GBSC 724 Advanced Special Topics in Metabolomics

POPULATION-SCALE CLINICAL METABOLOMICS: EXPANDED NEWBORN SCREENING

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Lecture overview

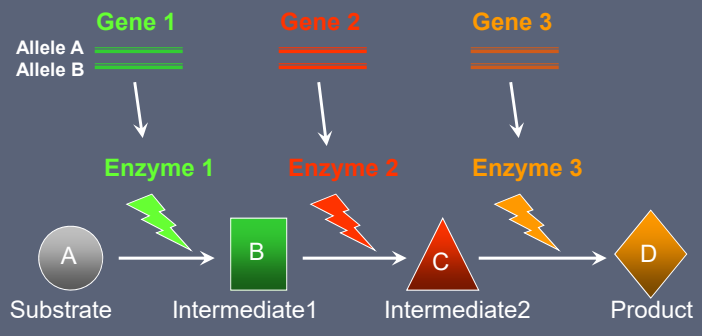
- Introduction and relevance
- Historical perspective
- Methodology
- Future prospects

Prologue: the impact of newborn screening

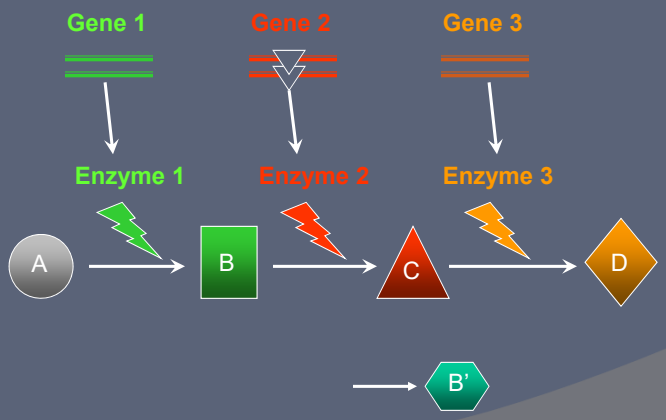
- JS was born in1942 with phenylketonuria (PKU). Undiagnosed, he developed severe intellectual disability and was institutionalized at the age of 20.
- JD was born in1962 with PKU. NBS was now available and led to a diagnosis at 2 weeks of age. She was placed on a special diet, and grew to be an adult with normal intelligence.

- ES was born in a state without medium chain acyl-CoA dehydrogenase (MCAD) deficiency screening in 1999. Undiagnosed, she died in her sleep at 15 months of age.
- RD was born on the same day, but 15 miles away, just across the border in a state where MCAD screening was offered. He was placed on dietary therapy and grew to be a normal adult.

Metabolic Pathways: Sequential Enzyme-Catalyzed Reactions



Consequences of Metabolic Enzyme Dysfunction



Newborn Screening: one of the ten great public health achievements worldwide, 2001–2010

"Improvements in technology and endorsement of a uniform newborn-screening panel of diseases have led to earlier life-saving treatment and intervention for at least [4000] additional newborns each year with selected genetic and endocrine disorders."

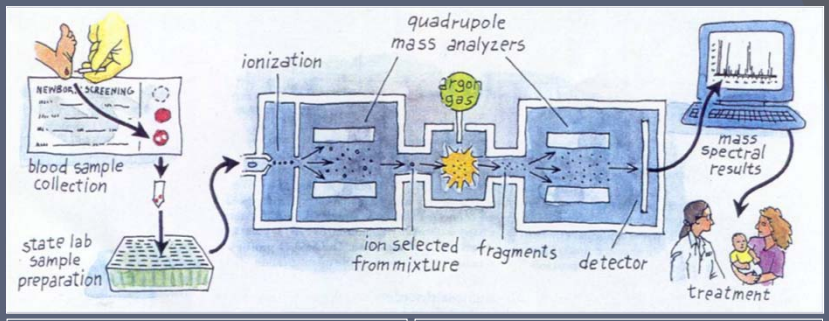
Morbidity & Mortality Weekly Report. 2011; 60(24):814-818

What is newborn screening (NBS)?

- Population scale screening of all newborns* for the presence of *treatable* conditions that are not otherwise evident at birth
  - Screening vs. diagnostic testing
- State – specific programs (no federal mandate) with significant variability
  - disorders detected
  - follow-up procedures

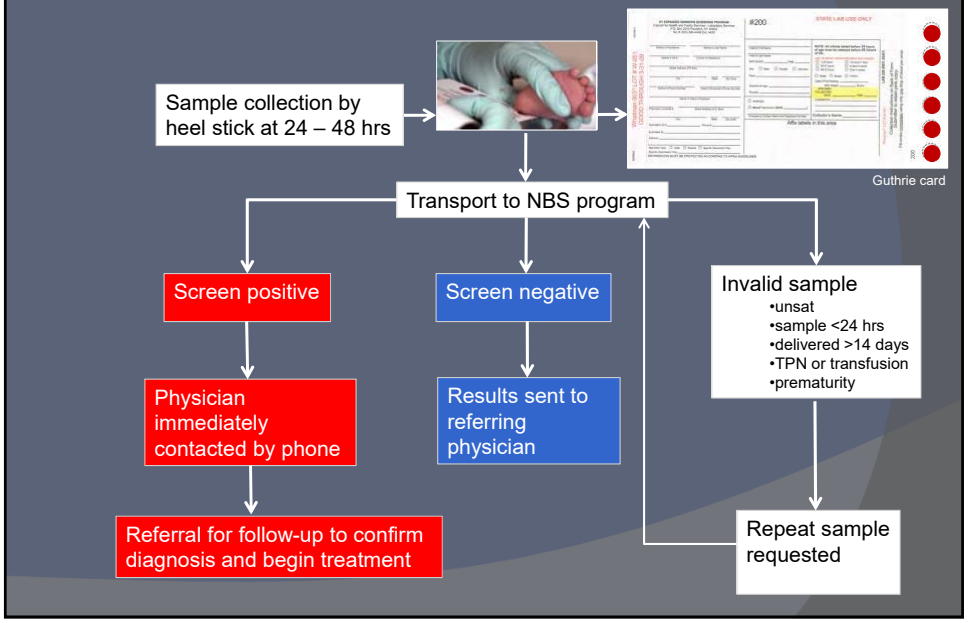
*USA: 4.3 million births/year

Modern newborn screening program



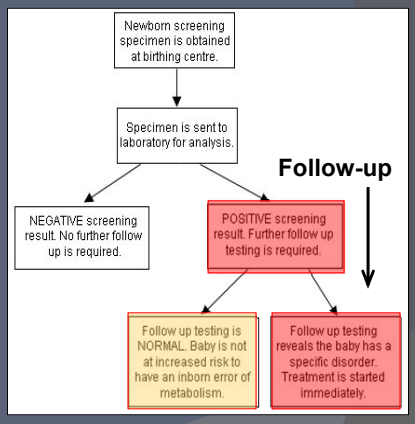
•Blood sample collected 24 – 48 hrs after birth (may be follow-up screen at 2 – 4 weeks)	•Analytical time: ~ 5 minutes •Metabolites detected: >20 •Conditions detected: >50

