

Newborn Screening



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Outline of Lecture

- Principles of newborn screening
- Logistics of newborn screening programs
- Review of selected diseases which are screened
- Tandem mass spectrometry and "expanded newborn screening"
- New additions to the screening panel
- Challenges and controversies in newborn screening

Newborn Screening Programs

- Most states in US began newborn screening in the 1960's
- Statewide screening began in Missouri in 1967
- State is mandated to offer screening
- Program composed of laboratory and follow-up office
- Located in Department of Health in Jefferson City

Newborn Screening **Missouri State Public Health Laboratory**



Patrick Hopkins; Newborn Screening Laboratory Manager

Goals of Newborn Screening

1. Decrease the morbidity, mortality, and burden to individuals and society from genetic diseases
2. Distinguish individuals who probably have treatable disorder from those who do not have the disorder ("screening")
3. Ensure that each individual with an abnormal result receives appropriate follow-up, evaluation, definitive diagnosis, and long-term management

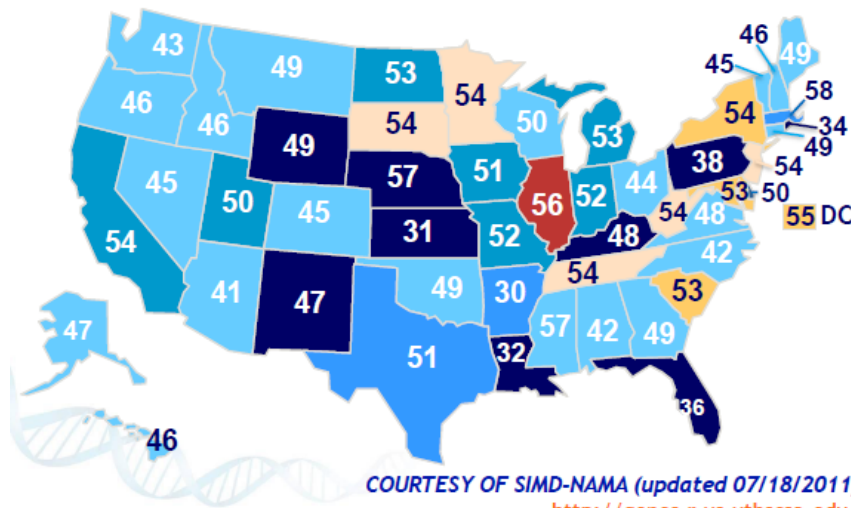
Selection Criteria for Disorders Screened in Program

- Relatively "high" incidence
- If untreated, severe morbidity or mortality
- Treatment available
- Treatment offers clear and immediate benefit to affected individual
- Good assay
 - Accurate (sensitive, specific, high positive predictive value)
 - Reproducible
 - Inexpensive
 - Cost effective (per positive result, per diagnosis)

Newborn Screening Programs

- Babies in most states are screened for:
 - Amino acid disorders
 - Organic acid disorders
 - Fatty acid oxidation disorders
 - Galactosemia
 - Biotinidase deficiency
 - Congenital hypothyroidism
 - Congenital adrenal hyperplasia (CAH)
 - Cystic fibrosis
 - Hemoglobinopathies
 - Hearing loss

Newborn screening - USA, 2011



77 Conditions Screened in Missouri

Disease	Incidence
• Hypothyroidism	1:2600
• PKU (and variants)	1:15,000
• Galactosemia	1:80,000
• Sickle Cell Anemia	1:400 (AA)
• CAH	1:13,000
• Cystic fibrosis	1:2500 (Caucasians)
• Biotinidase Deficiency	1:105,000
• Homocystinuria	1:140,000
• MSUD	1:104,000
• Fatty acid oxidation disorders	1: 16,000
• Organic acidurias	1: 16,000
• Urea cycle defects	1:30,000
• Tyrosinemia	1:120,000
• Lysosomal storage diseases	~1:3000 to 1:100,000

