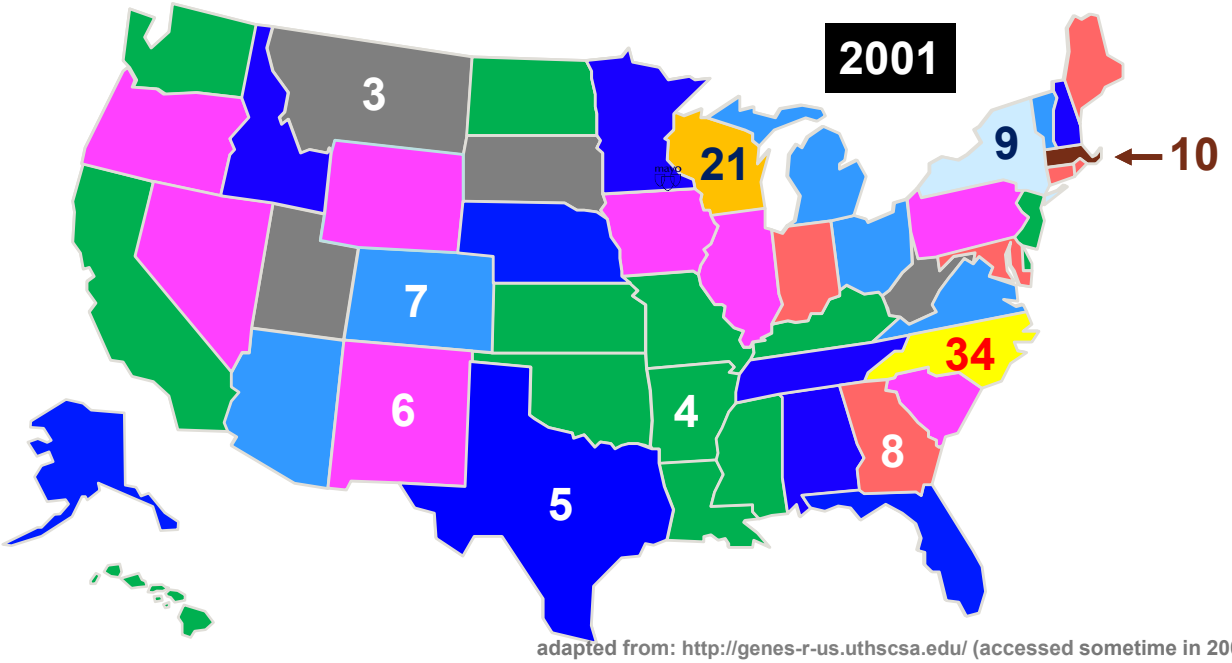
1993:	Chace et al describe NBS for PKU using MS/MS
1994:	**Molecular genetic analysis applied to CF screening (2nd tier test)**
1996:	**Naylor starts using MS/MS for NBS in a private lab (Neogen)**

18

Number of Conditions included in NBS Programs



adapted from: http://genes-r-us.uthscsa.edu/ (accessed sometime in 2001)

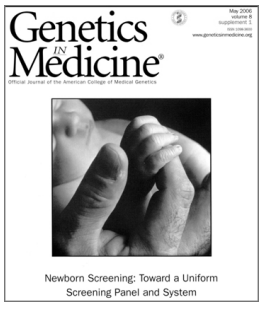
19

Expansion of Newborn Screening: Uniform Screening Panel

May 2006 · Vol. 8 · No. 5, Supplement

executive summary

Michael S. Watson, PhD, Marie Y. Mann, MD, MPH, Michele A. Lloyd-Puryear, MD, PhD, Piero Rinaldo, MD, PhD, and R. Rodney Howell, MD, editors



The Maternal and Child Health Bureau commissioned the American College of Medical Genetics to outline a process for the standardization of outcomes and guidelines for state newborn screening programs and to define responsibilities for collecting and evaluating outcome data, including a recommended uniform panel of conditions to include in state newborn screening programs. The expert panel identified 29 conditions for which screening should be mandated. An additional 25 conditions were identified because they are part of the differential diagnosis of a condition in the core panel, they are clinically significant and revealed with screening technology but lack an efficacious treatment, or they represent incidental findings for which there is potential clinical significance. The process of identification is described, and recommendations are provided. ***Genet Med* 2006:8(5, Supplement): 1S–11S.**

20

Newborn Screening Criteria

1968 - WHO (Wilson & Jungner)

- Treatable illness
- Detectable in newborn period
- Presymptomatic initiation of treatment is beneficial
- Available resources for diagnosis/treatment/follow-up
- Availability of a simple method for sample collection
- Evidence of substantial public benefit & acceptance
- Suitable and simple test methods
- Acceptable costs

2006 - ACMG Criteria

- Clinical characteristics (e.g., incidence, burden of disease if not treated, phenotype in the newborn)
- Analytical characteristics of the screening test (e.g., availability, features of the platform)
- Diagnosis, treatment and management of the condition in both acute and chronic forms (includes the availability of health professionals experienced in diagnosis, treatment, and management)

21

ACMG Panel: Final Scoring

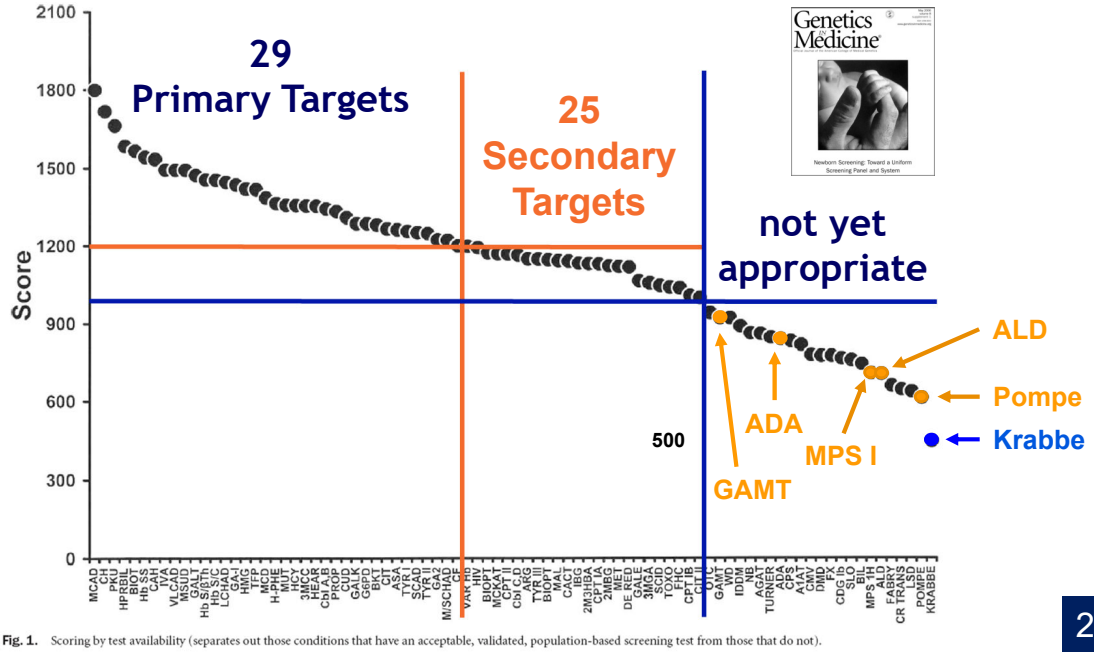
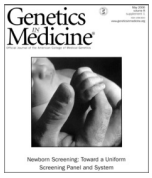


Fig. 1. Scoring by test availability (separates out those conditions that have an acceptable, validated, population-based screening test from those that do not).

2006

22



ACMG's Recommended NBS Panel

29 Core Conditions

25 Secondary Targets

Table 2
Newborn screening panel: core panel and secondary targets

MS/MS				
Acylcarnitines		Amino acids		
9 OA	5 FAO	6 AA	3 Hb Pathies	6 Others
CORE PANEL				
IVA	MCAD	PKU	Hb SS*	CH
GA I	VLCAD	MSUD	Hb S/βTh*	BIOT
HMG	LCHAD	HCY*	Hb S/C*	CAH*
MCD	TFP	CIT		GALT
MUT*	CUD	ASA		HEAR
3MCC*		TYR I*		CF
Cbl A,B*				
PROP				
BKT				

SECONDARY TARGETS				
6 OA	8 FAO	8 AA	1 Hb Pathies	2 Others
Cbl C,D*	SCAD	HYPER-PHE	Var Hb*	GALK*
MAL	GA2	TYR II		GALE
IBG	M/SCHAD	BIOPT (BS)		
2M3HBA	MCKAT	ARG		
2MBG	CPT II	TYR III		
3MGA	CACT	BIOPT (REG)		
	CPT IA	MET		
	DE RED	CIT II		

NOTE: Codes are as follows: OA, disorders of organic acid metabolism; FAO, disorders of fatty acid metabolism; AA, disorders of amino acid metabolism; Hb Pathies, hemoglobinopathies.
* Identifies conditions for which specific discussions of unique issues are found in the main report.

Watson MS et al. *Genet Med*. 2006; 8(5, Suppl 1): 1S-252S

23

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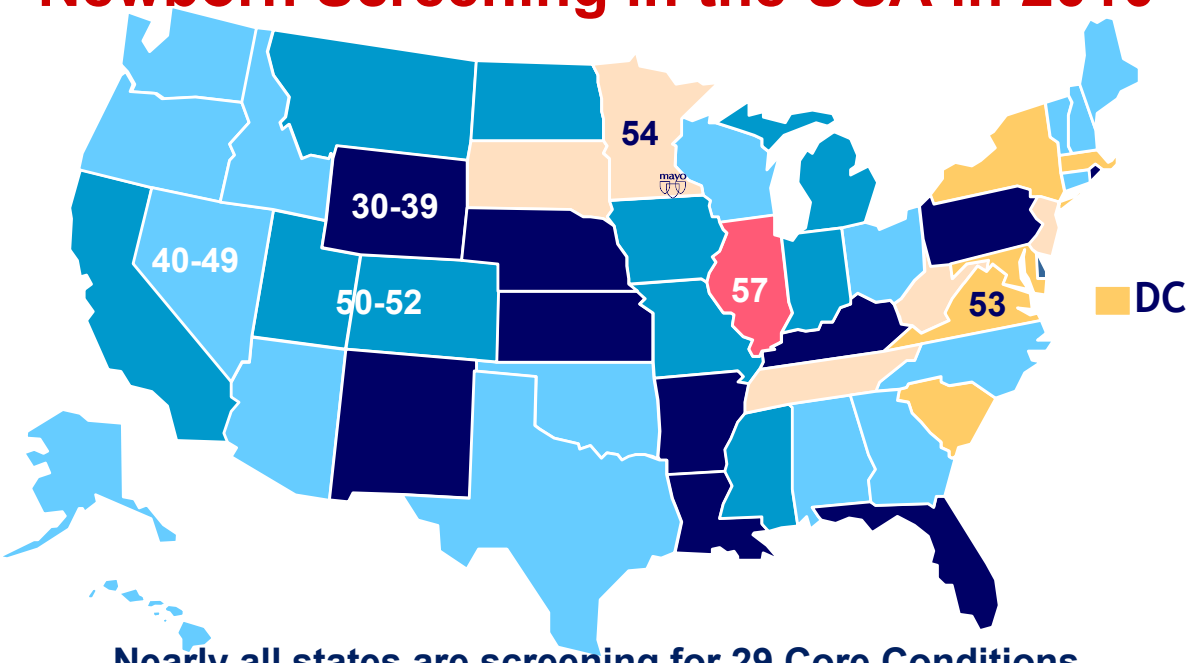
1996: Naylor starts using MS/MS for NBS in a private lab (Neogen)

2005: ACHDNC recommends adoption of ACMG recommended screening panel

2010: HHS Secretary adopts ACMG recommendation as "Recommended Uniform Screening Panel" (RUSP)