Logistics of newborn screening



Newborn screening follow-up programs: screening is only the beginning

- Required to confirm or refute screening results
- Follow-up programs vary significantly by state
  - Biochemical/molecular genetic laboratories
- Most infants with abnormal NBS results have normal follow-up (~90%)
  - Prematurity
  - TPN or certain formulas
- If disease is confirmed then treatment is initiated immediately



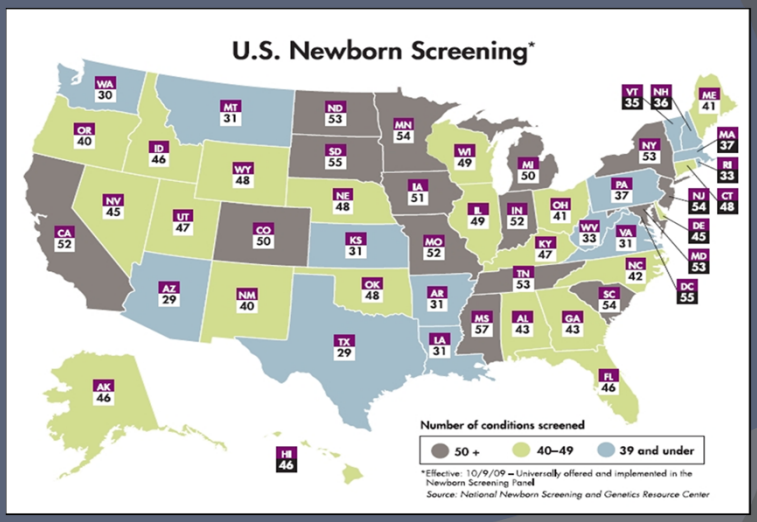
Western Australia Newborn Screening Program

Screened disorders in the United States

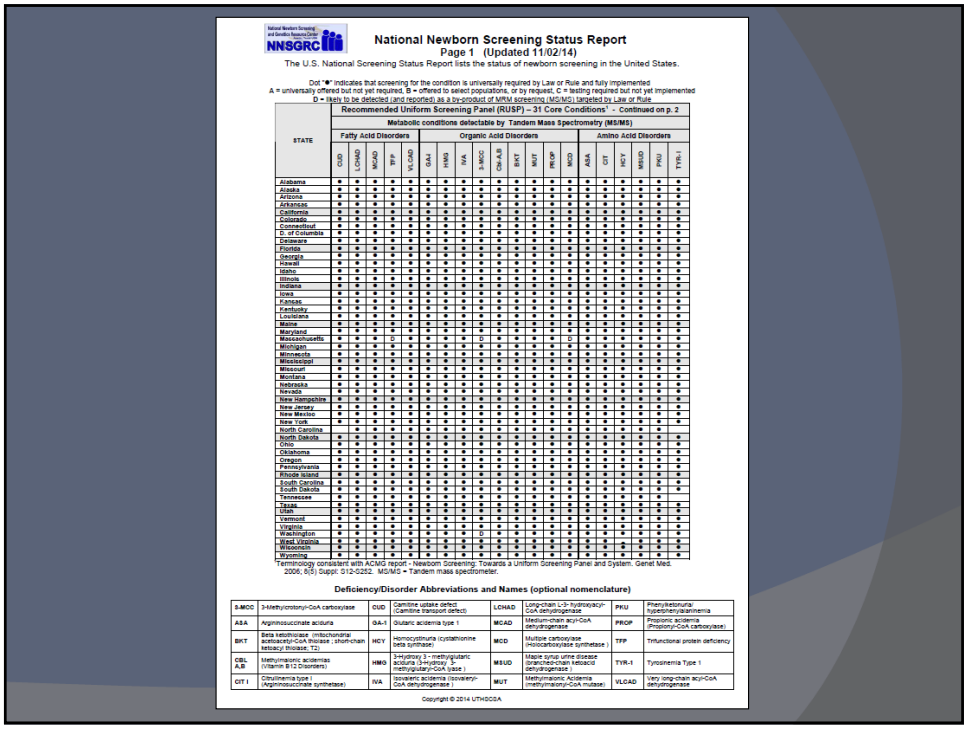
- Currently, 34 core conditions are recommended for newborn screening
  - 20 metabolic disorders (eg, PKU)
  - 2 endocrine disorders (eg, CAH)
  - 3 hemoglobin disorders (eg, sickle cell anemia)
  - 9 other conditions (eg, hearing loss, cystic fibrosis)
- Also 26 secondary conditions (may lack an effective therapy or have an unclear natural hx) that can be detected when screening for core disorders
  - 24 metabolic
  - 1 hemoglobinopathy
  - 3 other

National Newborn Screening & Global Resource Center (NNSGRC)

Conditions screened* by state



*Core + secondary conditions



National Newborn Screening Status Report
Page 1 (Updated 11/02/14)

The U.S. National Screening Status Report lists the status of newborn screening in the United States.