Newborn screening - Europe, 2011



Country	Number of Disorders Screened
Austria	26
Belgium	9 / 6
Denmark	13
France	1
Germany	12
Greece	2
Hungary	24
Ireland	4
Italy	1
Netherlands	14
Portugal	24
Spain	23
Sweden	3
United Kingdom	2

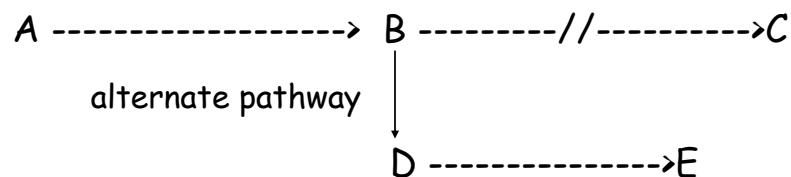
EU Network of Experts on NB Screening - 2011

Limitations of Screening Program

- Screening tests are not diagnostic
 - False positives
 - Usually involves screening for metabolites
 - Some specific gene testing is being done
- In many cases, the full clinical spectrum of disease is unknown
- Cannot always differentiate milder variants from severe variants based on biochemical parameters
- Does not diagnose all metabolic diseases or other genetic diseases

INBORN ERRORS OF METABOLISM

Simplified scheme of a blockage in a metabolic pathway:



Clinical effect of the enzymatic defect can be due to:

- (1) Increased A and B precursors
- (2) Decreased C product
- (3) Increased D and E from alternate pathway