24

Newborn Screening in the USA in 2010

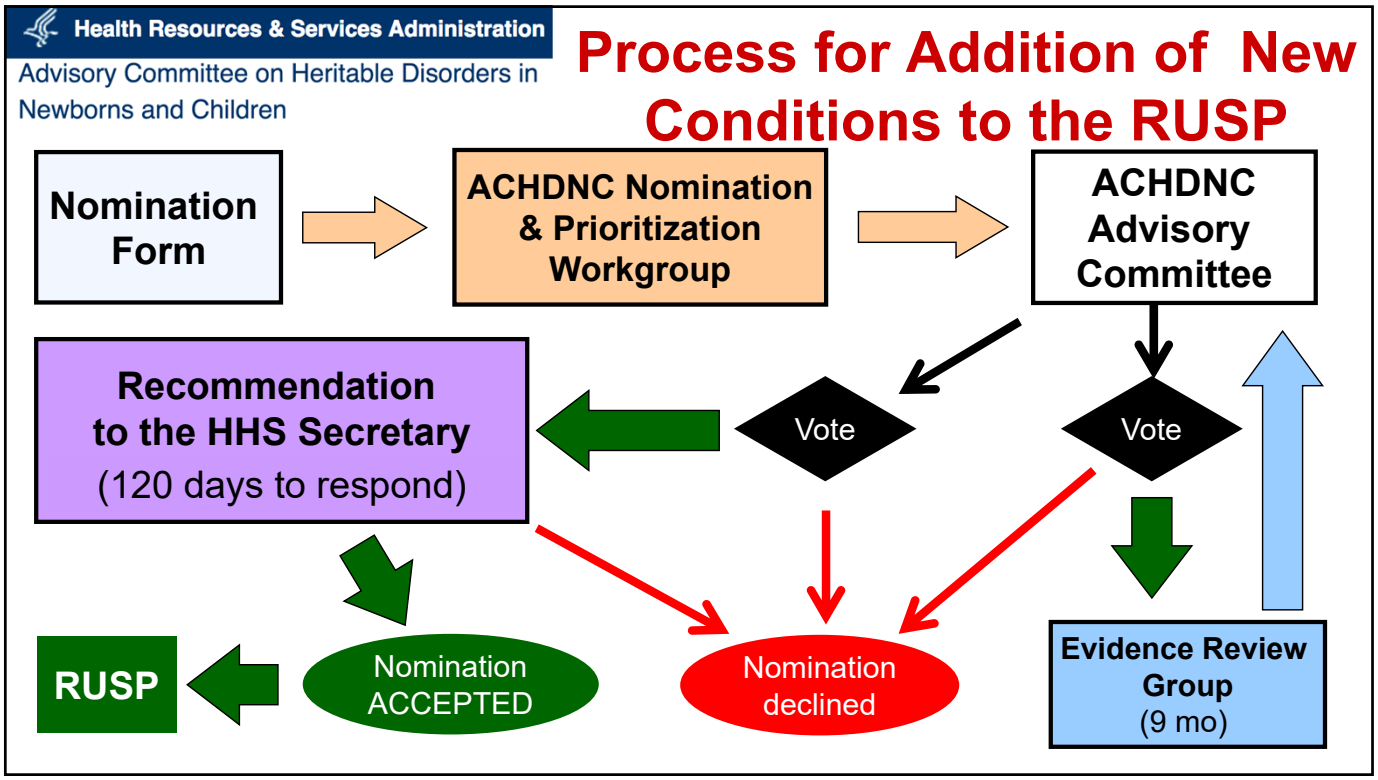


Nearly all states are screening for 29 Core Conditions

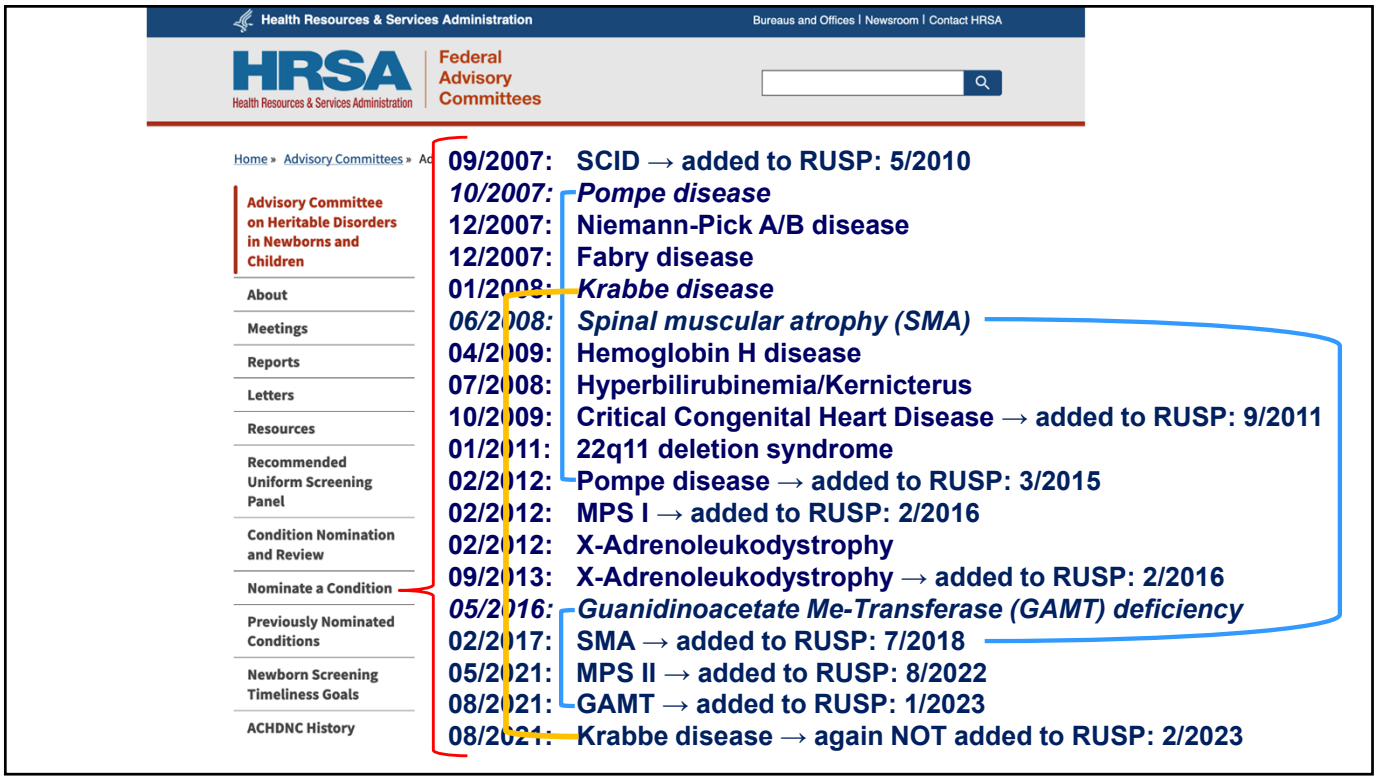
25

HRSA's ACHDNC

26

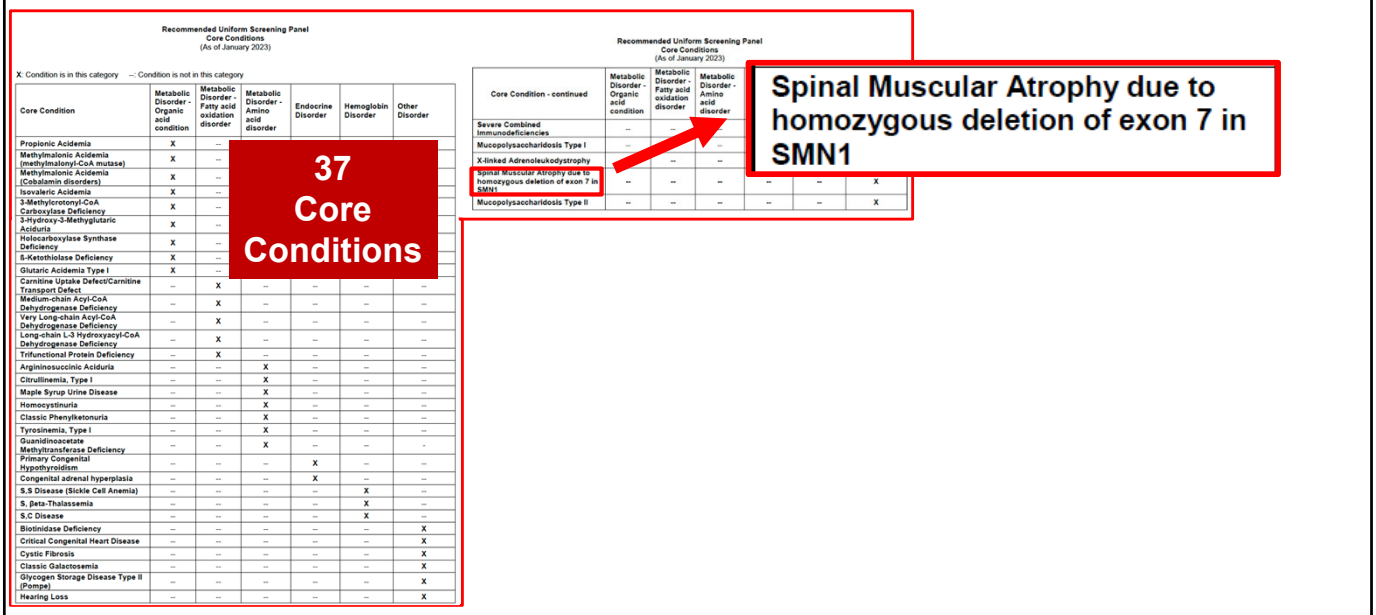

Health Resources & Services Administration
Advisory Committee on Heritable Disorders in Newborns and Children
Process for Addition of New Conditions to the RUSP
Nomination Form
ACHDNC Nomination & Prioritization Workgroup
ACHDNC Advisory Committee
Vote
Vote
Recommendation to the HHS Secretary
(120 days to respond)
RUSP
Nomination ACCEPTED
Nomination declined
Evidence Review Group
(9 mo)

27


Health Resources & Services Administration
Bureaus and Offices | Newsroom | Contact HRSA
HRSA
Health Resources & Services Administration
Federal Advisory Committees
Home » Advisory Committees »
Advisory Committee on Heritable Disorders in Newborns and Children
About
Meetings
Reports
Letters
Resources
Recommended Uniform Screening Panel
Condition Nomination and Review
Nominate a Condition
Previously Nominated Conditions
Newborn Screening Timeliness Goals
ACHDNC History
09/2007: SCID → added to RUSP: 5/2010
10/2007: Pompe disease
12/2007: Niemann-Pick A/B disease
12/2007: Fabry disease
01/2008: Krabbe disease
06/2008: Spinal muscular atrophy (SMA)
04/2009: Hemoglobin H disease
07/2008: Hyperbilirubinemia/Kernicterus
10/2009: Critical Congenital Heart Disease → added to RUSP: 9/2011
01/2011: 22q11 deletion syndrome
02/2012: Pompe disease → added to RUSP: 3/2015
02/2012: MPS I → added to RUSP: 2/2016
02/2012: X-Adrenoleukodystrophy
09/2013: X-Adrenoleukodystrophy → added to RUSP: 2/2016
05/2016: Guanidinoacetate Me-Transferase (GAMT) deficiency
02/2017: SMA → added to RUSP: 7/2018
05/2021: MPS II → added to RUSP: 8/2022
08/2021: GAMT → added to RUSP: 1/2023
08/2021: Krabbe disease → again NOT added to RUSP: 2/2023

28

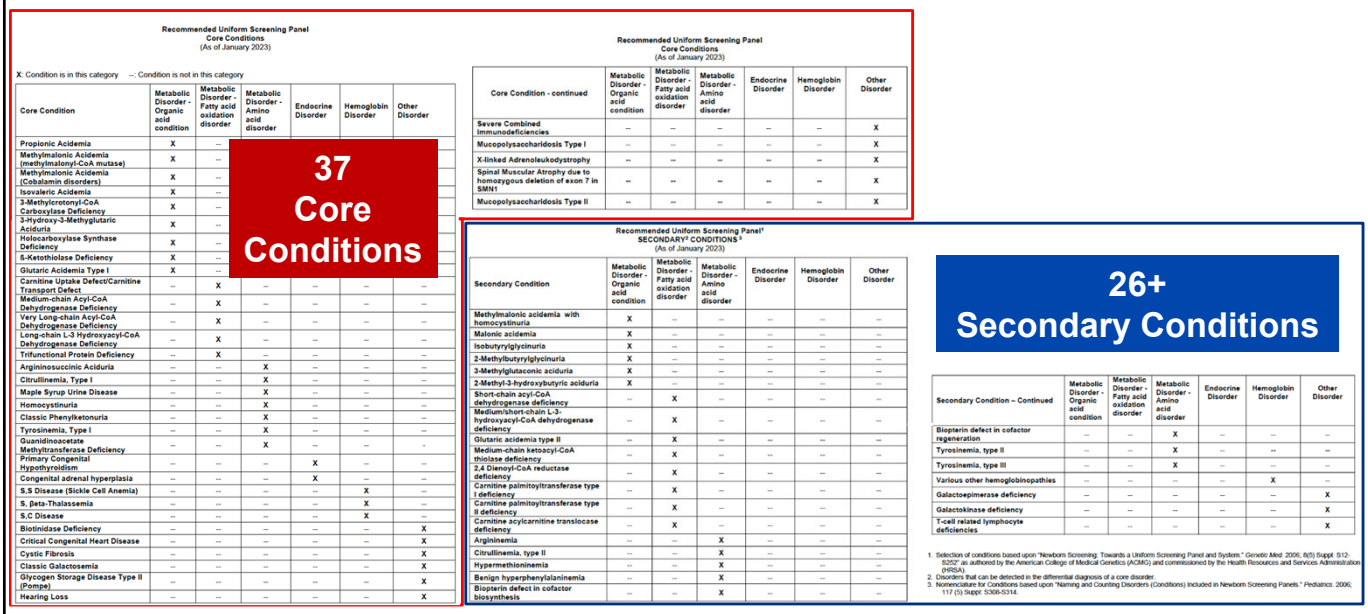
Recommended Uniform Screening Panel



https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp (last accessed: 4/2/2023)

29

Recommended Uniform Screening Panel



https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp (last accessed: 4/2/2023)

30

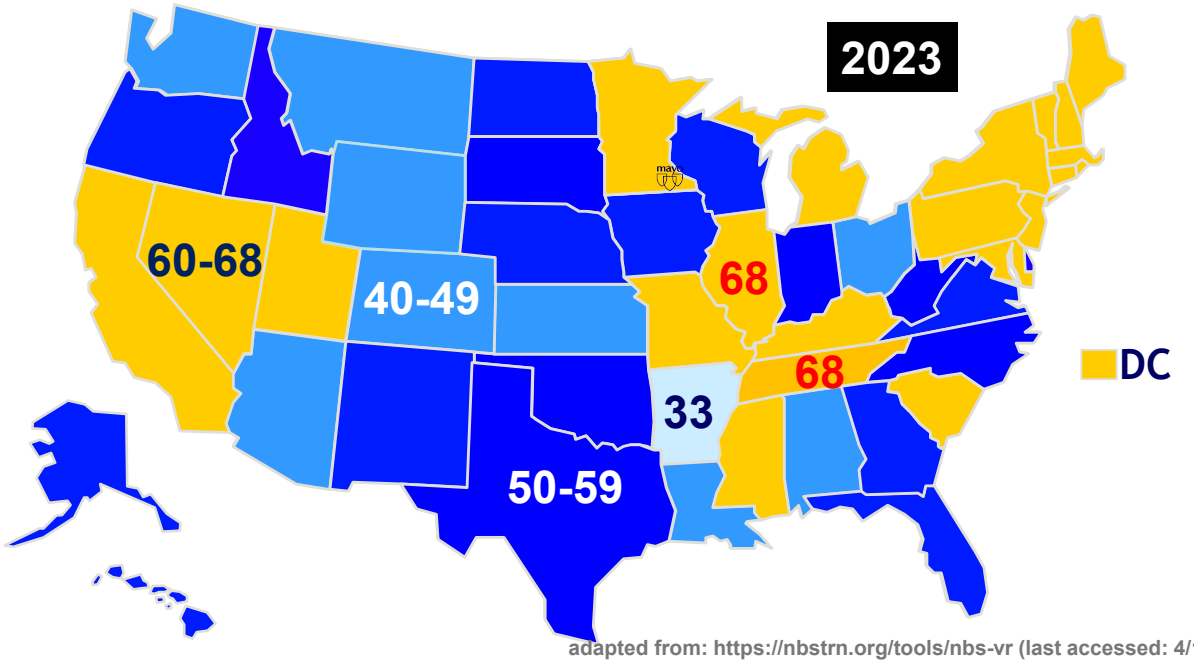
Newborn Screening

A public health program:

- Aimed at identification of conditions for which early intervention can prevent mortality, morbidity and disabilities.
- Performed by analysis of diagnostic markers in blood spots collected on filter paper on the second day of life (exception: hearing loss and congenital cyanotic heart disease).
- Extent of program is determined by each state independently.