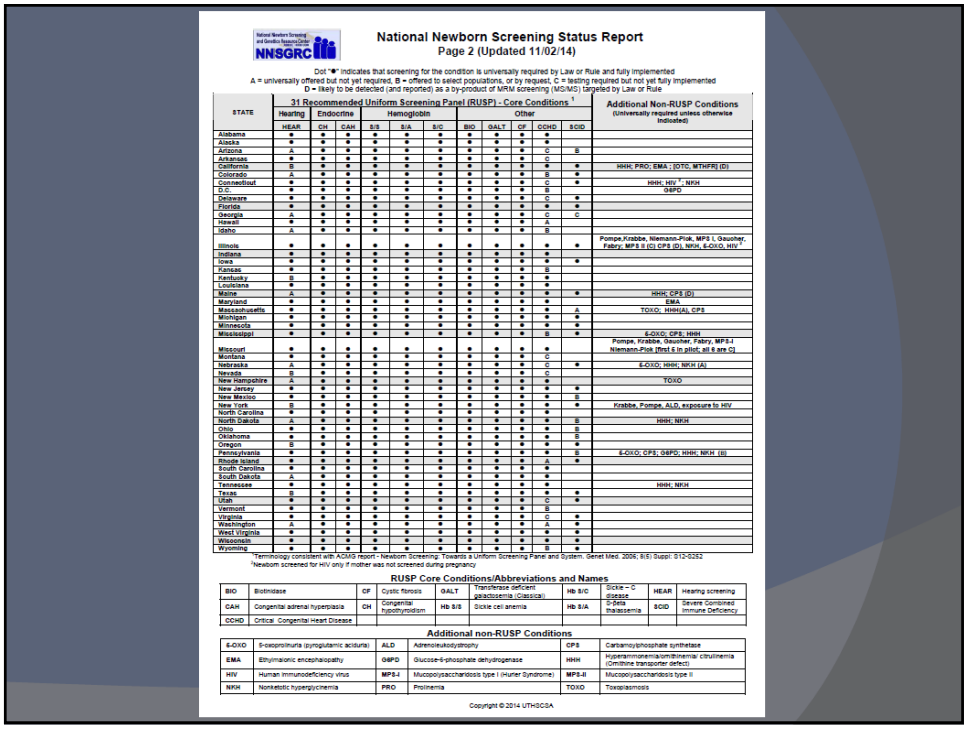
Dot "●" indicates that screening for the condition is universally required by Law or Rule and fully implemented
A = universally offered but not yet required, B = offered to select populations, or by request, C = testing required but not yet implemented
D = likely to be detected (and reported) as a by-product of MRM screening (MS/MS) targeted by Law or Rule

STATE	Recommended Uniform Screening Panel (RUSP) – 31 Core Conditions[1] – Continued on p. 2																		
	Metabolic conditions detectable by Tandem Mass Spectrometry (MS/MS)																		
	Fatty Acid Disorders					Organic Acid Disorders										Amino Acid Disorders			
	CUD	LCHAD	MCAD	TFP	VLCAD	GA-I	HMG	IVA	3-MCC	CBl-A,B	BKT	MUT	PROP	MCD	ASA	CIT	HCY	MSUD	PKU	TYR-I
Alabama	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Alaska	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Arizona	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Arkansas	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
California	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Colorado	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Connecticut	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
D. of Columbia	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Delaware	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Florida	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Georgia	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Hawaii	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Idaho	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Illinois	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Indiana	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Iowa	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Kansas	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Kentucky	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Louisiana	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Maine	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Maryland	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Massachusetts	●	●	●	D	●	●	●	●	D	●	●	●	●	D	●	●	●	●	●	●
Michigan	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Minnesota	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Mississippi	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Missouri	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Montana	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Nebraska	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Nevada	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
New Hampshire	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
New Jersey	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
New Mexico	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
New York	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
North Carolina	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
North Dakota	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Ohio	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Oklahoma	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Oregon	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Pennsylvania	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Rhode Island	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
South Carolina	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
South Dakota	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Tennessee	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Texas	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Utah	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Vermont	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Virginia	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Washington	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
West Virginia	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Wisconsin	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Wyoming	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

[1]Terminology consistent with ACMG report - Newborn Screening: Towards a Uniform Screening Panel and System. Genet Med. 2006; 8(5) Suppl: S12-S252. MS/MS = Tandem mass spectrometer.

Deficiency/Disorder Abbreviations and Names (optional nomenclature)

3-MCC	3-Methylcrotonyl-CoA carboxylase	CUD	Carnitine uptake defect (Carnitine transport defect)	LCHAD	Long-chain L-3- hydroxyacyl-CoA dehydrogenase	PKU	Phenylketonuria/ hyperphenylalaninemia
ASA	Argininosuccinate aciduria	GA-I	Glutaric acidemia type I	MCAD	Medium-chain acyl-CoA dehydrogenase	PROP	Propionic acidemia (Propionyl-CoA carboxylase)
BKT	Beta ketothiolase (mitochondrial acetoacetyl-CoA thiolase ; short-chain ketoacyl thiolase; T2)	HCY	Homocystinuria (cystathionine beta synthase)	MCD	Multiple carboxylase (Holocarboxylase synthetase)	TFP	Trifunctional protein deficiency
CBL A,B	Methylmalonic acidemias (vitamin B12 Disorders)	HMG	3-Hydroxy 3 - methylglutaric aciduria (3-Hydroxy 3- methylglutaryl-CoA lyase)	MSUD	Maple syrup urine disease (branched-chain ketoacid dehydrogenase)	TYR-I	Tyrosinemia Type I
CIT I	Citrullinemia type I (Argininosuccinate synthetase)	IVA	Isovaleric acidemia (Isovaleryl-CoA dehydrogenase)	MUT	Methylmalonic Acidemia (methylmalonyl-CoA mutase)	VLCAD	Very long-chain acyl-CoA dehydrogenase