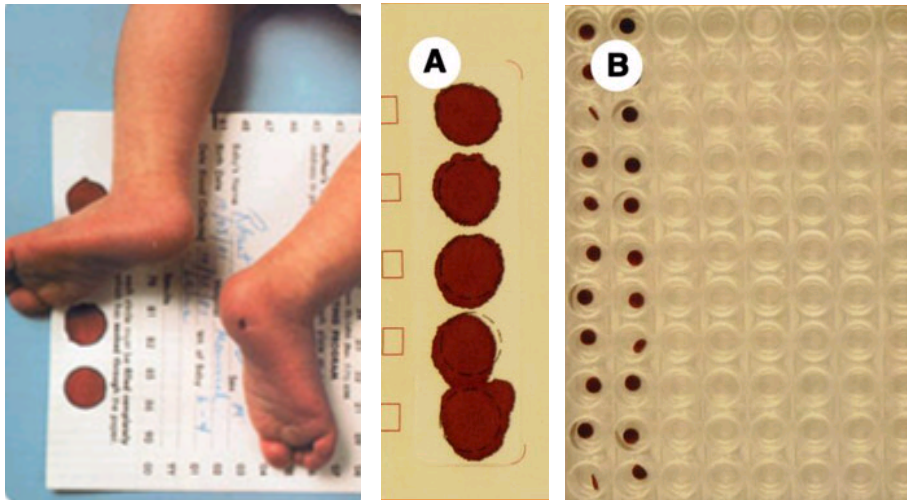
Analysis Methods

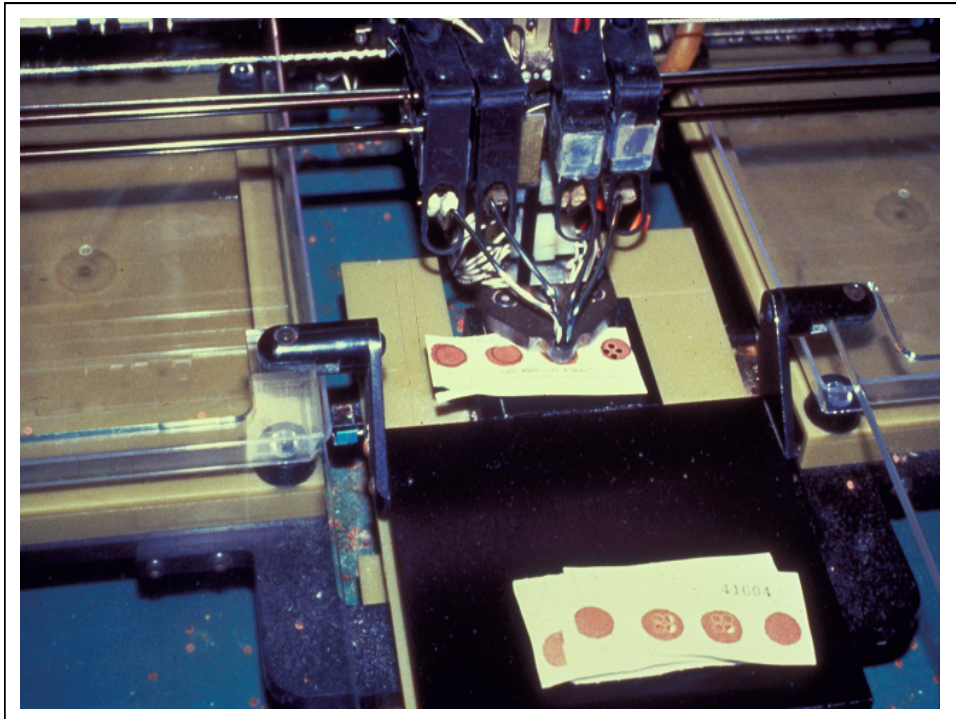
- Direct Enzyme Assay
 - Biotinidase (colorimetric)
 - Galactose-1-P Uridyltransferase (fluorescent)
- Hormone Levels (immunoassays)
 - TSH and T4 for hypothyroidism
 - 17-OH progesterone for congenital adrenal hyperplasia
- Hemoglobin electrophoresis
 - Isoelectric focusing
- Tandem mass spectrometry
 - Amino acidopathies (eg. PKU, MSUD)
 - Some urea cycle defects (eg. citrullinemia)
 - Organic acidurias (eg. MMA, GA 1)
 - Fatty acid oxidation defects (eg. MCAD)
- DNA analysis
 - Cystic fibrosis

Screening Program in Missouri

- Initial screen
 - Mandatory testing of all newborns
 - Newborn should be >24 hours of age
 - \$65 current cost (cost varies by state)
 - Consent is passive, but parents can refuse testing in writing for religious or other reasons
- Second screen
 - At the discretion of the physician
 - If initial screen is abnormal
 - Picks up additional cases of later onset congenital hypothyroidism

Newborn Screening

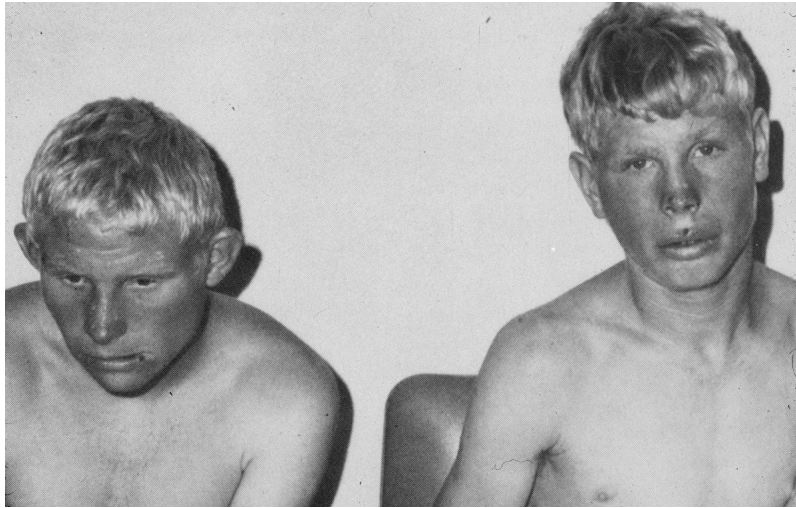




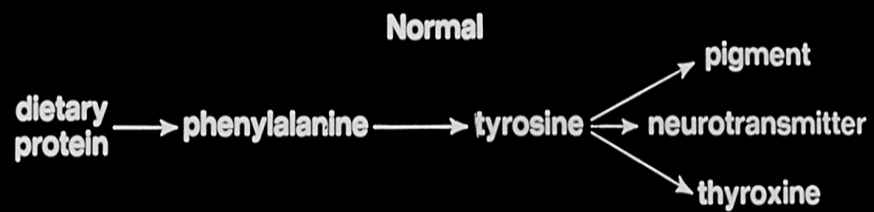
Phenylketonuria (PKU) (1967)