31

Number of Conditions included in NBS Programs



adapted from: https://nbstrn.org/tools/nbs-vr (last accessed: 4/1/2023)

32

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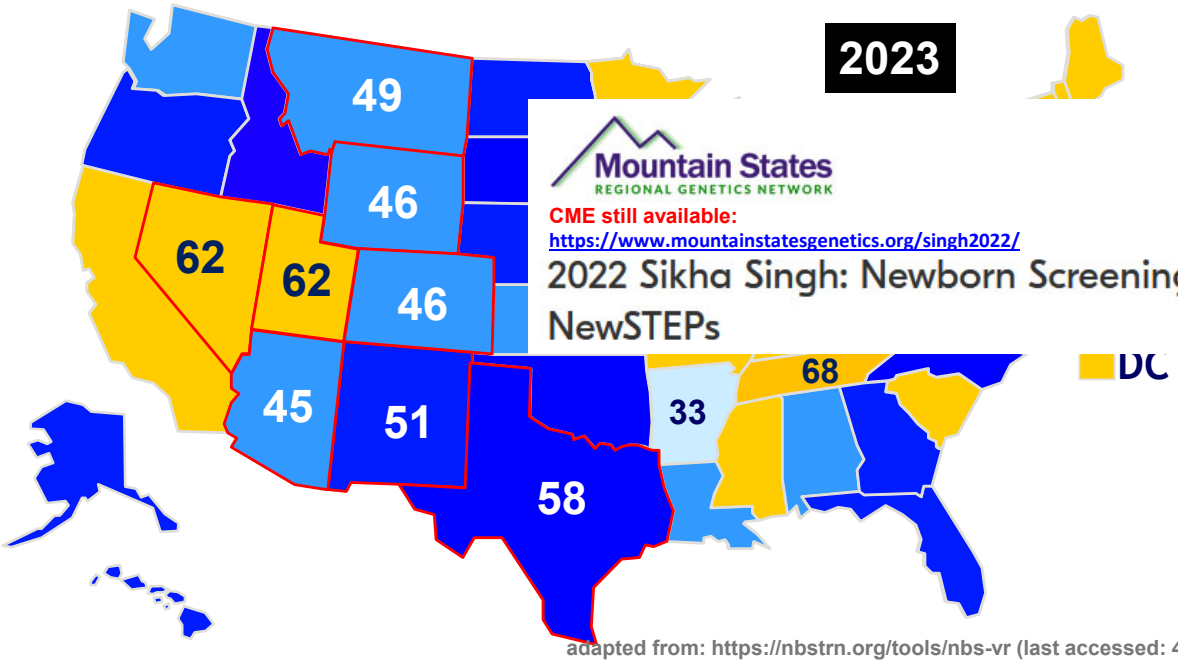

adapted from: https://nbstrn.org/tools/nbs-vr (last accessed: 4/1/2023)

33

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A public health program:

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- **Performed by analysis of diagnostic markers in blood spots collected on filter paper on the second day of life**
  (exception: hearing loss and congenital cyanotic heart disease).
- **Extent of program determined by each state independently.**
- **Administered by each state but testing may be out of state**

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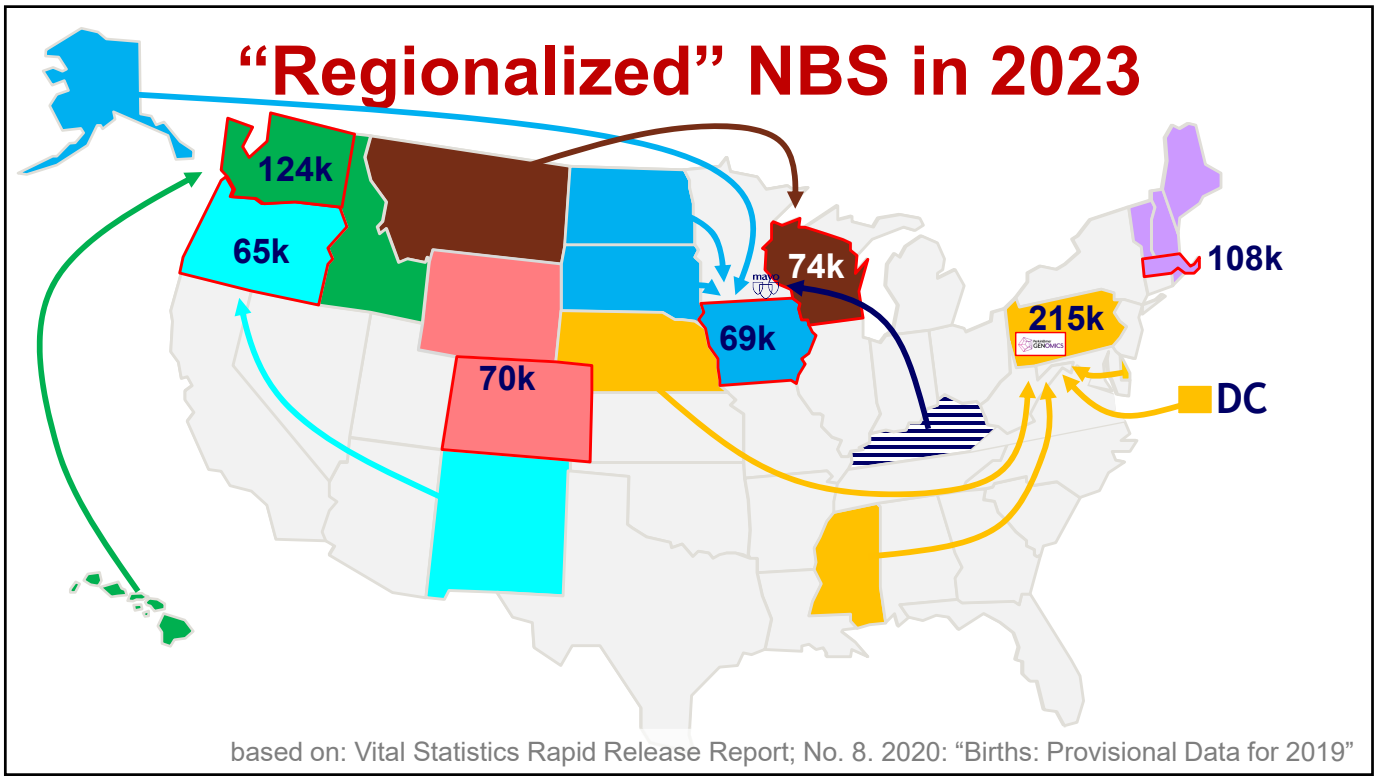
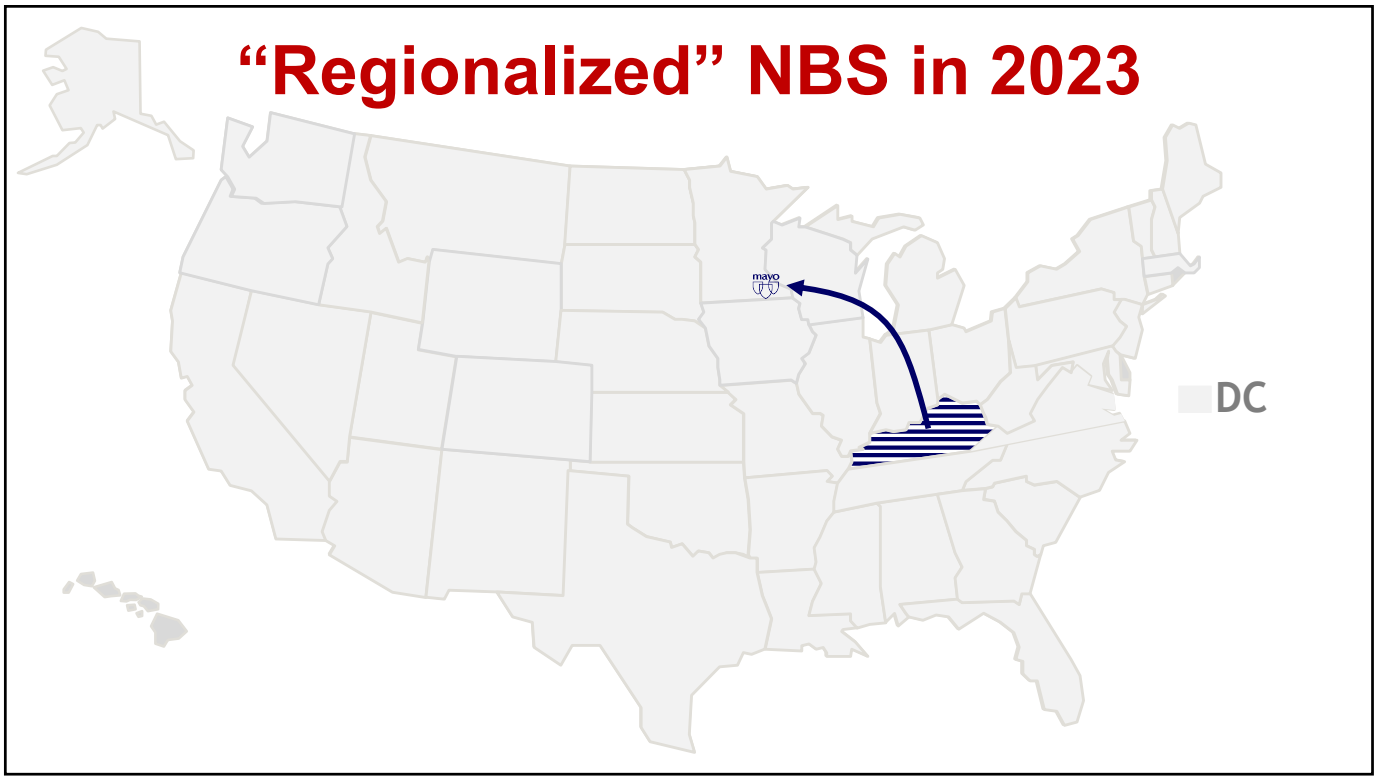
34

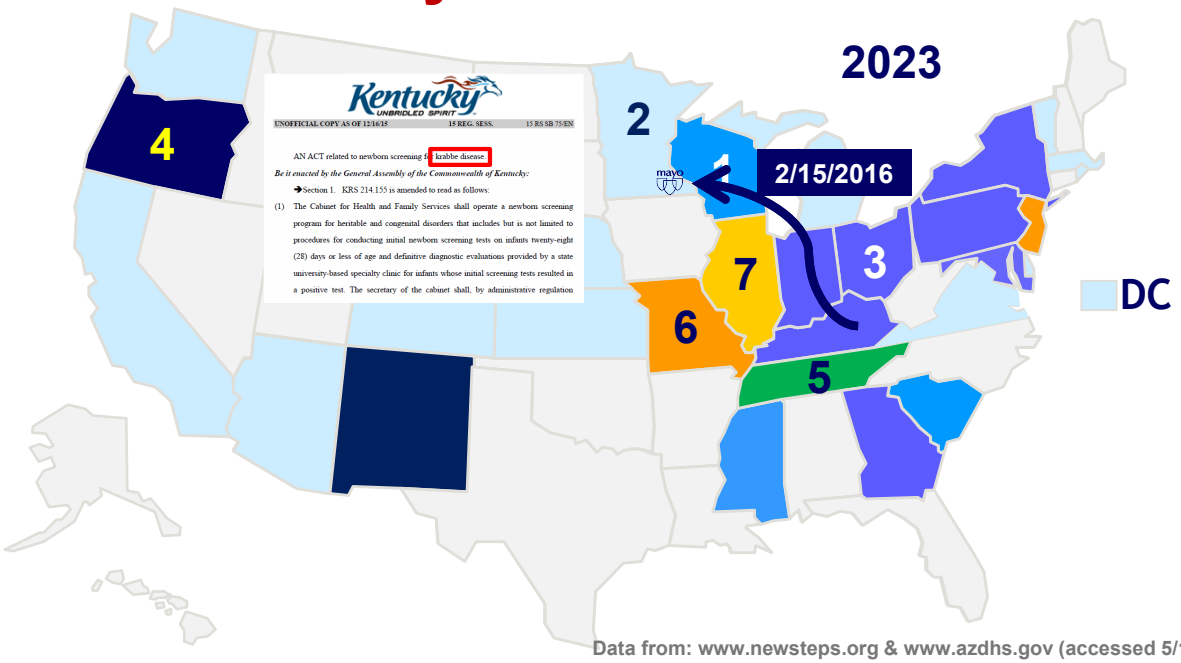

"Regionalized" NBS in 2023
124k
65k
74k
69k
70k
108k
215k
DC
based on: Vital Statistics Rapid Release Report; No. 8. 2020: "Births: Provisional Data for 2019"

35


"Regionalized" NBS in 2023
DC

36

NBS for Lysosomal Disorders

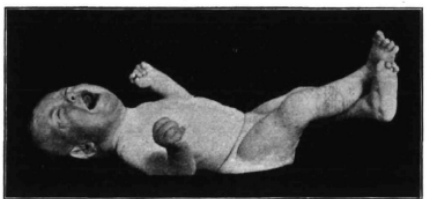


Data from: www.newsteps.org & www.azdhs.gov (accessed 5/16/2023)

37

Krabbe Disease

- **Infantile Krabbe disease (IKD) presents in first 12 months:**
  - **Extreme irritability**
  - **Spasticity**
  - **Developmental regression**
  - **Median survival: <2 yrs of age**



Krabbe K. *Brain*, 1916; 39: 74-114

- **Late Infantile KD (LIKD):**
  - **onset of irreversible and progressive symptoms between 1-3 yrs**
  - **Median survival: 7 yrs**
- **Juvenile KD (JKD): progressive symptoms as of 4-17 yrs**
- **Adult KD (AKD): progressive symptoms as of 18 or more yrs**
- **JKD and LKD can present with weakness, spasticity, ataxia, vision loss, and/or as neuropsychiatric disease in adults**

38

Why NBS for Krabbe Disease

Clinical Chemistry 50:10
1785–1796 (2004)

Automation and
Analytical Techniques

Direct Multiplex Assay of Lysosomal Enzymes in Dried Blood Spots for Newborn Screening

Yijun Li,[1] C. Ronald Scott,[2] Nestor A. Chamoles,[3] Ahmad Ghavami,[4] B. Mario Pinto,[4] Frantisek Turecek,[1] and Michael H. Gelb[1,5]*

Background: **Newborn screening for deficiency in the lysosomal enzymes that cause Fabry, Gaucher, Krabbe, Niemann–Pick A/B, and Pompe diseases is warranted because treatment for these syndromes is now available or anticipated in the near feature. We describe a multiplex screening method for all five lysosomal enzymes that uses newborn-screening cards containing dried blood spots as the enzyme source.**

Methods: **We used a cassette of substrates and internal standards to directly quantify the enzymatic activities, and tandem mass spectrometry for enzymatic product detection. Rehydrated dried blood spots were incubated with the enzyme substrates. We used liquid-liquid extracti**

gel to r

inhibito

neutrop

plicated

Results:

with Gaucher, 5 with Niemann–Pick A/B, 11 with Pompe, 5 with Fabry, and 12 with Krabbe disease, and in all cases the enzyme activities were below the minimum activities measured in a collection of heterozygous carriers and healthy noncarrier individuals. The enzyme activities measured in 5–9 heterozygous carriers were approximately one-half those measured with 15–32 healthy individuals, but there was partial overlap of each condition between the data sets for carriers and healthy individuals.