National Newborn Screening Status Report
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STATE	31 Recommended Uniform Screening Panel (RUSP) - Core Conditions [1]											Additional Non-RUSP Conditions (universally required unless otherwise indicated)
	Hearing	Endocrine	Hemoglobin			Other						
	HEAR	CH	CAH	S/S	S/A	S/C	BIO	GALT	CF	CCHD	SCID	
Alabama	●	●	●	●	●	●	●	●	●	●		
Alaska	●	●	●	●	●	●	●	●	●	●		
Arizona	A	●	●	●	●	●	●	●	●	C	B	
Arkansas	●	●	●	●	●	●	●	●	●	C		
California	B	●	●	●	●	●	●	●	●	●	●	HHH; PRO; EMA ; [OTC, MTHFR] (D)
Colorado	A	●	●	●	●	●	●	●	●	B	●	
Connecticut	●	●	●	●	●	●	●	●	●	C	●	HHH; HIV [2]; NKH
D.C.	●	●	●	●	●	●	●	●	●	B		G6PD
Delaware	●	●	●	●	●	●	●	●	●	C	●	
Florida	●	●	●	●	●	●	●	●	●	●	●	
Georgia	A	●	●	●	●	●	●	●	●	C	C	
Hawaii	●	●	●	●	●	●	●	●	●	A		
Idaho	A	●	●	●	●	●	●	●	●	B		
Illinois	●	●	●	●	●	●	●	●	●	●	●	Pompe,Krabbe, Niemann-Pick, MPS I, Gaucher, Fabry; MPS II (C) CPS (D), NKH, 6-OXO, HIV [2]
Indiana	●	●	●	●	●	●	●	●	●	●		
Iowa	●	●	●	●	●	●	●	●	●	●	●	
Kansas	●	●	●	●	●	●	●	●	●	E		
Kentucky	B	●	●	●	●	●	●	●	●	●		
Louisiana	●	●	●	●	●	●	●	●	●	●		
Maine	A	●	●	●	●	●	●	●	●	●	●	HHH; CPS (D)
Maryland	●	●	●	●	●	●	●	●	●	●		EMA
Massachusetts	●	●	●	●	●	●	●	●	●	●	A	TOXO; HHH(A), CPS
Michigan	●	●	●	●	●	●	●	●	●	●	●	
Minnesota	●	●	●	●	●	●	●	●	●	●	●	
Mississippi	●	●	●	●	●	●	●	●	●	B	●	
Missouri	●	●	●	●	●	●	●	●	●	●		6-OXO; CPS; HHH Pompe, Krabbe, Gaucher, Fabry, MPS-I Niemann-Pick (Pmt I in pilot; all 6 are D)
Montana	●	●	●	●	●	●	●	●	●	C		
Nebraska	●	●	●	●	●	●	●	●	●	C	●	6-OXO; HHH; NKH (A)
Nevada	B	●	●	●	●	●	●	●	●	C		
New Hampshire	A	●	●	●	●	●	●	●	●	●		TOXO
New Jersey	●	●	●	●	●	●	●	●	●	●	●	
New Mexico	●	●	●	●	●	●	●	●	●	●	B	
New York	B	●	●	●	●	●	●	●	●	●	●	Krabbe, Pompe, ALD, exposure to HIV
North Carolina	●	●	●	●	●	●	●	●	●	●		
North Dakota	A	●	●	●	●	●	●	●	●	●	B	HHH; NKH
Ohio	●	●	●	●	●	●	●	●	●	●	B	
Oklahoma	●	●	●	●	●	●	●	●	●	●	B	
Oregon	B	●	●	●	●	●	●	●	●	●	●	
Pennsylvania	●	●	●	●	●	●	●	●	●	●	B	6-OXO; CPS; G6PD; HHH; NKH (B)
Rhode Island	●	●	●	●	●	●	●	●	●	●	●	
South Carolina	●	●	●	●	●	●	●	●	●	A		
South Dakota	A	●	●	●	●	●	●	●	●	●		
Tennessee	●	●	●	●	●	●	●	●	●	●		HHH; NKH
Texas	B	●	●	●	●	●	●	●	●	●	●	
Utah	●	●	●	●	●	●	●	●	●	C	●	
Vermont	●	●	●	●	●	●	●	●	●	B		
Virginia	●	●	●	●	●	●	●	●	●	C	●	
Washington	A	●	●	●	●	●	●	●	●	A	●	
West Virginia	●	●	●	●	●	●	●	●	●	●	●	
Wisconsin	●	●	●	●	●	●	●	●	●	●	●	
Wyoming	●	●	●	●	●	●	●	●	●	B	●	

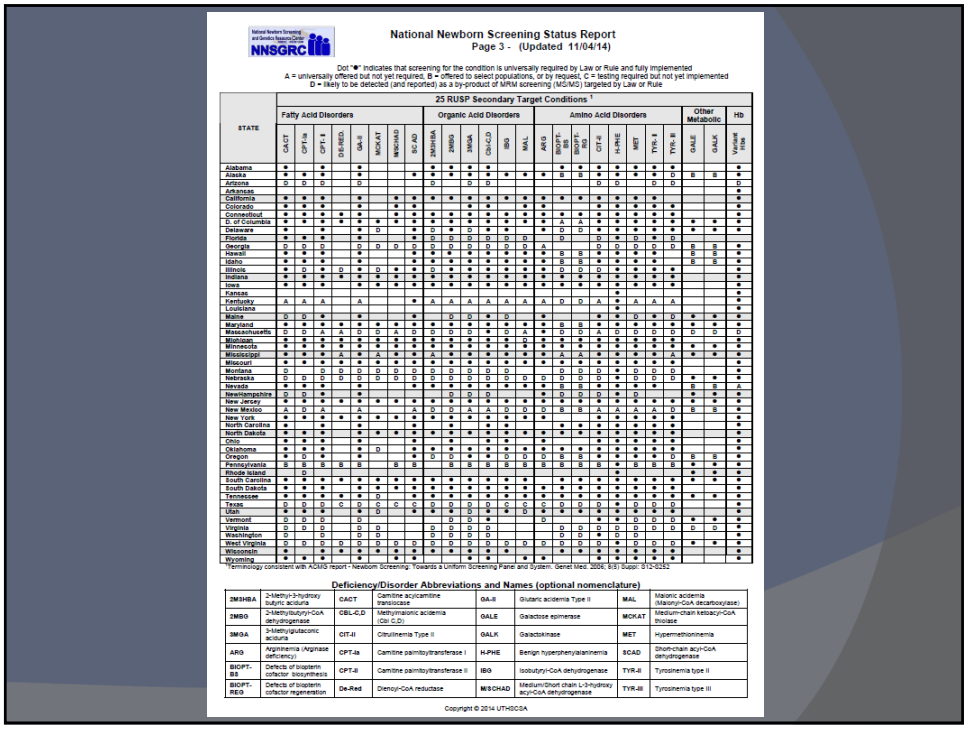
[1]Terminology consistent with ACMG report - Newborn Screening: Towards a Uniform Screening Panel and System. Genet Med. 2006; 8(5) Suppl: S12-S252
[2]Newborn screened for HIV only if mother was not screened during pregnancy

RUSP Core Conditions/Abbreviations and Names

BIO	Biotinidase	CF	Cystic fibrosis	GALT	Transferase deficient galactosemia (Classical)	Hb S/C	Sickle – C disease	HEAR	Hearing screening
CAH	Congenital adrenal hyperplasia	CH	Congenital hypothyroidism	Hb S/S	Sickle cell anemia	Hb S/A	S-Beta thalassemia	SCID	Severe Combined Immune Deficiency
CCHD	Critical Congenital Heart Disease								

Additional non-RUSP Conditions

6-OXO	5-oxoprolinuria (pyroglutamic aciduria)	ALD	Adrenoleukodystrophy	CPS	Carbamoylphosphate synthetase
EMA	Ethylmalonic encephalopathy	G6PD	Glucose-6-phosphate dehydrogenase	HHH	Hyperammonemia/orn/thinemia/ citrullinemia (Ornithine transporter defect)
HIV	Human immunodeficiency virus	MPS-I	Mucopolysaccharidosis type I (Hurler Syndrome)	MPS-II	Mucopolysaccharidosis type II
NKH	Nonketotic hyperglycinemia	PRO	Prolinemia	TOXO	Toxoplasmosis