- Incidence $\sim 1/12,000$ - $1/15,000$
- If untreated, symptoms include:
 - Mental retardation
 - Microcephaly and seizures
 - Eczematous rash and mousy odor
- To prevent mental retardation, dietary treatment must be started by 1 month of age
- Life-long treatment allows normal growth and development
- If diet therapy is stopped later, neuropsychiatric complications often occur

Brothers with Untreated PKU



PHENYLALANINE METABOLISM



Phenylketonuria (PKU)



Congenital Hypothyroidism (1979)

- Incidence = 1 in 2600
- Screening of TSH by immunofluorescent assay method
- If untreated, symptoms include:
 - Feeding problems and lethargy in infancy
 - Enlarged fontanelle and macroglossia
 - Hair loss
 - Mental retardation
 - Short stature
- Early identification and treatment prevent irreversible brain damage

Galactosemia (1985)

- Incidence = 1 in 80,000
- Carbohydrate metabolic disorder
- Screened for by galactose-1-PO4-uridylyltransferase enzyme activity
- If untreated, will result in liver dysfunction, cataracts, mental retardation or death
- Treated with galactose-free diet (soy milk)
- Will detect classic cases, some heterozygotes and most Duarte-galactosemia (DG) cases
- Will not detect epimerase or galactokinase deficiencies

Hemoglobinopathies (1989)

- Incidence of sickling disorders is 1/400 in African-American infants
- Other hemoglobin disorders detected as well
- Early identification of sickle cell disease and initiation of antibiotics prevents 11% mortality rate in first 6 months of life
- Possible results
 - Normal (FA)
 - Sickle cell trait (FAS) or disease (FS)
 - Possible thalassemia (F) or sickle beta thal (FSA)
 - Other variants (hemoglobin C)
- Transfusion invalidates test

Tandem Mass Spectrometry Expanded Newborn Screening

- Method started in Illinois in 2001 and in Missouri in 2005
- Method measures acylcarnitine species and amino acids on a blood spot
- Additional amino acid levels detect more amino acid disorders than just PKU
- Acylcarnitine analysis detect organic acidemias and fatty acid oxidation disorders

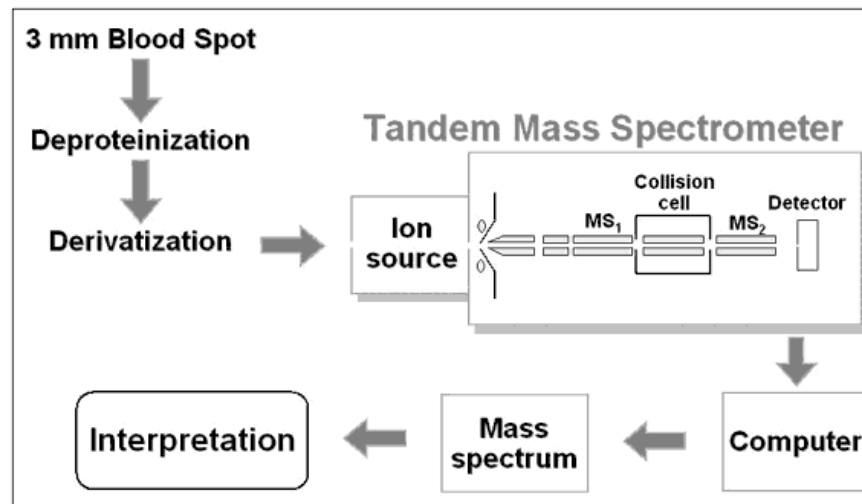
Tandem Mass Spectrometry (MS/MS)