Conclusion: **For all five diseases, the affected individuals were detected. The assay can be readily automated, and the anticipated reagent and supply costs are well within the budget limits of newborn-screening centers.**

Screening test available

The application of mass spectrometry in disease diagnostics is rapidly advancing. Major applications include (*a*) quantification of metabolites in dried blood spots (DBS)[6] by tandem mass spectrometry (*1*); (*b*) identification and quantification of protein biomarkers in serum by surface-enhanced laser desorption/ionization mass spectrometry (*2*); (*c*) analysis of PCR-amplified DNA by MassArray to identify mutated genes (*3*); and (*d*) quantification of enzyme activities in cell lysates by affinity capture/

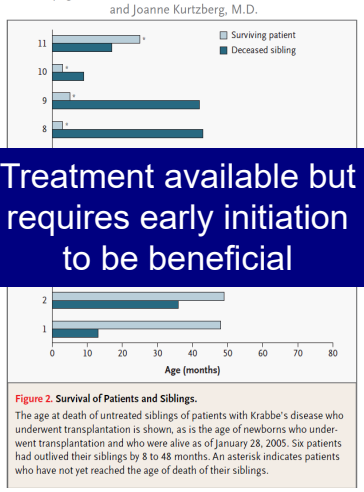
ders that affect both somatic organs and the central nervous system. Depending on the disorder, they can manifest from birth to adulthood. Until recently, there has been minimal therapeutic intervention available to alter the natural course of the disorders, but with the recent availability of commercial preparations of recombinant enzymes, selective lysosomal disorders are now amenable to therapeutic intervention (*7–9*). There is excellent documentation that enzyme replacement therapy for Gaucher disease [acid β-glucocerebrosidase (ABG) deficiency] and Fabry disease [acid α-galactosidase A (GLA) deficiency] may alter the natural progression of the disorders and improve the clinical phenotype. Clinical trials are currently underway for Hurler syndrome and Pompe disease [lysosomal acid α-glucosidase (GAA) deficiency], disor-

Departments of [1] Chemistry, [2] Pediatrics, and [5] Biochemistry, University of Washington, Seattle, WA.
[3] Laboratory of Neurochemistry, Buenos Aires, Argentina.
[4] Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada.
*Address correspondence to this author at: Department of Chemistry, Campus Box 351700, University of Washington, Seattle, WA 98195. Fax 206-685-8665; e-mail gelb@chem.washington.edu.
Received April 20, 2004; accepted June 29, 2004.
Previously published online at DOI: 10.1373/clinchem.2004.035907

[6] Nonstandard abbreviations: DBS, dried blood spot; ABG, acid β-glucocerebrosidase; GLA, acid α-galactosidase A; GAA, lysosomal acid α-glucosidase; ASM, acid sphingomyelinase; GALC, galactocerebroside β-galactosidase; PMN, polymorphonuclear leukocyte; CID, collision-induced dissociation; and RAAG, renal acid α-glucosidase.

Transplantation of Umbilical-Cord Blood in Babies with Infantile Krabbe's Disease

Maria L. Escolar, M.D., Michele D. Poe, Ph.D., James M. Provenzale, M.D., Karen C. Richards, M.D., June Allison, R.N., Susan Wood, P.N.P., David A. Wenger, Ph.D., Daniel Pietryga, M.D., Donna Wall, M.D., Martin Champagne, M.D., Richard Morse, M.D., William Krivit, M.D., Ph.D., and Joanne Kurtzberg, M.D.



Treatment available but requires early initiation to be beneficial

Figure 2. Survival of Patients and Siblings.

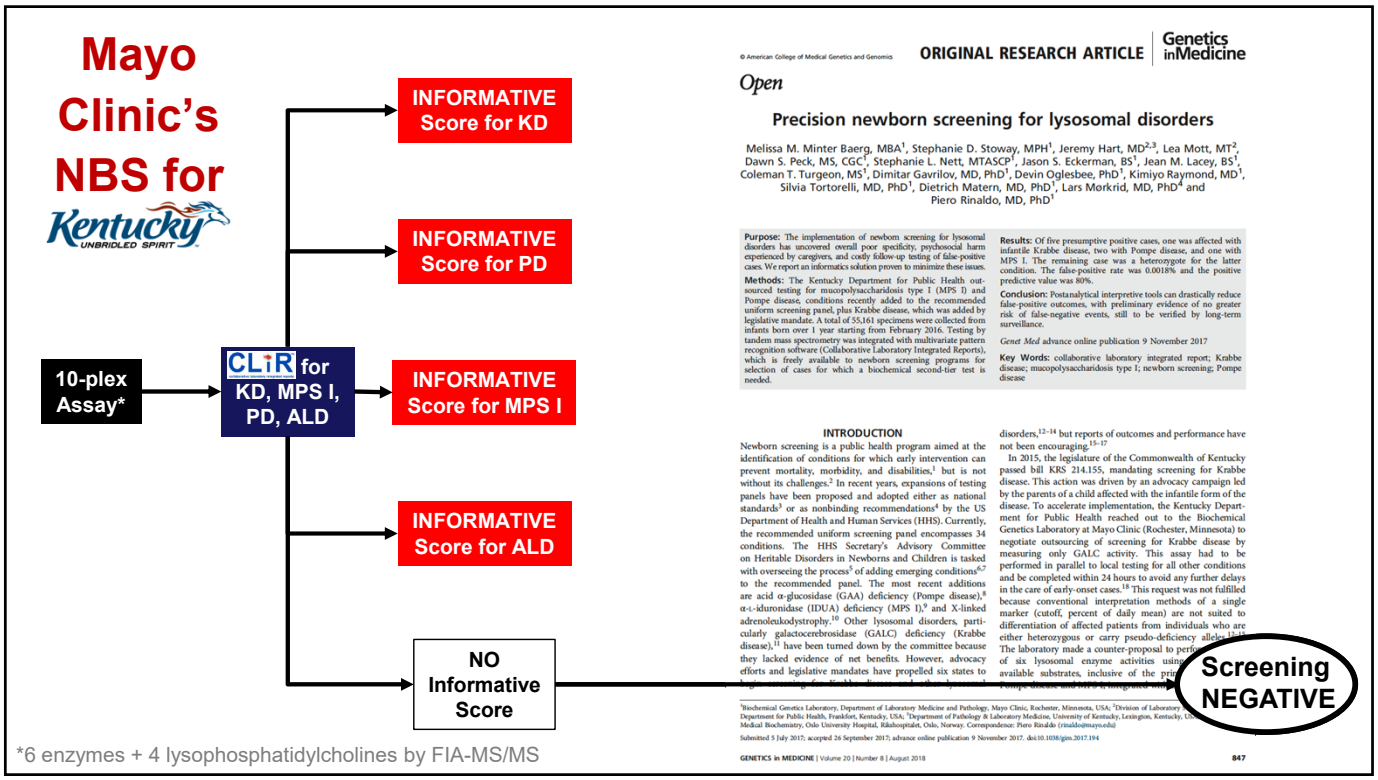
The age at death of untreated siblings of patients with Krabbe's disease who underwent transplantation is shown, as is the age of newborns who underwent transplantation and who were alive as of January 28, 2005. Six patients had outlived their siblings by 8 to 48 months. An asterisk indicates patients who have not yet reached the age of death of their siblings.

39

Mayo Clinic's Supplemental NBS for Kentucky

- **Public/private partnership after law to add Krabbe disease to KY NBS panel became effective on 6/24/2015.**
- **KY NBS Lab started sending DBS to Mayo (MN) for screening for Krabbe disease, Pompe disease and MPS I on 2/15/16; ALD was added on 7/9/18.**
- **DBS are separated in KY & shipped to Mayo; demographic data transmitted electronically.**
- **KY operates 6 days a week, Mayo operates 7 days a week.**
- **Samples received are analyzed overnight; results reported next day.**
- **Leftover DBS are returned to KY every other week.**

40



© American College of Medical Genetics and Genomics ORIGINAL RESEARCH ARTICLE Genetics inMedicine
Open

Precision newborn screening for lysosomal disorders

Melissa M. Minter Baerg, MBA[1], Stephanie D. Stoway, MPH[1], Jeremy Hart, MD[2,3], Lea Mott, MT[2], Dawn S. Peck, MS, CGC[1], Stephanie L. Nett, MTASCP[1], Jason S. Eckerman, BS[1], Jean M. Lacey, BS[1], Coleman T. Turgeon, MS[1], Dimitar Gavrilov, MD, PhD[1], Devin Oglesbee, PhD[1], Kimiyo Raymond, MD[1], Silvia Tortorelli, MD, PhD[1], Dietrich Matern, MD, PhD[1], Lars Mørkrid, MD, PhD[4] and Piero Rinaldo, MD, PhD[1]

Purpose: The implementation of newborn screening for lysosomal disorders has uncovered overall poor specificity, psychosocial harm experienced by caregivers, and costly follow-up testing of false-positive cases. We report an informatics solution proven to minimize these issues.

Methods: The Kentucky Department for Public Health outsourced testing for mucopolysaccharidosis type I (MPS I) and Pompe disease, conditions recently added to the recommended uniform screening panel, plus Krabbe disease, which was added by legislative mandate. A total of 55,161 specimens were collected from infants born over 1 year starting from February 2016. Testing by tandem mass spectrometry was integrated with multivariate pattern recognition software (Collaborative Laboratory Integrated Reports), which is freely available to newborn screening programs for selection of cases for which a biochemical second-tier test is needed.

Results: Of five presumptive positive cases, one was affected with infantile Krabbe disease, two with Pompe disease, and one with MPS I. The remaining case was a heterozygote for the latter condition. The false-positive rate was 0.0018% and the positive predictive value was 80%.

Conclusion: Postanalytical interpretive tools can drastically reduce false-positive outcomes, with preliminary evidence of no greater risk of false-negative events, still to be verified by long-term surveillance.

Genet Med advance online publication 9 November 2017

Key Words: collaborative laboratory integrated report; Krabbe disease; mucopolysaccharidosis type I; newborn screening; Pompe disease

INTRODUCTION

Newborn screening is a public health program aimed at the identification of conditions for which early intervention can prevent mortality, morbidity, and disabilities,[1] but is not without its challenges.[2] In recent years, expansions of testing panels have been proposed and adopted either as national standards[3] or as nonbinding recommendations[4] by the US Department of Health and Human Services (HHS). Currently, the recommended uniform screening panel encompasses 34 conditions. The HHS Secretary's Advisory Committee on Heritable Disorders in Newborns and Children is tasked with overseeing the process[5] of adding emerging conditions[6,7] to the recommended panel. The most recent additions are acid α-glucosidase (GAA) deficiency (Pompe disease),[8] α-L-iduronidase (IDUA) deficiency (MPS I),[9] and X-linked adrenoleukodystrophy.[10] Other lysosomal disorders, particularly galactocerebrosidase (GALC) deficiency (Krabbe disease),[11] have been turned down by the committee because they lacked evidence of net benefits. However, advocacy efforts and legislative mandates have propelled six states to

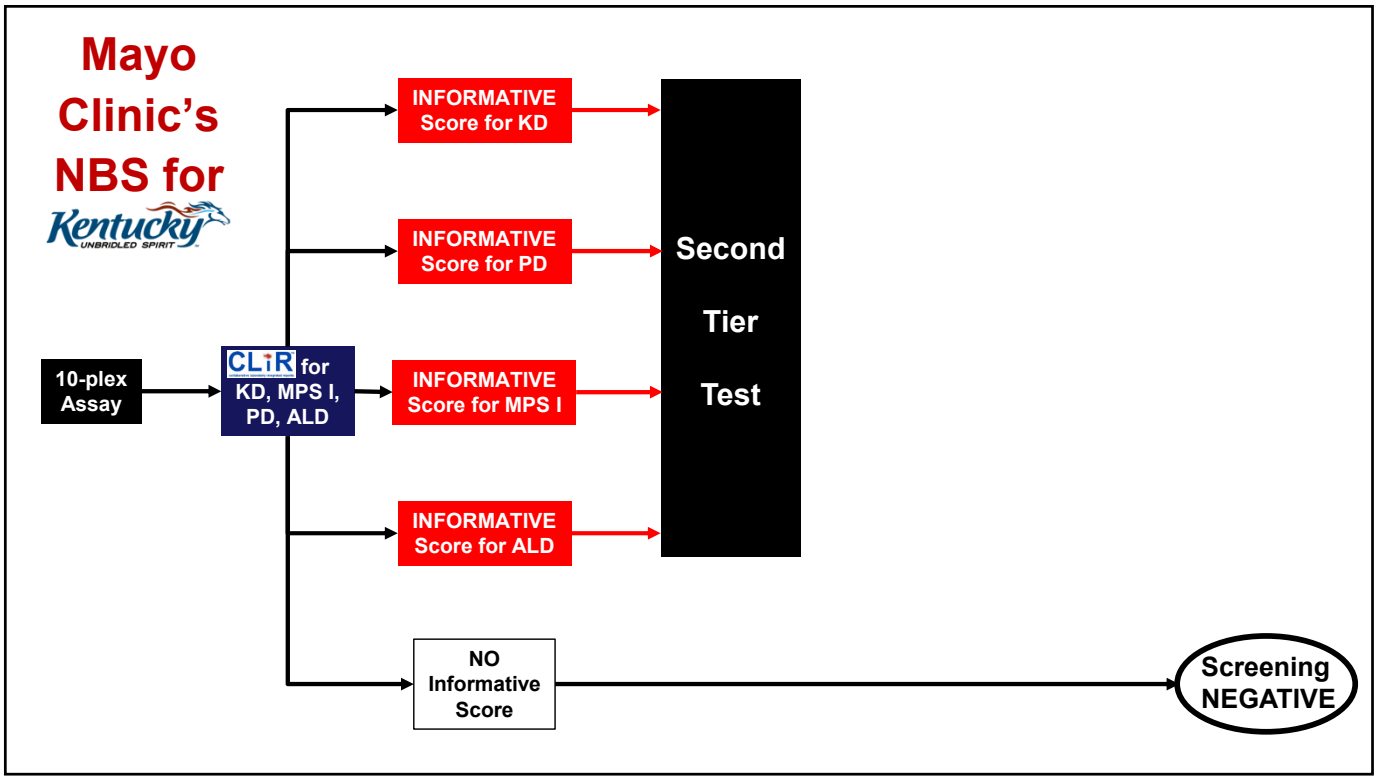
disorders.[12–14] but reports of outcomes and performance have not been encouraging.[15–17]

In 2015, the legislature of the Commonwealth of Kentucky passed bill KRS 214.155, mandating screening for Krabbe disease. This action was driven by an advocacy campaign led by the parents of a child affected with the infantile form of the disease. To accelerate implementation, the Kentucky Department for Public Health reached out to the Biochemical Genetics Laboratory at Mayo Clinic (Rochester, Minnesota) to negotiate outsourcing of screening for Krabbe disease by measuring only GALC activity. This assay had to be performed in parallel to local testing for all other conditions and be completed within 24 hours to avoid any further delays in the care of early-onset cases.[18] This request was not fulfilled because conventional interpretation methods of a single marker (cutoff, percent of daily mean) are not suited to differentiation of affected patients from individuals who are either heterozygous or carry pseudo-deficiency alleles.[19–21] The laboratory made a counter-proposal to perform [illegible] of six lysosomal enzyme activities using [illegible] available substrates, inclusive of the pri[illegible] assays, tandem and UHPLC integrated with

[1]Biochemical Genetics Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA; [2]Division of Laboratory [illegible], Department for Public Health, Frankfort, Kentucky, USA; [3]Department of Pathology & Laboratory Medicine, University of Kentucky, Lexington, Kentucky, USA; [4]Department of Medical Biochemistry, Oslo University Hospital, Rikshospitalet, Oslo, Norway. Correspondence: Piero Rinaldo (rinaldo@mayo.edu)

Submitted 5 July 2017; accepted 26 September 2017; advance online publication 9 November 2017. doi:10.1038/gim.2017.194

41



42

What are 2nd Tier Tests?