National Newborn Screening Status Report
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STATE	25 RUSP Secondary Target Conditions †																												
	Fatty Acid Disorders							Organic Acid Disorders						Amino Acid Disorders											Other Metabolic		Hb
	CACT	CPT-Ia	CPT-II	DE-RED	GA-II	MCKAT	MKSCHAD	SCAD	2M3HBA	3MBG	3MGA	CBL-C,D	IBG	MAL	ARG	BIOPT-BS	BIOPT-REG	CIT-II	H-PHE	MET	TYR-I	TYR-II	GALE	GALK	Variant Utah
Alabama	●		●		●				●	●	●	●				●	●	●	●	●	●	●			●
Alaska	●	●	●		●			●	●	●	●	●	●	●	●	B	B	●	●	●	●	●	B	B	●
Arizona	D	D	D		D			D			D	D							D	D		D	D		D
Arkansas																									●
California	●	●	●		●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●				●
Colorado	●	●	●		●		●				●	●		●	●			●	●	●	●	●			●
Connecticut	●	●	●	●	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●			●
D. of Columbia	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	A	A	●	●	●	●	●	●	●	●
Delaware	●		●		●	D		●	D	●	D	●	●		●	D	D	●	●	●	●	●	●	●	●
Florida	●	●	●		●			●	D	D	D	D	D	D		D		D	●	D	●	D			
Georgia	D	D	D		D	D	D	D	D	D	D	D	D	D	A			D	D	D	D	D	B	B	●
Hawaii	●	●	●		●			●	●	●	●	●	●	●		B	B	●	●	●	●		B	B	●
Idaho	●	●	●		●			●	●	●	●	●	●	●		B	B	●	●	●	●		B	B	●
Illinois	●	D	●	D	●	D	●	●	D	●	●	●	●	●		D	D	D	●	●	●	●			●
Indiana	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●			●
Iowa	●	●	●		●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●			●
Kansas																			●						●
Kentucky	A	A	A		A			●	A	A	A	A	A	A	A	D	D	A	●	A	A	A			●
Louisiana																			●						●
Maine	D	D	●		●			●		D	D	●	D		●				●	●	D	●	D	●	●
Maryland	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	B	B	●	●	●	●	●	●	●	●
Massachusetts	D	D	A	A	D	D	A	D	D	D	D	D	D	A	●	D	D	D	D	D	D	D	D	D	D
Michigan	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●			●
Minnesota	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Mississippi	●	●	●	A	●	A	●	●	A	●	●	●	●	●	●	A	A	●	●	●	●	A	●	●	●
Missouri	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●			●
Montana	D		D	D	D	D	D	D	D	D	D	D				D	D	D	●	D	D	D			●
Nebraska	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	●	D	D	D	●	●	●
Nevada	●	●	●		●			●	●	●	●	●	●	●	●	B	B	●	●	●	●		B	B	A
NewHampshire	D	D	●		●				D	D	D				●	D	D	D	●	D			●	●	●
New Jersey	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
New Mexico	A	D	A		A			A	D	C	A	A	C	D	D	B	B	A	A	A	A	D	B	B	●
New York	●	●	●	●	●	●	●	●	●	●	●	●	●	●				●	●	●	●	●			●
North Carolina	●		●		●			●			●							●	●	●	●	●			●
North Dakota	●	●	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●			●
Ohio	●	●	●		●			●			●								●	●	●	●			●
Oklahoma	●	●	●		●	D		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●			●
Oregon	●	D	●		●			●	D	D	●	●	D	D	D	B	B	●	●	●	●	D	B	B	●
Pennsylvania	B	B	B	B	B		B	B		B	B	B	B	B	B	B	B	B	●	B	B	●	●	●	●
Rhode Island		D																	●				●	●	●
South Carolina	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●	●	●
South Dakota	●	●	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●			●
Tennessee	●	●	●	●	●	D		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Texas	D	D	D	C	D	C	C	D	D	D	D	C	C	C	C	D	D	D	●	D	D	D			●
Utah	●	●	●		●	D		●	●	●	D	●	●	D	●	●	●	●	●	●	●	●			●
Vermont	D	D	D		D				D	D	●				D			●	●	D	D	D	●	●	●
Virginia	D	D	D		D	D			D	D	D	D				D	D	D	●	D	D	D	D	D	●
Washington	D		D		D	D			D	D	D	D				D	D	●	D	D					●
West Virginia	D	D	D	B	D	D	B	D	D	C	D	D	D	D	D	D	D	D	●	D	D	D	●	●	●
Wisconsin	●		●	●	●	●	●	●	●	●	●	●	●			●	●	●	●	●	●	●			●
Wyoming	●	●	●		●	●	●	●			●	●	●	●	●			●	●	●	●	●			●

†Terminology consistent with ACMG report - Newborn Screening: Towards a Uniform Screening Panel and System. Genet Med. 2006; 8(5) Suppl: 01S-252S

Deficiency/Disorder Abbreviations and Names (optional nomenclature)

2M3HBA	2-Methyl-3-hydroxy butyric aciduria	CACT	Carnitine acylcarnitine translocase	GA-II	Glutaric acidemia Type II	MAL	Malonic acidemia (Malonyl-CoA decarboxylase)
2MBG	2-Methylbutyryl-CoA dehydrogenase	CBL-C,D	Methylmalonic acidemia (Cbl C,D)	GALE	Galactose epimerase	MCKAT	Medium-chain ketoacyl-CoA thiolase
3MGA	3-Methylglutaconic aciduria	CIT-II	Citrullinemia Type II	GALK	Galactokinase	MET	Hypermethioninemia
ARG	Argininemia (Arginase deficiency)	CPT-Ia	Carnitine palmitoyltransferase I	H-PHE	Benign hyperphenylalaninemia	SCAD	Short-chain acyl-CoA dehydrogenase
BIOPT-BS	Defects of biopterin cofactor biosynthesis	CPT-II	Carnitine palmitoyltransferase II	IBG	Isobutyryl-CoA dehydrogenase	TYR-II	Tyrosinemia type II
BIOPT-REG	Defects of biopterin cofactor regeneration	De-Red	Dienoyl-CoA reductase	MSCHAD	Medium/Short chain L-3-Hydroxy acyl-CoA dehydrogenase	TYR-III	Tyrosinemia type III

Tangible benefits of newborn screening

- Improved health outcomes:
  - estimated that 4000 – 5000 newborns/yr experience significantly improved health outcomes as a result of early detection and initiation of treatment[1]
  - prevents diagnostic odysseys
- Cost-effective:
  - For one condition (congenital hypothyroidism) estimated annual economic benefit (eg, avoiding cost of treating an affected individual) is nearly 20 fold greater than the cost of screening ($400 M vs. $20 M)[2]

1. http://www.councilforresponsiblegenetics.org/genewatch/GeneWatchPage.aspx?pageId=450#endnotes