- All states now use MS/MS to perform "expanded newborn screening"
- Screens for more disorders & with greater accuracy than the traditional methods
- Can be used to screen and to diagnose
- Identifies molecules by the relative weight and quantity of individual ions

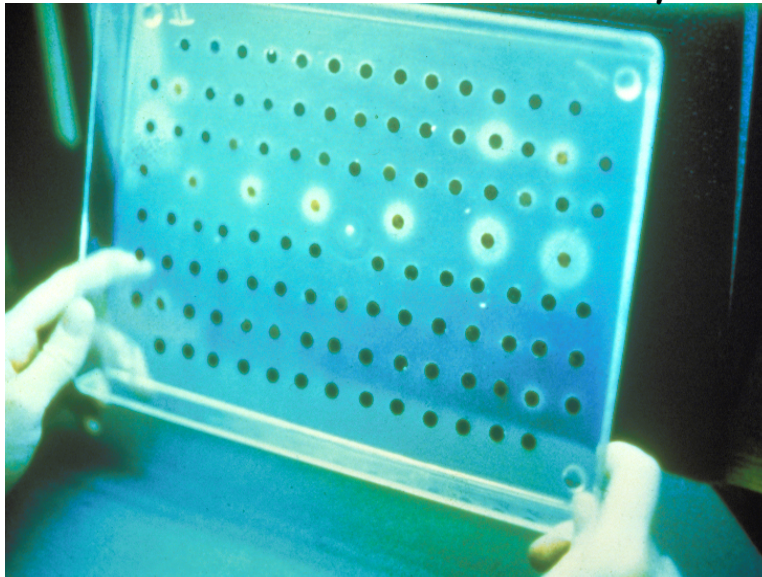
Tandem Mass Spectrometer



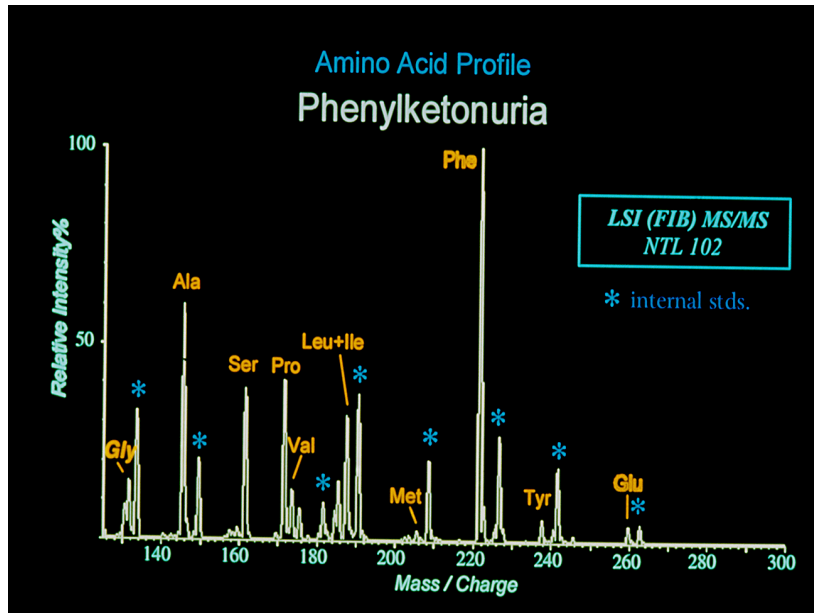
Basic scheme of an MS/MS analysis



Old PKU Method Bacterial Inhibition Assay



New PKU Method - MS/MS



Advantages of MS/MS

- Shorter turnaround time
 - 2 minutes in the machine
 - 2 hours from preparation to result
 - Less than 24 hours in the laboratory
- Fewer false positives
- Increased sensitivity
- Earlier start of therapy
- More conditions detected by a single test

Tandem Mass Spectrometry Amino Acid Abnormalities

- Phenylalanine
 - PKU
- Tyrosine
 - Tyrosinemia
- Methionine
 - Homocystinuria
 - Hypermethionemia
- Leucine
 - MSUD
- Citrulline
 - Citrullinemia
 - Argininosuccinate (lyase) deficiency
- Arginine
 - Argininemia