- A cost effective approach to reduce false positive results when normal population and disease range overlap (poor specificity)
- After primary screen
- Same specimen, no additional patient contact
- Normal 2nd tier test result overrules primary screen

  ➔ reduction of false positive results
- Examples: - biochemical (CAH, MSUD, PA, MMA, RMD, HCU, SCAD, GA I, GA II, Pompe, Krabbe, MPS I, MPS II),
  - molecular (CF)

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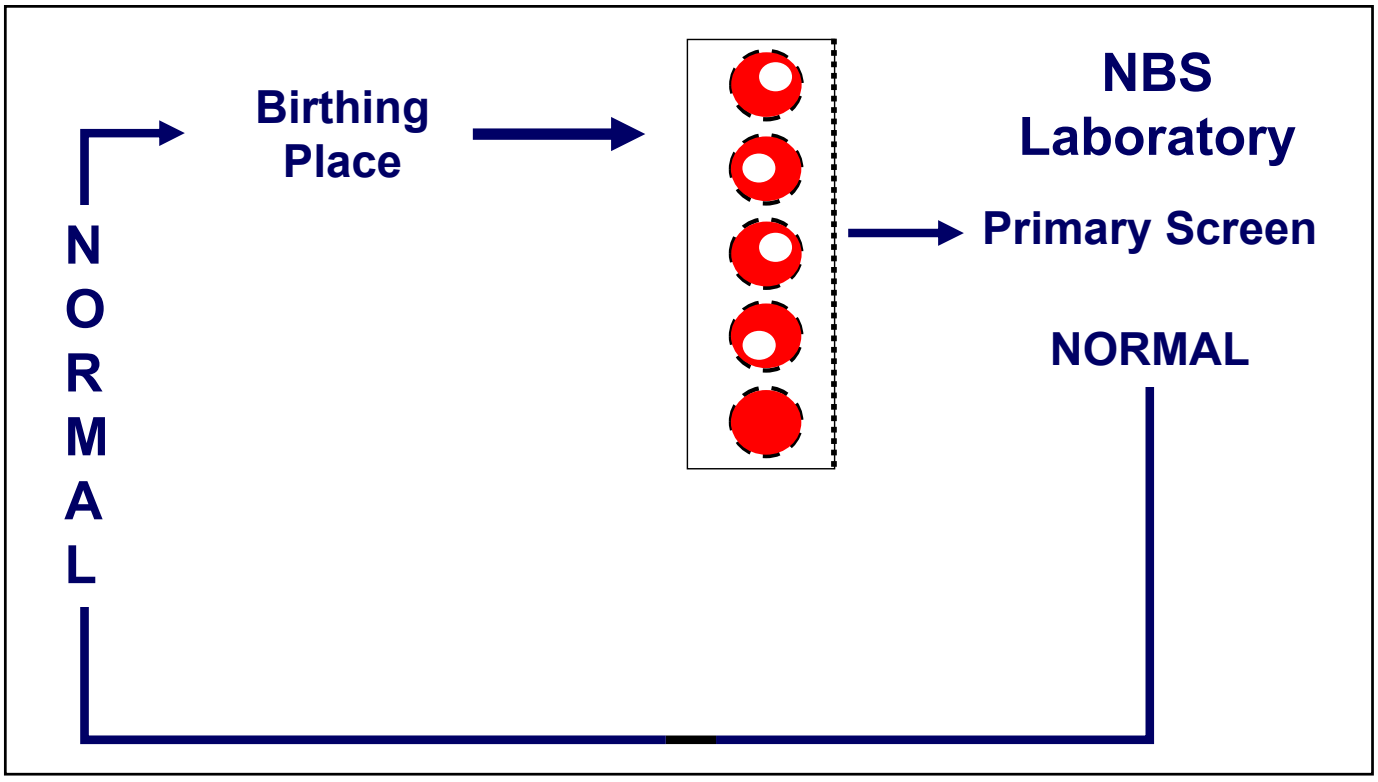
43



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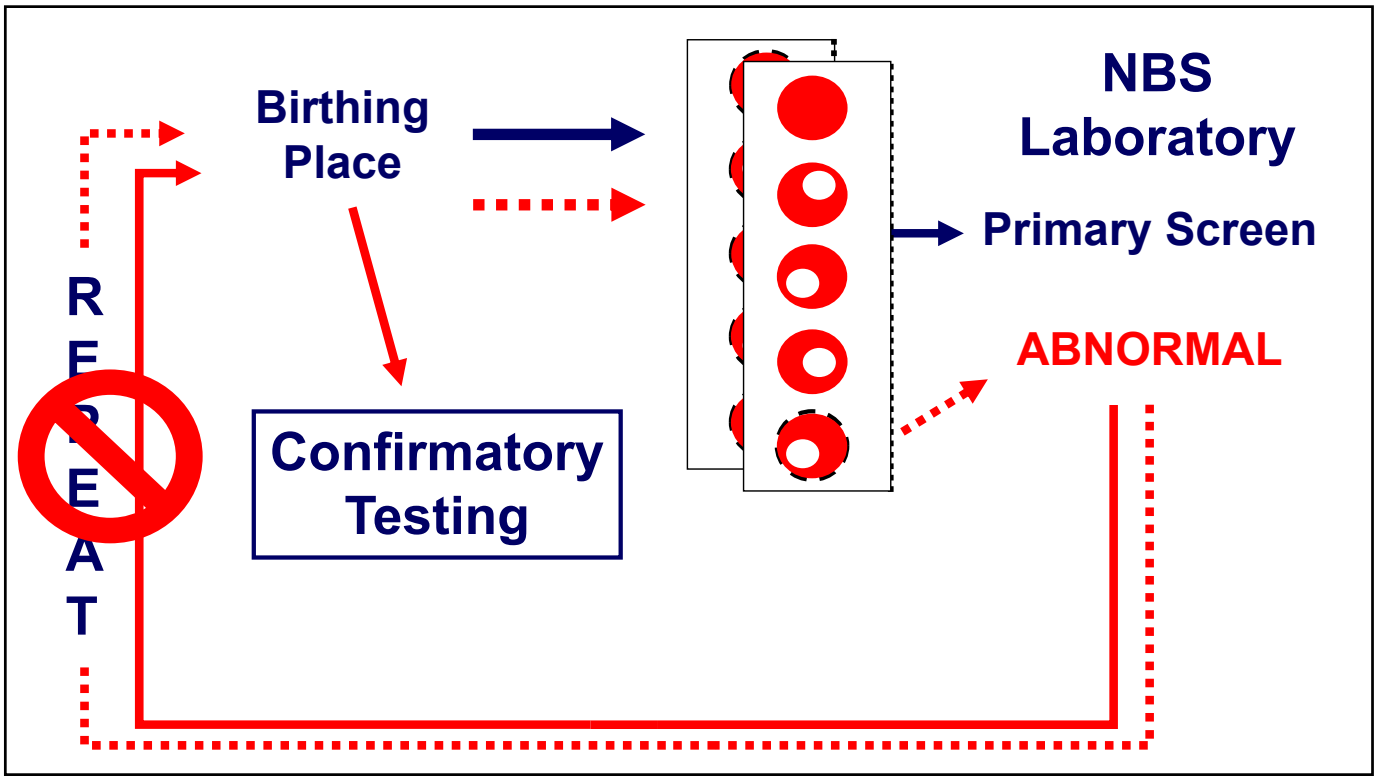
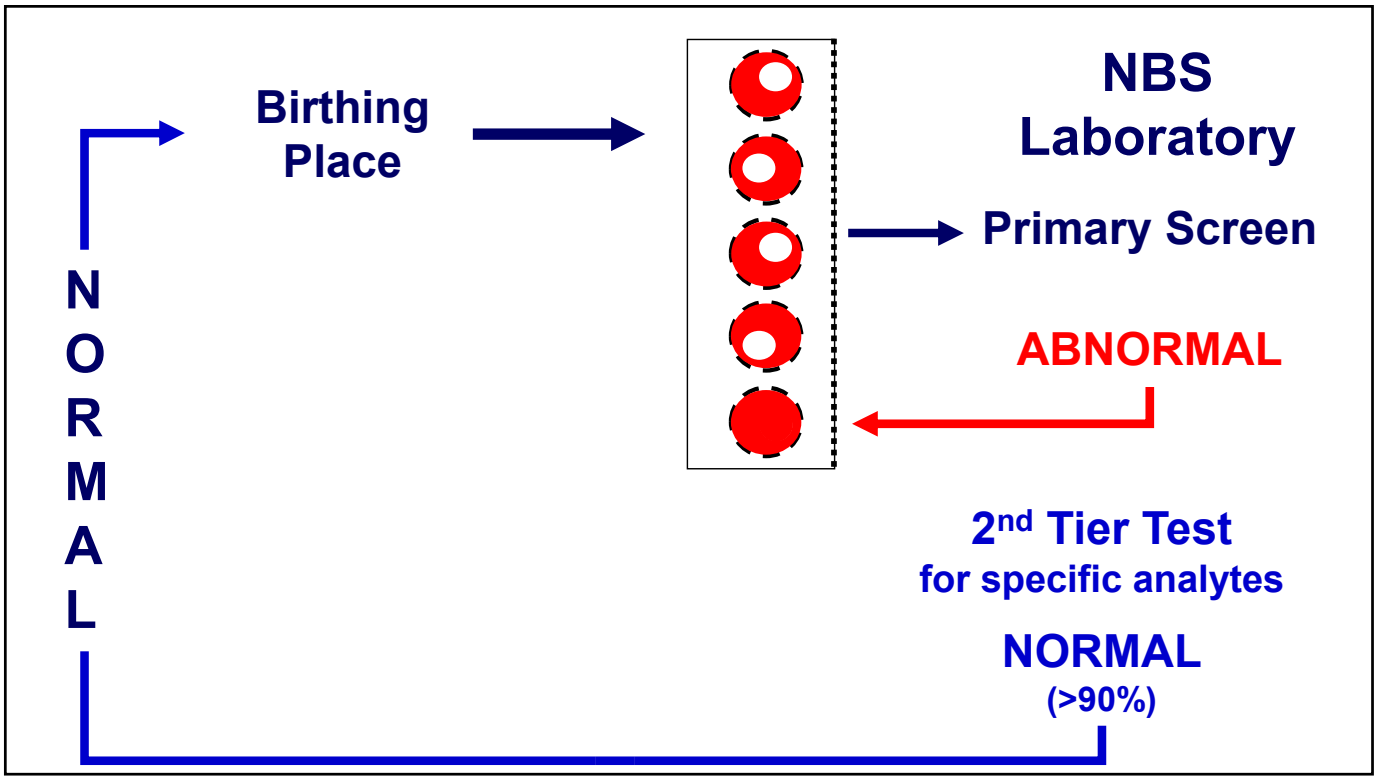
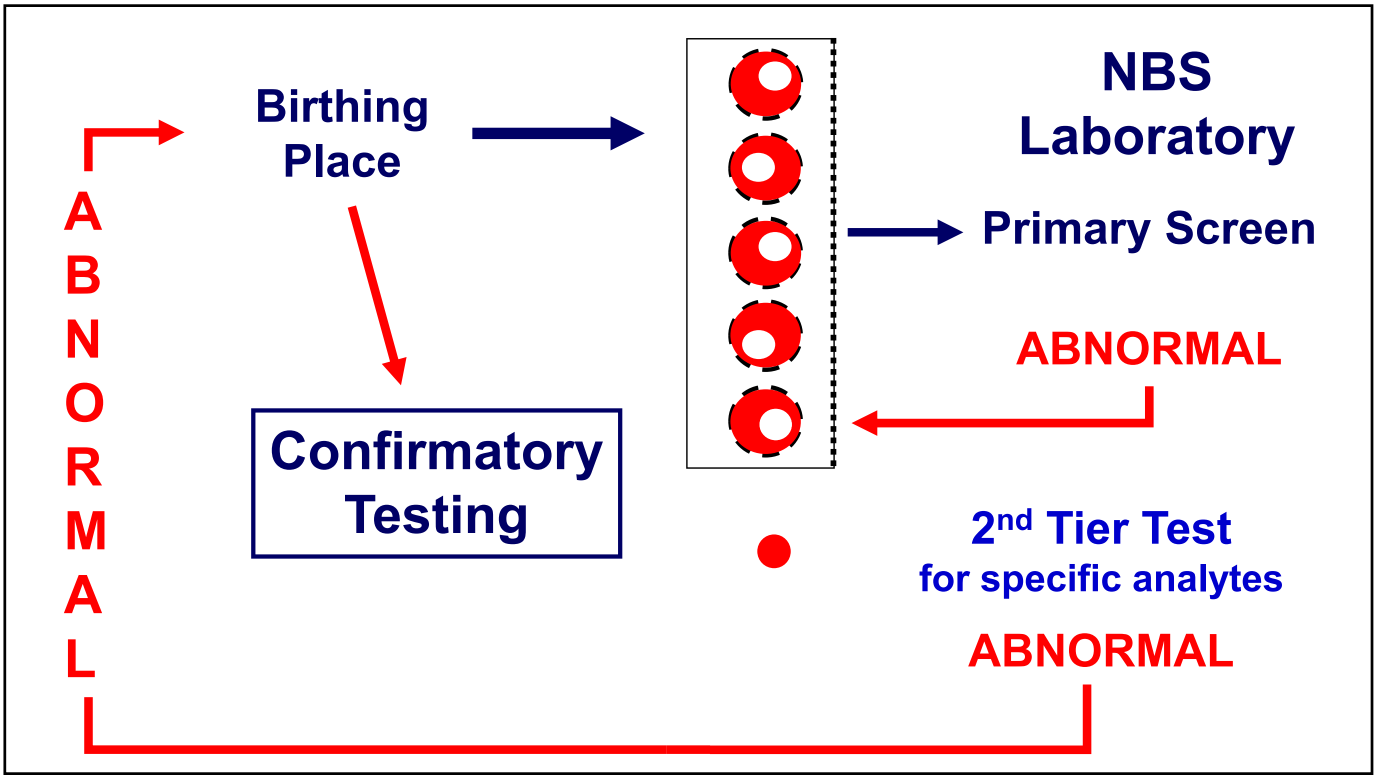