2.CDC. MMWR 2004; 53(3):57–59
Grosse SD. AERE Newsletter. 2007; 27(2):17-21Grosse, SD et al. Med Care. 2009; 47(7 Suppl1):S94–S103

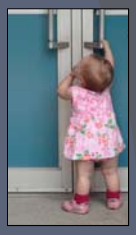
Limitations of NBS

- False negatives
- False positives
  - create significant stress for families
- Many types of metabolic disorders are not screened
- Questionable clinical utility for some screened disorders
- Lack of clinical and laboratory expertise
- Significant financial constraints

Newborn screening: ethical issues

- Privacy
  - Sample retention and security of stored data
- Clinical utility is questionable for some screened disorders
  - Severe forms of certain disorders that may present before NBS results are available
  - Very rare disorders with small numbers of affected patients, making outcomes uncertain
  - Very mild, ill-defined phenotypes
  - Lack of treatment options

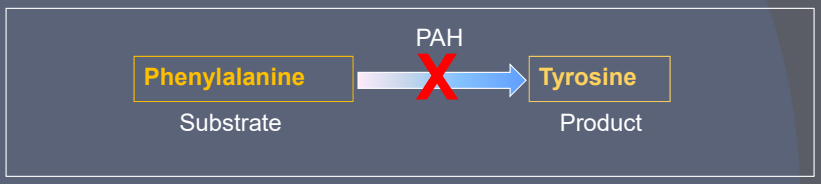
Criteria for inclusion in the ACMG ENS core screening panel (2006)



- An effective treatment is available
- Demonstrated benefits of early detection and treatment (clinical utility)
- The condition does not usually produce symptoms within 24 – 48 hrs after birth
- A sensitive, specific, and cost-effective test is available that can detect the condition within this time frame
- See http://mchb.hrsa.gov/screening/ for more about the ENS task force

Historical Perspective

The origins of NBS: phenylketonuria (PKU)



- Etiology: impaired phenylalanine metabolism, with resulting CNS toxicity
- Treatment: reduction of dietary phenylalanine, but requires early detection
  - Development of a phenylalanine-free formula (Lofenalac)
  - Development of a simple test to detect PKU soon after birth

Robert Guthrie pioneered the first newborn screening test for PKU

- BIA: filter paper containing blood from newborns applied to an agar plate
- Bacteria only grow in the presence of phenylalanine
- Large colonies = PKU
- Paradigm: one test for one disorder

A brief history of newborn screening: the early years

- 1961: Robert Guthrie develops screening test for PKU
- 1962: Massachusetts pilots state-wide PKU screening
- 1965: Over 50% of states have mandated PKU screening
- 1968: WHO publishes *Principles and Practices of Screening for Disease*
  - Wilson-Jungner principles (early screening criteria)
- 1970s - 1980s: most states screen for ~6 conditions

A brief history of newborn screening: the era of MSMS expansion

- 1990s – early 2000s: Development and implementation of MSMS for newborn screening ⟹
  - Paradigm: one test for multiple disorders
- 2002: Maternal and Child Health Bureau commissions ACMG to recommend a uniform panel of conditions for NBS
  - 2005: ACMG ENS report identifies 29 core conditions and 25 secondary conditions (designated by HHS as the national standard for NBS – but not federally mandated) ⇩
- 2009: All states screen for at least 29 disorders; approximately 20 states screen for 40+ disorders

Methodology

Acylcarnitines are biomarkers for fatty acid oxidation disorders

- Deficient fatty/organic acid oxidation enzyme activity results in accumulation of one or more size-specific acylcarnitines in blood
- Effectively measured via MSMS; basis for expanded newborn screening (fatty/organic acid oxidation defects)

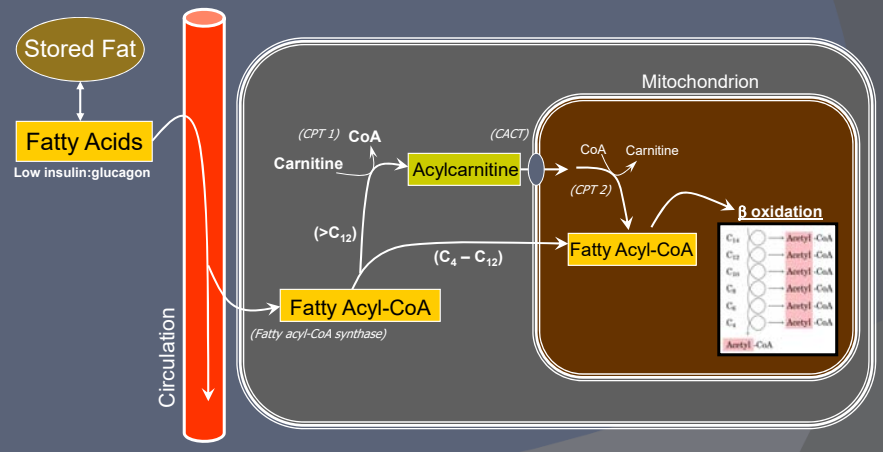
Acylcarnitines

- Disorders detected
  - Fatty acid oxidation disorders
  - Organic acid disorders
  - Other conditions identified
    - Ketosis, acidosis, catabolism, liver disease, renal disease, MCT feeding, etc
- Methodology
  - MSMS analysis of butylated acylcarnitines
  - Quantification of >30 acylcarnitines
  - Analytical time: ~2 hrs

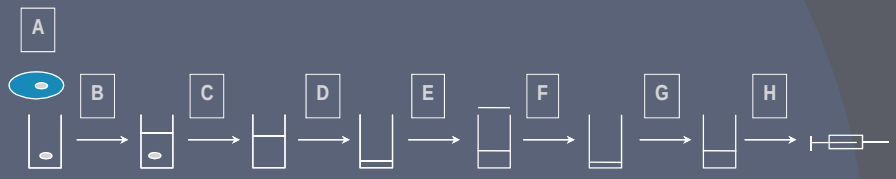
Acylcarnitines, continued

- Sample requirements
  - Plasma (≥1 cc)
    - 20 ul used in assay
- Limitations
  - Interfering substances
  - Results generally not considered to be diagnostic (enzyme activity and/or sequence analysis)
- Confounders
  - Liver/kidney disease (AC-DCs)
  - Ketosis (C2, C4-OH, C12:1, C14:1)
  - MCT oil (C8, C10)
  - Valproate (C0, C8, C10)
  - Carnitine supplements (short chain ACs)
  - Cefotaxime (C14:1, C16:1-OH)
  - Cheese (C3)