Tandem Mass Spectrometry Acylcarnitines

- Organic acidurias
 - Propionic acidemia
 - Methylmalonic acidemia
 - Isovaleric acidemia
 - Glutaric aciduria, type 1
 - Others

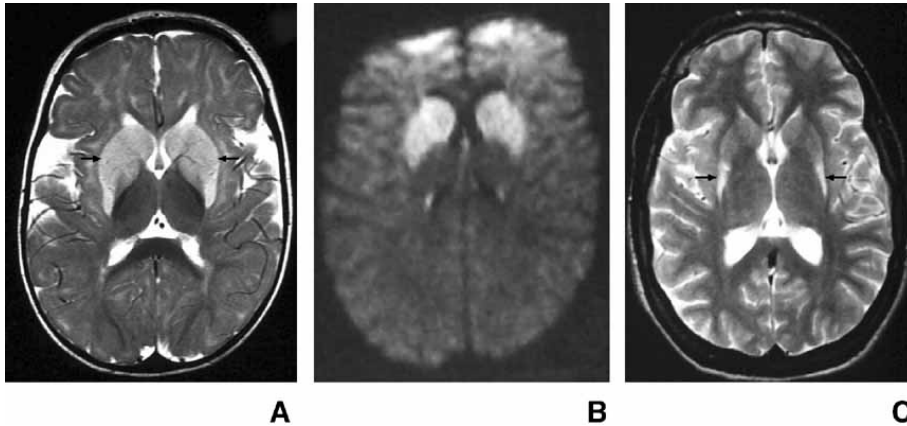
Classical Organic Acid Disorders (MMA, PA, IVA)

- Encephalopathy, lethargy, poor feeding
- Dehydration
- Ketoacidosis, hyperammonemia, pancytopenia
- Seizures
- Coma and death if unrecognized
- Some forms with vitamin cofactor responsiveness
(B12 in MMA, biotin in PA)

Glutaric Aciduria Type I

- Defect in lysine, hydroxylysine, and tryptophan metabolism
- If untreated, symptoms can include:
 - Progressive macrocephaly (may be present at birth)
 - Acute metabolic encephalopathy
 - Basal ganglia strokes and movement disorder
 - Subdural and/or retinal hemorrhages
- Should be considered in the differential diagnosis of shaken baby syndrome
- With treatment, >90% have no brain degeneration