Birthing Place
NBS Laboratory
Primary Screen
ABNORMAL
Confirmatory Testing
REPEAT

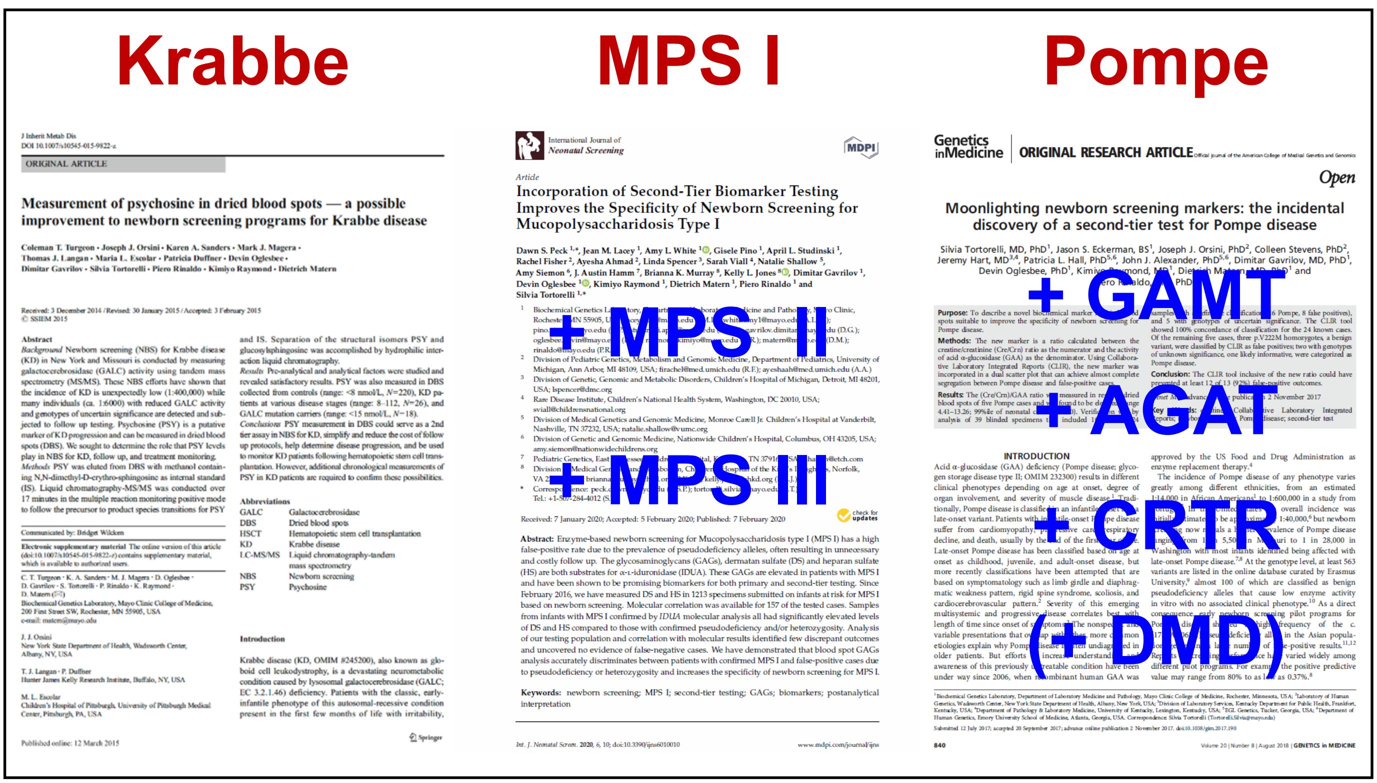
45


Birthing Place
NBS Laboratory
Primary Screen
ABNORMAL
2nd Tier Test
for specific analytes
NORMAL
(>90%)
NORMAL

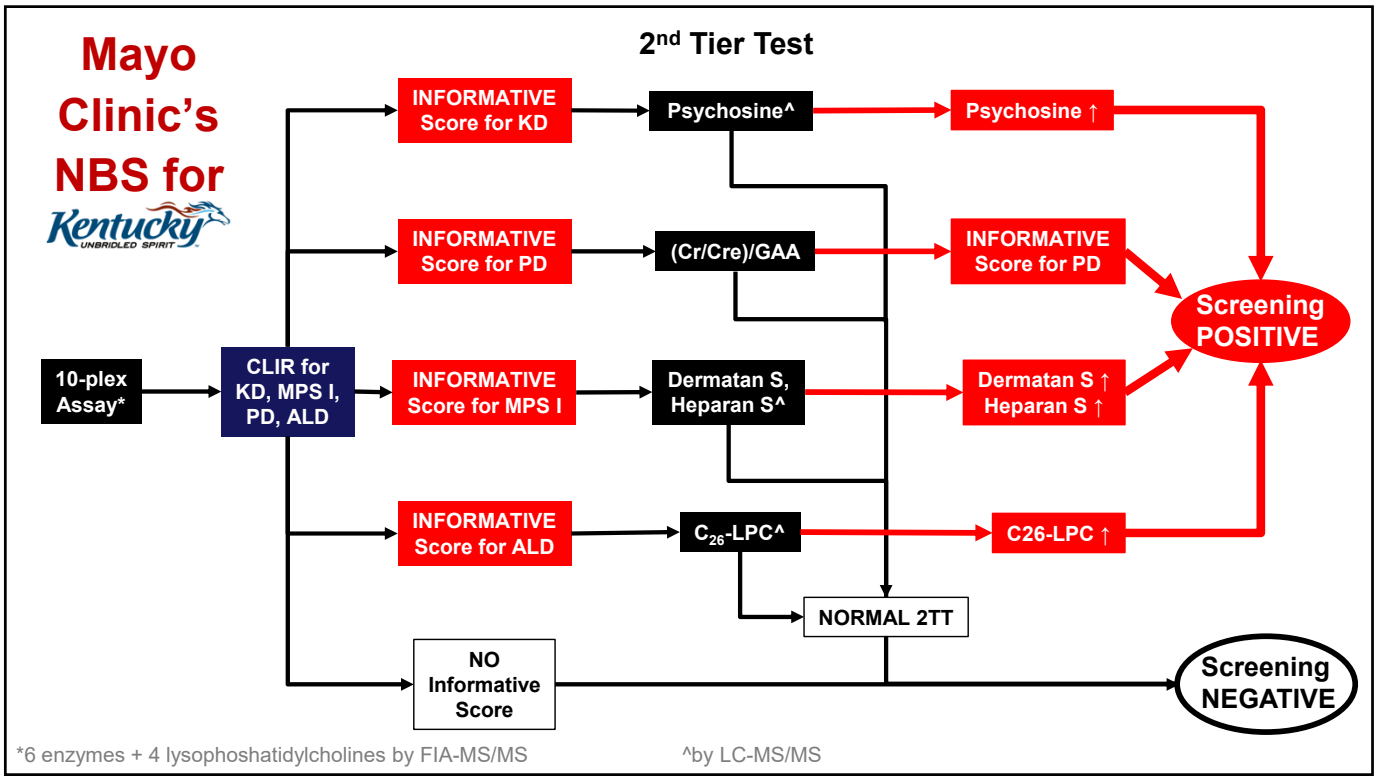
46


Birthing Place
NBS Laboratory
Primary Screen
ABNORMAL
Confirmatory Testing
2nd Tier Test
for specific analytes
ABNORMAL
ABNORMAL

47


Krabbe
MPS I
Pompe
Measurement of psychosine in dried blood spots — a possible improvement to newborn screening programs for Krabbe disease
Incorporation of Second-Tier Biomarker Testing Improves the Specificity of Newborn Screening for Mucopolysaccharidosis Type I
Moonlighting newborn screening markers: the incidental discovery of a second-tier test for Pompe disease
+ MPS II
+ MPS III
+ GAMT
+ AGAT
+ CRTR
(+ DMD)

48



49

Kentucky Status

Newborns screened: 379,507 (2/15/2016 – 2/28/2023)*

Condition	2nd Tier Test	True Positive (TP)	False Positive (FP)	False Positive Rate	Positive Predictive Value	Final Diagnosis
Krabbe	116 (0.031%)	2	0	0%	100%	Infantile KD
Pompe	188 (0.049%)	24	14	0.004%	63%	Late onset PD
MPS I	149 (0.039%)	10	7	0.002%	59%	MPS I Hurler/Scheie
ALD*	1,368* (0.556%*)	8	0	0%	100%	6 ALD, 2 Zellweger
TOTAL		**44**	**21**	**0.006%**	**68%**	

*Newborns screened for ALD (7/9/2018 – 2/28/2023): 246,137

False Positive Rate: (FP/Total)×100%; Positive Predictive Value: (TP/[TP+FP])×100%

50

A Case of Krabbe Disease from KY

NBS collected	DBS arrives in KY Lab	DBS arrives at Mayo			Patient admitted at Duke		HSCT
		Sample prep and incubation	Informative for Krabbe ➔ initiate rpt + 2TTs (PSY & 30kbDel)	GALC ↓ PSY ↑ 30kbDel -/- ➔ initiate follow up, initiate GALC sequencing	GALC in WBC, PSY, Parental DNA, HLA typing, Neurologic exam, MRI brain with DTI, BAER, VEP, EEG, NCV, Neurocognitive testing, CSF protein, Psy in CSF	Diagnosis confirmed by: GALC in WBC PSY in DBS and CSF Genotype: 1 MUT + 1 VUS + 2 Pseudo	
		Sat	Sun	Mon			
2nd DOL	3rd DOL	4th DOL	5th DOL	6th DOL	7th DOL	9th DOL	24th DOL

51

1st KY Case with Krabbe Disease

2 yrs old

- PSY not normalized (at least not by 2 y/o)
- Sitting, but not walking
- Non-verbal, but expressive
- recurrent autoimmune hemolytic anemia


4 years old

52

The Newest NBS Model

- Many conditions
- Many tests
- Many markers
- Pattern recognition (disease risk)

$(IEM, etc.)_n$

↓

MS/MS, etc.

↓

AA, AC, TSH, GALT, etc.

↓

CLiR™ collaborative laboratory integrated reports

Bioinformatics (no cut offs)

53

The Newest NBS Model

ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

Open

Postanalytical tools improve performance of newborn screening by tandem mass spectrometry

Patricia L. Hall, PhD[1], Gregg Marquardt, MSS[1], David M.S. McHugh[1], Robert J. Currier, PhD[2], Hao Tang, PhD[2], Stephanie D. Stoway, BS[1] and Piero Rinaldo, MD, PhD[1]

Purpose: The purpose of this study was to compare performance metrics of postanalytical interpretive tools of the Region 4 Stork collaborative project to the actual outcome based on cutoff values for

Results: In the study period, the actual false-positive rate and positive predictive value were 0.26 and 10%, respectively. Utilization of the Region 4 Stork tools, simple interpretation rules, and second-tier tests

Conclusion: Region 4 Stork interpretive tools, second-tier tests, and other evidence-based interpretation rules could have reduced false-positive cases by up to 90% in California.

for individual target conditions were deemed informative when equal or greater to the value representing the first percentile rank of known true-positive cases (17,099 cases in total).

Key Words: cutoff values; newborn screening; postanalytical interpretive tools; second-tier test; tandem mass spectrometry

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54

Newborn Screening

A public health program:

- Aimed at identification of conditions for which early intervention can prevent mortality, morbidity and disabilities.
- Performed by analysis of diagnostic markers in blood spots collected on filter paper on the second day of life
  (exception: hearing loss and congenital cyanotic heart disease).
- Extent of program determined by each state independently.
- Administered by each state but testing may be out of state
- States may screen twice: by 2 days old + 1-2 weeks old

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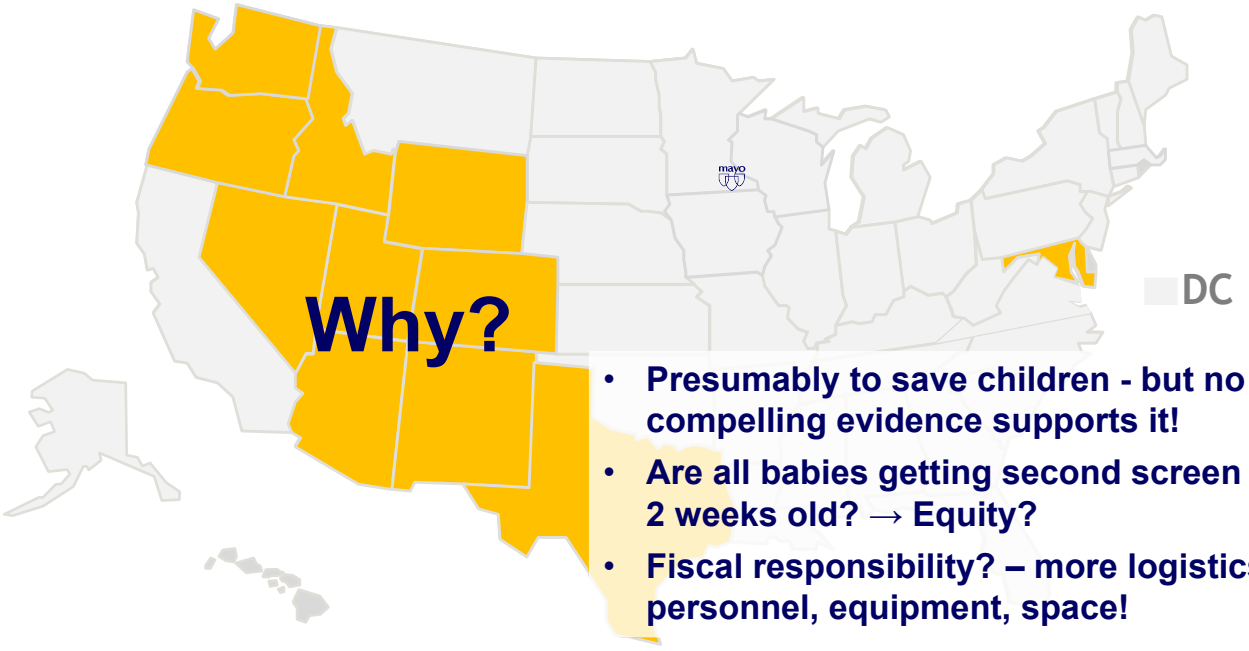
55

"Two Screen" States in 2023



Why?