Overview of fatty acid oxidation



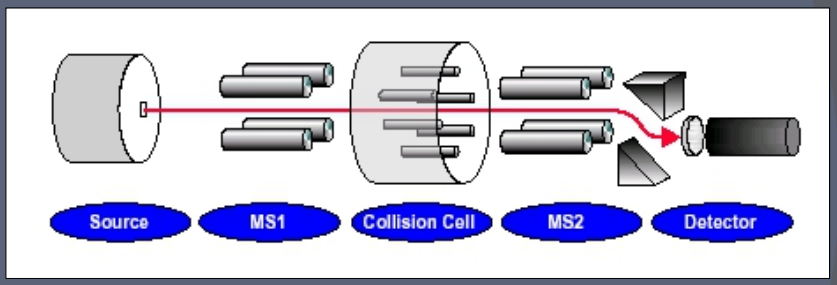
Blood Spot Sample Preparation



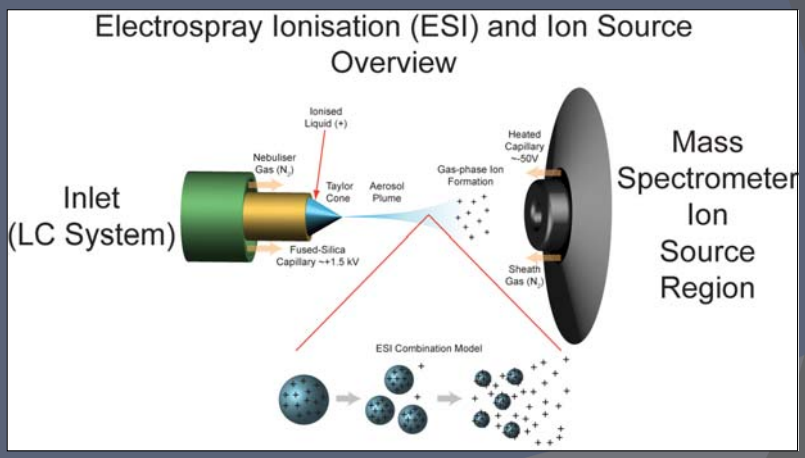
A. Punch out one spot from Guthrie card (typically 3/16" or 3mm).
B. Add 100 µL MeOH (with internal standards) and extact for 30 minutes
C. Transfer supernatant into second plate.
D. Evaporate to dryness under nitrogen with mild (40°C) heating.
E. Add 100 µL 3 N Butanolic HCl to each sample and heat at 60°C for 15 minutes for butylation.
F. Evaporate to dryness under nitrogen with mild (40°C) heating.
G. Add 100 µL 80% MeCN to dissolve each sample.
H. Inject 10 µL into mobile phase

Waters Quattro Micro LC-MSMS

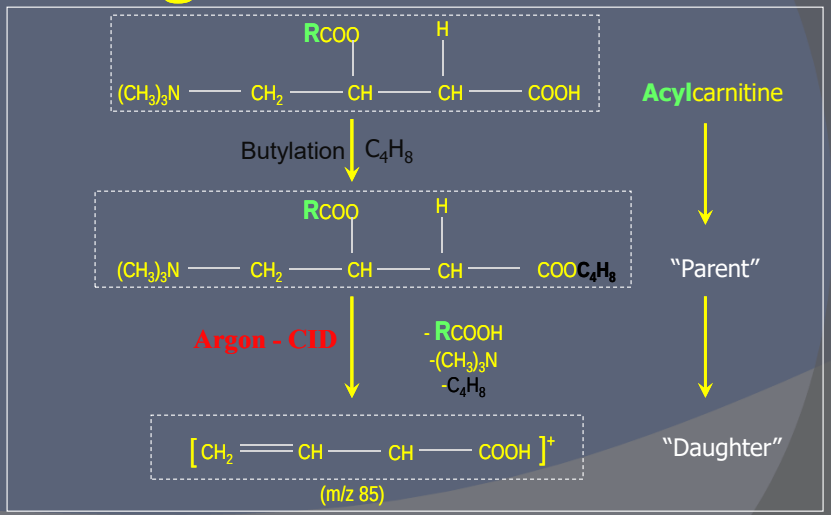
Schematic of a triple quadrupole tandem mass spectrometer



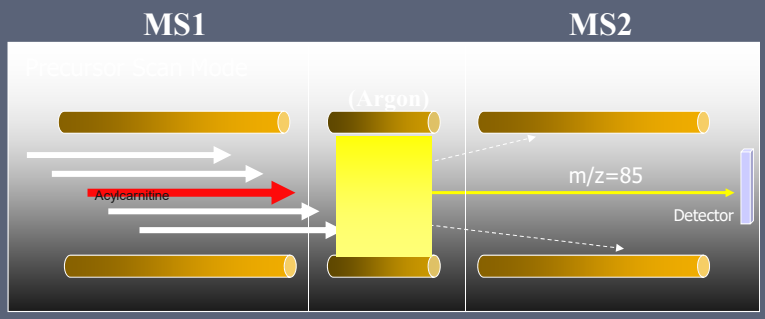
Electrospray ionization



Acylcarnitines: derivatization and fragmentation



Analysis of plasma acylcarnitines using precursor scanning ("parents of 85")

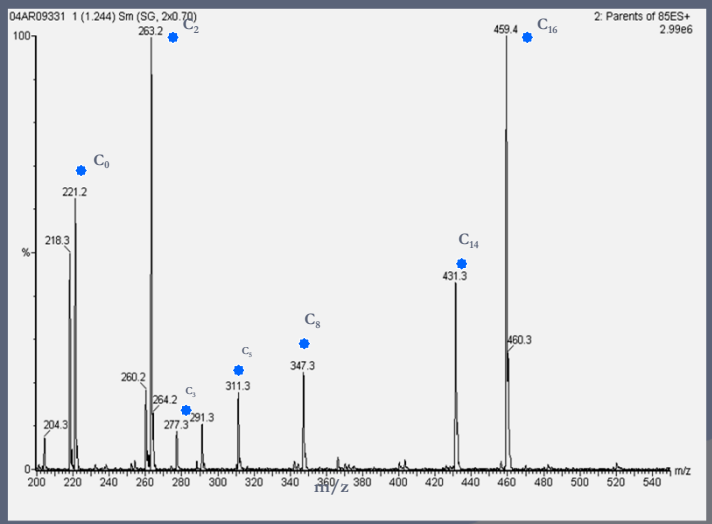


Scanning:
-sequential passage of all masses

CID

Static
-specific daughter mass only; refer back to parent precursor of m/z=85

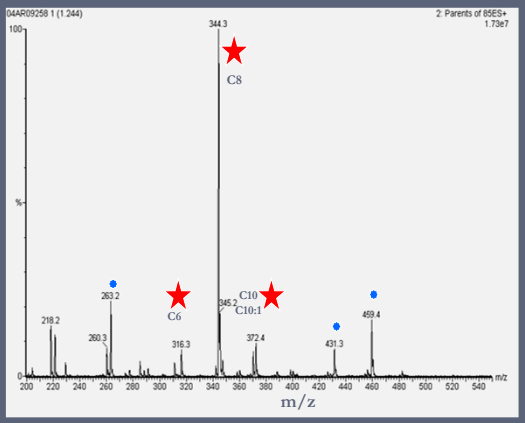
Plasma acylcarnitine profile



Normal profile

= internal standard peak

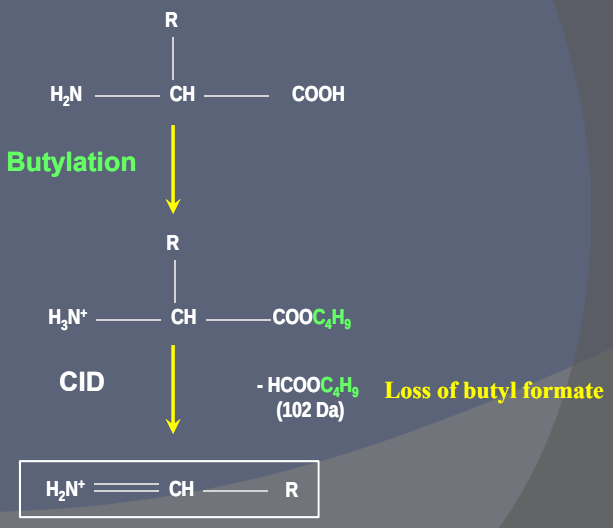
Abnormal acylcarnitine profile



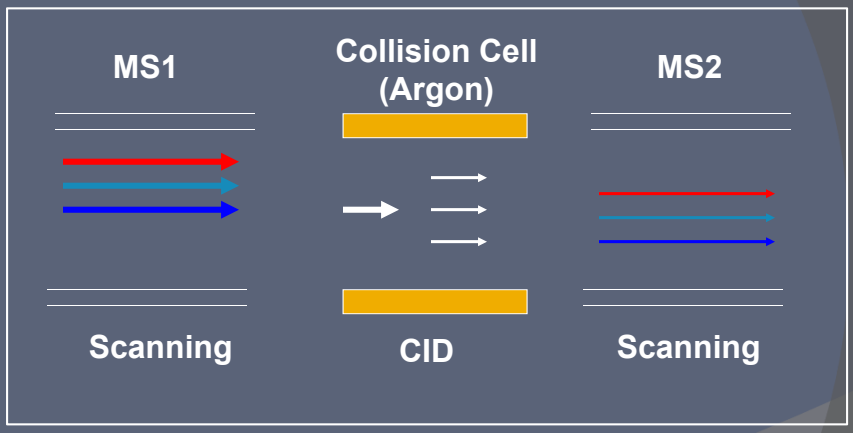
MCAD

- Medium Chain Acyl-CoA Dehydrogenase (MCAD) deficiency
- Most common defect of mitochondrial FAO (1:15,000)
- Lethargy, seizures, hypoketotic hypoglycemia, sudden death
- Diagnosis allows for treatment (avoidance of fasting)
  - Clinical utility

Neutral and acidic amino acids: derivatization and fragmentation

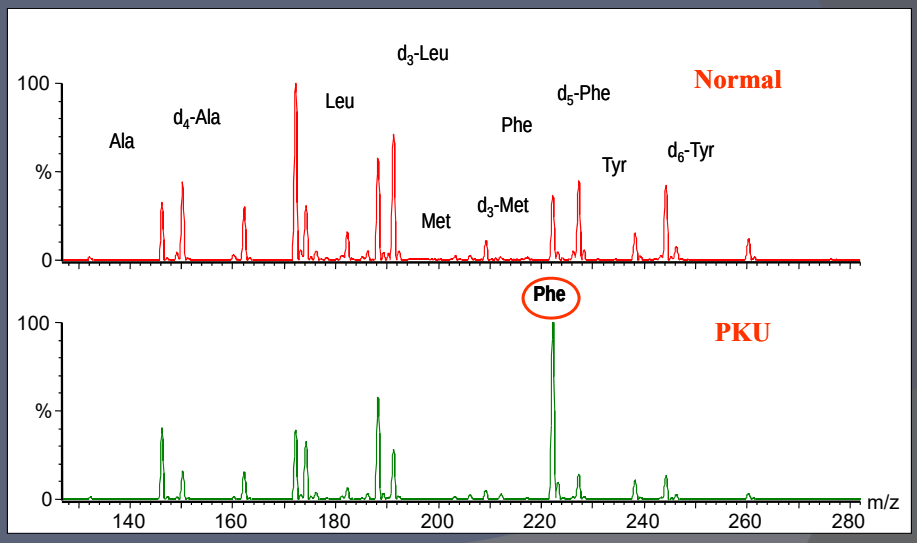


Neutral Loss Scan for Amino Acids

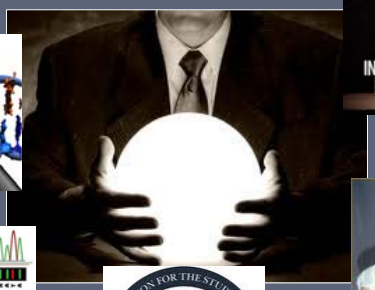


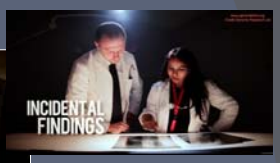
- Loss of 119 Da for basic amino acids
- Loss of 102 Da for acidic and neutral amino acids

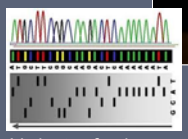
Phenylketonuria (PKU)



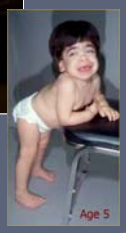
The Future of Newborn Screening







Variants of unknown significance



Genzyme

Google images

Where do we go from here?

- The existing NBS model continues to evolve
  - More conditions (eg, selected lysosomal storage diseases) being added or considered for screening
  - Changes to screening criteria proposed
- Next generation sequencing: the new screening paradigm?
  - Potential for massive expansion of genetic screening

Altering the paradigm: should we screen for diseases without an effective therapy?

- Cornerstone of traditional screening: must be an effective treatment available
- However, it has been suggested that future screening should consider other benefits:
  - avoiding diagnostic odysseys
  - making preparations for disease
  - reproductive decisions
  - early access to promising new therapies

Alexander and van Dyck, 2006
Tarini 2008

Thank You!

UAB DEPARTMENT OF GENETICS