- Presumably to save children - but no compelling evidence supports it!
- Are all babies getting second screen at 2 weeks old? → Equity?
- Fiscal responsibility? – more logistics, personnel, equipment, space!

56

Newborn Screening

What do screening laboratories like to do on Friday afternoons?

Inform you of a presumptive positive result on one of your newborn patients!

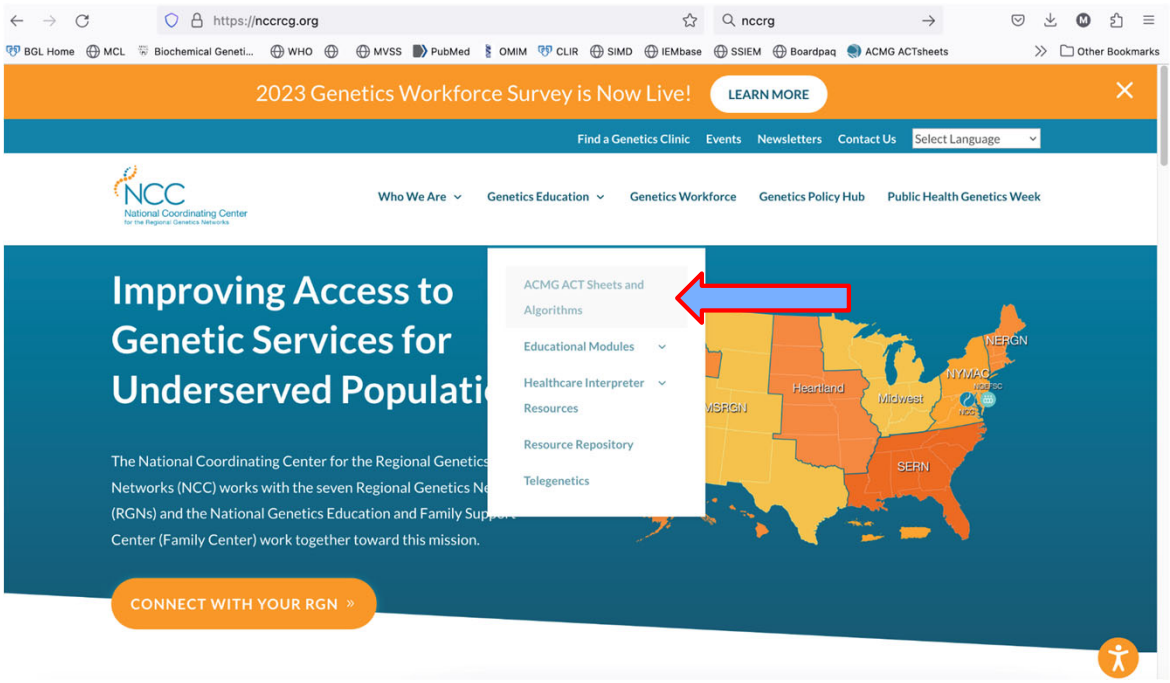
MAYO CLINIC

57

What to do when you get a call about an abnormal "*PKU* test"*?*

A. I tell myself not to panic!
B. I take the necessary information but make sure they mean an elevated Phe, not an abnormal NBS for Pompe disease or something else.
C. I ask around if any of my colleagues knows what to do.
D. I call the colleague who did not fall asleep during Dr. Matern's presentation.
E. I'm self-sufficient and Google for ideas.
F. I'm self-sufficient and go to the NCCRCG or ACMG website.

58



59

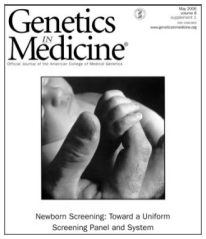
The ACMG Newborn Screening Diagnosis & Follow-up Work Group

- **Geneticists, other specialists and primary care providers involved in NBS for endocrine, hematologic, genetic and metabolic diseases**
- **ACT sheets and diagnostic algorithms**
- **ACT sheets include:**
  - **Information about the analytes and their clinical significance**
  - **Links to informational resources, if needed**
  - **Links to websites that allow identification of regional subspecialists for consultation and referral if desired**

60

Development of ACT Sheet Concept

- Began when NBS expansion was apparent
- Too many "fact" sheets
- Need was for tools to support clinical decision-making for all NBS conditions
  - Point of care education and decision support
  - EMR compatibility
    - Pop-up education
    - Clinical decision support (CDS) (i.e. directive support)



From: Michael Watson, PhD; American College of Medical Genetics and National Coordinating Center for Regional Genetics and Newborn Screening Collaboratives; May 7, 2007

61

NBS ACT(ion) Sheets and Diagnostic Algorithms

- For all conditions in uniform panel
- ACMG Board approved and AAP Board endorsed
- Posted on ACMG website
  - Genetics Home Reference, NNSGRC, and many others link to these
- Distributed to NBS labs and programs
  - To accompany all "screen positive" lab reports
- Distributed to RCs to coordinate use with local and regional plans
- Survey of utility presently being conducted

From: Michael Watson, PhD; American College of Medical Genetics and National Coordinating Center for Regional Genetics and Newborn Screening Collaboratives; May 7, 2007

62

The ACMG Newborn Screening Diagnosis & Follow-up Work Group

Harvey Levy, MD (Chair)

Endocrinology
Stephen LaFranchi, MD (OR)
Phyllis Speiser, MD (NY)
Kelly Leight, JD (CARES Found.)

Hematology
James Eckman, MD (GA)
Peter Lane, MD (GA)
Carolyn Hoppe, MD (CA)

Genetics
Gary Cutting, MD (MD)
Cynthia Morton, PhD (MA)
Richard Smith, MD (IA)

HRSA
Marie Mann, MD, MPH (DC)
Michelle Lloyd-Puryear, MD, PhD (DC)

Michael Watson, PhD (Project Dir.)

Metabolism
Gerard Berry, MD (PA)
Stephen Goodman, MD (CO)
Harvey Levy, MD (MA)
Deborah Marsden, MD (MA)
Dietrich Matern, MD, PhD (MN)
William Nyhan, MD (CA)

Primary Care
Danielle Laraque, MD (NY)
Barbara Yawn, MD (MN)

Newborn Screening
Julie Miller, MS (NE)
Kenneth Pass, PhD (NY)
Bradford Therrell, PhD (TX)

From: Michael Watson, PhD; American College of Medical Genetics and National Coordinating Center for Regional Genetics and Newborn Screening Collaboratives; May 7, 2007

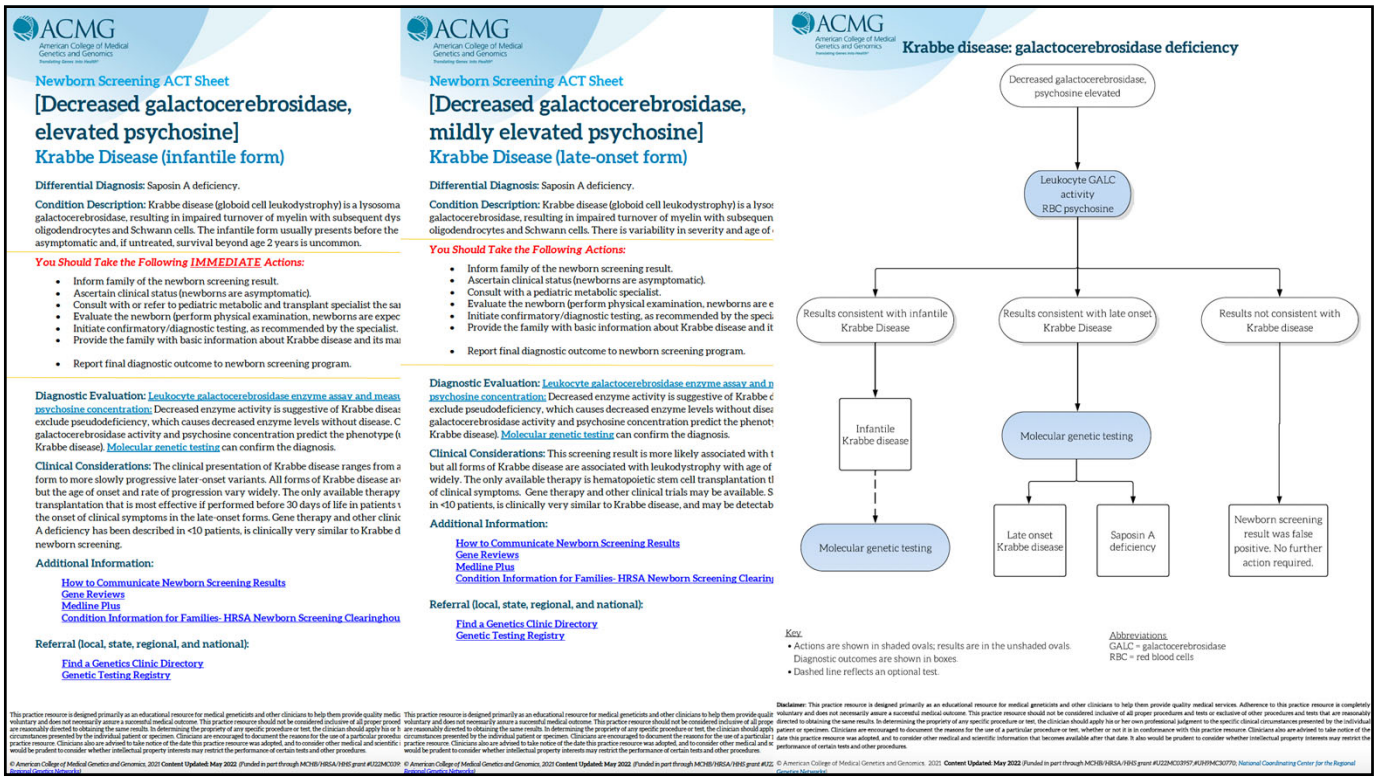
63

Conclusions

- **ACT sheets and algorithms were designed to aid in the most appropriate and timely evaluation and treatment of newborns with abnormal newborn screening results**
- **Algorithms probably work best when clinician and laboratorians "talk"**
- **Constructive feedback to the ACMG is encouraged for continued improvement**
- **ACT sheets and algorithms are freely available for adaptation to regional needs (practice, hospital, screening program)**
- **Updates must be communicated effectively to users and adopters**

From: Michael Watson, PhD; American College of Medical Genetics and National Coordinating Center for Regional Genetics and Newborn Screening Collaboratives; May 7, 2007

64



ACMG
American College of Medical Genetics and Genomics

Newborn Screening ACT Sheet

[Decreased galactocerebrosidase, elevated psychosine]

Krabbe Disease (infantile form)

Differential Diagnosis: Saposin A deficiency.

Condition Description: Krabbe disease (globoid cell leukodystrophy) is a lysosoma galactocerebrosidase, resulting in impaired turnover of myelin with subsequent dys oligodendrocytes and Schwann cells. The infantile form usually presents before the asymptomatic and, if untreated, survival beyond age 2 years is uncommon.

You Should Take the Following IMMEDIATE Actions:

- Inform family of the newborn screening result.
- Ascertain clinical status (newborns are asymptomatic).
- Consult with or refer to pediatric metabolic and transplant specialist the sa
- Evaluate the newborn (perform physical examination, newborns are expec
- Initiate confirmatory/diagnostic testing, as recommended by the specialist.
- Provide the family with basic information about Krabbe disease and its ma

- Report final diagnostic outcome to newborn screening program.

Diagnostic Evaluation: Leukocyte galactocerebrosidase enzyme assay and meas psychosine concentration: Decreased enzyme activity is suggestive of Krabbe disea exclude pseudodeficiency, which causes decreased enzyme levels without disease. C galactocerebrosidase activity and psychosine concentration predict the phenotype (Krabbe disease). Molecular genetic testing can confirm the diagnosis.

Clinical Considerations: The clinical presentation of Krabbe disease ranges from a form to more slowly progressive later-onset variants. All forms of Krabbe disease ar but the age of onset and rate of progression vary widely. The only available therapy transplantation that is most effective if performed before 30 days of life in patients the onset of clinical symptoms in the late-onset forms. Gene therapy and other clinic A deficiency has been described in <10 patients, is clinically very similar to Krabbe d newborn screening.

Additional Information:

How to Communicate Newborn Screening Results
Gene Reviews
Medline Plus
Condition Information for Families- HRSA Newborn Screening Clearinghou

Referral (local, state, regional, and national):

Find a Genetics Clinic Directory
Genetic Testing Registry

ACMG
American College of Medical Genetics and Genomics

Newborn Screening ACT Sheet

[Decreased galactocerebrosidase, mildly elevated psychosine]

Krabbe Disease (late-onset form)

Differential Diagnosis: Saposin A deficiency.

Condition Description: Krabbe disease (globoid cell leukodystrophy) is a lyso galactocerebrosidase, resulting in impaired turnover of myelin with subsequen oligodendrocytes and Schwann cells. There is variability in severity and age of

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Clinical Considerations: This screening result is more likely associated with t but all forms of Krabbe disease are associated with leukodystrophy with age of widely. The only available therapy is hematopoietic stem cell transplantation t of clinical symptoms. Gene therapy and other clinical trials may be available. S in <10 patients, is clinically very similar to Krabbe disease, and may be detectab

Additional Information:

How to Communicate Newborn Screening Results
Gene Reviews
Medline Plus
Condition Information for Families- HRSA Newborn Screening Clearin

Referral (local, state, regional, and national):

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Genetic Testing Registry

ACMG
American College of Medical Genetics and Genomics

Krabbe disease: galactocerebrosidase deficiency

Key
- Actions are shown in shaded ovals; results are in the unshaded ovals.
- Diagnostic outcomes are shown in boxes.
- Dashed line reflects an optional test.

Abbreviations
GALC = galactocerebrosidase
RBC = red blood cells

65

Newborn Screening Going Forward

OPINION

Neonatal screening by DNA microarray: spots and chips

Nancy S. Green and Kenneth A. Pass

2005

NATURE REVIEWS | GENETICS VOLUME 6 | FEBRUARY 2005 | 149

66

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NEWS RELEASES

Wednesday, September 4, 2013 **2013**

NIH program explores the use of genomic sequencing in newborn healthcare

Can sequencing of newborns' genomes provide useful medical information beyond what current newborn screening already provides? Pilot projects to examine this important question are being funded by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) and the National Human Genome Research Institute (NHGRI), both parts of the National Institutes of Health. Awards of $5 million to four grantees have been made in fiscal year 2013 under the Genomic Sequencing and Newborn Screening Disorders research program. The program will be funded at $25 million over five years, as funds are made available.

Each of the new awards will consist of three parts: Genomic sequencing and analysis; research related to patient care; and the ethical, legal and social implications of using genomic information in the newborn period. Teams of researchers will work to further the understanding of disorders that appear in newborns and to improve treatments for these diseases using genomic information.

67

Parental Views on Expanded Newborn Screening Using Whole-Genome Sequencing

Galen Joseph, PhD, Flavia Chen, MPH, Julie Harris-Wai, PhD, MPH,[a] Jennifer M. Puck, MD, Charlotte Young, BS, Barbara A. Koenig, PhD

Pediatrics. 2016;137(s1):epeds.20153731H

abstract

BACKGROUND AND OBJECTIVE: The potential application of whole-genome sequencing (WGS) to state-mandated standard newborn screening (NBS) challenges the traditional public health approach to NBS and raises ethical, policy, and clinical practice issues. This article examines the perspectives and values of diverse healthy pregnant women and parents of children diagnosed with a primary immunodeficiency disorder about traditional NBS and expanded NBS with the use of WGS.

METHODS: We conducted 4 focus groups (3 in English and 1 in Spanish) with socioeconomically and ethnically diverse pregnant women ($n = 26$), and a comparison group with parents of children diagnosed with a primary immunodeficiency disorder ($n = 5$).

RESULTS: Pediatric policy–relevant themes that emerged from our analysis of the focus group data are presented within 4 categories: (1) perspectives on traditional NBS, (2) informed consent, (3) return of results, and (4) storage and retrieval of results. Analyses indicate that study participants desired greater inclusion in the NBS process. Despite an optimistic orientation to the potential benefits and limited harms likely to result from genomic applications of NBS, parents voiced concerns about privacy and control over test results. Limited trust in the medical system and the state-run NBS program informed these concerns.

CONCLUSIONS: Expanded NBS with WGS for pediatricians may require management of more genetic conditions, including mutations that convey risk to both the child and parents for adult-onset disorders, and an informed-consent process to manage the genomic data and storage of blood spots. Attention to how these technologies are understood in diverse populations is needed for effective implementation.

Interpretation of Genomic Sequencing Results in Healthy and Ill Newborns: Results from the BabySeq Project

Ozge Ceyhan-Birsoy,[1,2] Jaclyn B. Murry,[2,4] Kalotina Machini,[2,4] Matthew S. Lebo,[2,3,4,11] Timothy W. Yu,[4,5,6] Shawn Fayer,[7] Casie A. Genetti,[3] Talia S. Schwartz,[3] Pankaj B. Agrawal,[4,5,8] Richard B. Parad,[4,9] Ingrid A. Holm,[4,5] Amy L. McGuire,[10] Robert C. Green,[4,7,11] Heidi L. Rehm,[2,3,4,11,12] Alan H. Beggs,[4,5,*] and The BabySeq Project Team

Genomic sequencing provides many opportunities in newborn clinical care, but the challenges of interpreting and reporting newborn genomic sequencing (nGS) results need to be addressed for its broader and effective application. The BabySeq Project is a pilot randomized clinical trial that explores the medical, behavioral, and economic impacts of nGS in well newborns and those admitted to a neonatal intensive care unit (NICU). Here we present childhood-onset and actionable adult-onset disease risk, carrier status, and pharmacogenomics findings from nGS of 159 newborns in the BabySeq Project. nGS revealed a risk of childhood-onset disease in 15/159 (9.4%) newborns; none of the disease risks were anticipated based on the infants' known clinical or family histories. nGS also revealed actionable adult-onset disease risk in 3/85 (3.5%) newborns whose parents consented to receive this information. Carrier status for recessive diseases and pharmacogenomics variants were reported in 88% and 5% of newborns, respectively. Additional indication-based analyses were performed in 29/32 (91%) NICU newborns and 6/127 (5%) healthy newborns who later had presentations that prompted a diagnostic analysis. No variants that sufficiently explained the reason for the indications were identified; however, suspicious but uncertain results were reported in five newborns. Testing parental samples contributed to the interpretation and reporting of results in 13/159 (8%) newborns. Our results suggest that nGS can effectively detect risk and carrier status for a wide range of disorders that are not detectable by current newborn screening assays or predicted based on the infant's known clinical or family history, and the interpretation of results can substantially benefit from parental testing.

The American Journal of Human Genetics *104*, 76–93, January 3, 2019

Perceived Benefits, Risks, and Utility of Newborn Genomic Sequencing in the BabySeq Project

Stacey Pereira, PhD,[a] Jill Oliver Robinson, MA,[a] Amanda M. Gutierrez, BA,[a] Devan K. Petersen, MPH,[a] Rebecca L. Hsu, BA,[a] Caroline H. Lee,[a] Talia S. Schwartz, BA,[b,c] Ingrid A. Holm, MD, MPH,[b,c] Alan H. Beggs, PhD,[b,c] Robert C. Green, MD, PhD,[c,d,e,f] Amy L. McGuire, JD, PhD,[a] on behalf of The BabySeq Project Group

Pediatrics 2019;143;S6

abstract

BACKGROUND AND OBJECTIVES: There is interest in applying genomic sequencing (GS) to newborns' clinical care. Here we explore parents' and clinicians' attitudes toward and perceptions of the risks, benefits, and utility of newborn GS compared with newborn screening (NBS) prior to receiving study results.

METHODS: The BabySeq Project is a randomized controlled trial used to explore the impact of integrating GS into the clinical care of newborns. Parents ($n = 493$) of enrolled infants ($n = 309$) and clinicians ($n = 144$) completed a baseline survey at enrollment. We examined between-group differences in perceived utility and attitudes toward NBS and GS. Open-ended responses about risks and benefits of each technology were categorized by theme.

RESULTS: The majority of parents (71%) and clinicians (51%) agreed that there are health benefits of GS, although parents and clinicians agreed more that there are risks associated with GS (35%, 70%) than with NBS (19%, 39%; all $P < .05$). Parents perceived more benefit and less risk of GS than did clinicians. Clinicians endorsed concerns about privacy and discrimination related to genomic information more strongly than did parents, and parents anticipated benefits of GS that clinicians did not.

CONCLUSIONS: Parents and clinicians are less confident in GS than NBS, but parents perceive a more favorable risk/benefit ratio of GS than do clinicians. Clinicians should be aware that parents' optimism may stem from their perceived benefits beyond clinical utility.

Also identified one patient with:

- **nonsyndromic hearing loss**
- **nonclassic CAH**
- **partial Biotinidase deficiency.**

NBS was normal in all 3 infants.

68

LETTERS
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The role of exome sequencing in newborn screening for inborn errors of metabolism

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Public health newborn screening (NBS) programs provide population-scale ascertainment of rare, treatable conditions that require urgent intervention. Tandem mass spectrometry (MS/MS) is currently used to screen newborns for a panel of rare inborn errors of metabolism (IEMs)[1–4]. The NBSeq project evaluated whole-exome sequencing (WES) as an innovative methodology for NBS. We obtained archived residual dried blood spots and data for nearly all IEM cases from the 4.5 million infants born in California between mid-2005 and 2013 and from some infants who screened positive by MS/MS, but were unaffected upon follow-up testing. WES had an overall sensitivity of 88% and specificity of 98.4%, compared to 99.0% and 99.8%, respectively for MS/MS, although effectiveness varied among individual IEMs. Thus, WES alone was insufficiently sensitive or specific to be a primary screen for most NBS IEMs. However, as a secondary test for infants with abnormal MS/MS screens, WES could reduce false-positive results, facilitate timely case resolution and in some instances even suggest more appropriate or specific diagnosis than that initially obtained. This study represents the largest, to date, sequencing effort of an entire population of IEM-affected cases, allowing unbiased assessment of current capabilities of WES as a tool for population screening.

Effective population-level NBS must rapidly identify the few individuals at risk of disease with extraordinary sensitivity, high specificity and limited manual review. In California, NBS for 48 different IEMs performed with MS/MS[1–4] achieved 99.0% sensitivity and >99.8% specificity[5]. For some disorders, MS/MS nonetheless has a low positive predictive value and results may be nonspecific (Fig. 1).

Genomic sequencing, now commonly used for diagnosis of rare disorders[6–8], has been recommended for nearly all seriously ill children in intensive care units[9,10], proposed for all newborns to personalize their medical care[11] and marketed for screening newborns[12]. Yet, population-scale studies to establish performance characteristics of sequencing for NBS have not been reported. NBS IEMs provide an ideal model for evaluating the role of sequencing in population screening because most are Mendelian disorders affecting well-understood biochemical pathways and many have been studied extensively. Moreover, sensitivity and specificity of sequence-based detection of IEMs can be directly compared to those of current MS/MS screening. Studying WES to identify IEMs already included in NBS can also suggest its potential utility for further treatable disorders not amenable to detection by MS/MS[13].

We quantified the performance of WES, were it to have been the primary NBS for IEMs in California. Between July 2005, and December 2013, the Genetic Disease Screening Program (GDSP) of the California Department of Public Health (CDPH) screened dried blood spots (DBSs) from nearly 4.5 million neonates for 48 IEMs using a multiplex MS/MS platform. We obtained a comprehensive set of 1,728 residual, de-identified, archived DBSs representing all cases with IEMs, as well as a select set that were screened as positive but later were determined to be unaffected. We performed WES for 1,416 DBSs[14] and determined that DBS exomes passing quality controls (Methods) were comparable to exomes from fresh blood (Extended Data Figs. 1–4). We analyzed 1,190 high-quality exomes from 805 IEM-affected individuals and 385 MS/MS false positives (Table 1); exomes were divided into validation and test sets, with 178 and 1,012 individuals, respectively (Table 1).

We analyzed variants within an 'exome slice'[15] of 78 genes associated with the 48 IEMs ascertained by NBS in California (Methods; Supplementary Table 1). We systematically explored pipeline parameters on the validation set to derive a customized, robust, sensitive pipeline for reporting potential disease-causing variants in IEM genes in a screening context (Fig. 2a; Methods). The automated pipeline had three arms. The curation arm reported all variants curated as pathogenic in existing disease databases (Human Gene

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69

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ARTICLE

A genome sequencing system for universal newborn screening, diagnosis, and precision medicine for severe genetic diseases

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and informed by root cause analysis. In 119 affected children who had been diagnosed by rWGS, 87% were positive by NBS-rWGS. The diagnostic sensitivity of NBS-rWGS can be further increased by inclusion of variants identified by

Cost effectiveness studies of NBS-rWGS have not yet been performed. While NBS-rWGS is intended to supplement NBS-MS, not replace it, the current cost of NBS-MS for the 35 core disorders on the RUSP provides a reference point

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for what is likely to be acceptable for public-health-funded NBS-rWGS. Most states publish the fees charged for NBS-MS, which represent part of the total cost. The highest such fee is $220 per newborn. Diagnostic rWGS costs RCIGM ~$8,500 per newborn. However, the interpretation burden of NBS-rWGS is about one thousandth that of Dx-rWGS and several biotechnology companies have indicated that $100 rWGS will be possible in the relatively near future.[20] The prerequisites for inexpensive NBS-rWGS are performance at massive scale and near complete automation.

were achieved by combining screening, diagnosis, large genome-phenotype datasets, and learning feedback loops. We invite groups worldwide to join the BeginNGS (Newborn Genomic Sequencing) consortium in implementation studies of NBS-rWGS in diverse populations.

Data and code availability

Consented proband and parent data analyzed in this study and non-human subjects data generated during this study are available at the Longitudinal Pediatric Data Resource (LPDR) under acces-

Screening Panel (RUSP)—increased from 27 to 35, and the number of affected infants identified increased from 6,439 to 6,466.[8,9] However, there are ~7,200 known genetic diseases and hundreds of targeted treatments that

informed methods for newborn screening, diagnosis, and virtual, acute management guidance for 388 diseases with effective treatments and report analytic performance and clinical utility in large retrospective datasets.

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70

Genomic Newborn Screening?

- Who should do this? Public Health NBS Labs? Contracted academic or private labs? Partnership: NBS lab does test, consultant or AI interprets results?
- What to report?
  - Actionable genotype* for disease with onset in infancy only?
  - Actionable genotype* for disease with onset at any age (e.g. cancer predisposition, pharmacogenomics)?
  - Any 'pathogenic' genotype* for a disease treatable or not?
  - Any genotype (pathogenic vs. variants of uncertain significance)?
- What platform or reagent to use (performance not uniform)?

*likely not equitable for most genes and for several more years!

71

Realistic NBS Forecast to the 2030s

- Conditions nominated/added to the RUSP and the relevant screening strategies will be (better) defined, also paying attention to equity.
- Conditions could be added to RUSP at a pace of 1 – 2 per year (<20 by 2032) facilitated by grouping 'like' conditions (e.g. Mucopolysaccharidoses), but current ACHDNC voting members seem to put the brakes on.
- More (gene) therapies will become available, but at moderate pace.
- Genetic/genomic testing will become more commonplace in NBS, but biochemical testing will not be replaced.
- Rational (!) regionalization of NBS in the US, despite advantages, will not happen.

72

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- Rational (!) regionalization of NBS in the US, despite advantages, will not happen.

But remember: addition to RUSP ≠ state implementation

73

SUMMARY

- Newborn screening is one of the most successful public health programs.
- In the US, mechanisms are in place to expand the RUSP based on evidence (although evidence usually emerges only once screening has started).
- The most efficient and effective approach to newborn screening relies primarily on biochemical genetic assays – for 1st and 2nd tier testing.
- Molecular genetic testing/genomics for newborn screening is currently of limited value given high cost, time to complete analysis, and the frequency of genotypes of uncertain significance.
- The biggest limitation to expansion of newborn screening is not laboratory testing and result interpretation but the lack of treatment options.

74

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75