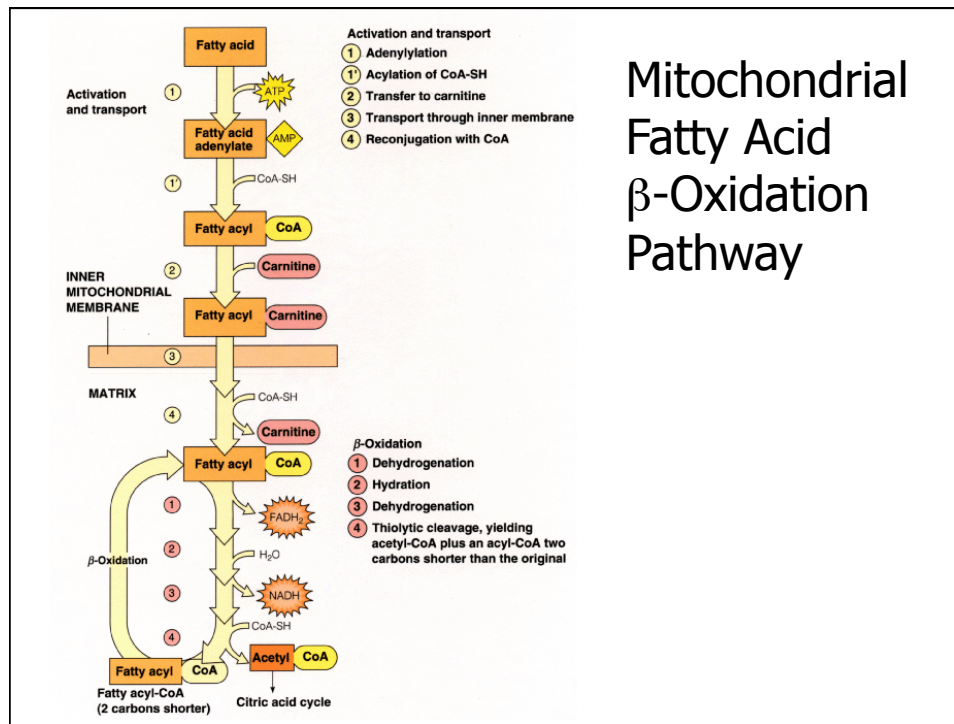
Glutaric Aciduria Type I



Courtesy of Richard Kelley, MD, PhD

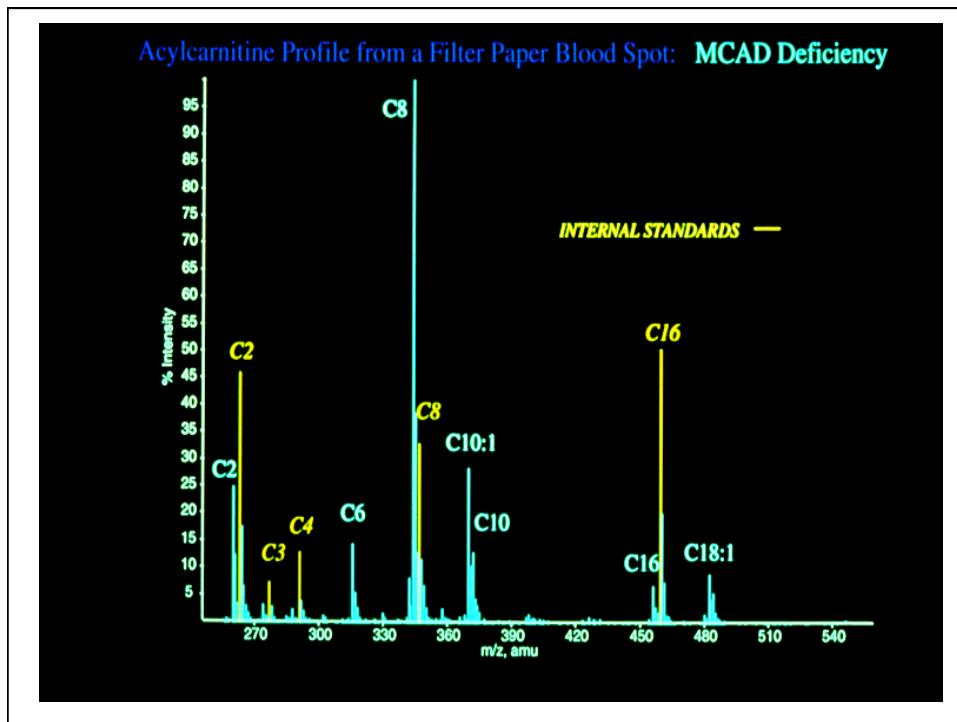
Tandem Mass Spectrometry Acylcarnitines

- Fatty acid oxidation disorders
- Large group of conditions
- Abnormal metabolism of fats leads to:
 - Hypoglycemia
 - Hypoketosis
 - Acidosis
 - Encephalopathy
 - Liver dysfunction
 - Cardiomyopathy
 - Skeletal muscle myopathy



Medium Chain Acyl CoA dehydrogenase (MCAD) deficiency

- Most common of the 11 fatty acid oxidation defects detectable by screening
- Incidence ~1:10,000 Northern Europeans
- Usual presentation is at 1-3 years of age with hypoketotic hypoglycemia, vomiting, dehydration and encephalopathy during intercurrent illness
- 25% of children die with first presentation
- Many suffer irreversible brain injury
- Treatment is to avoid prolonged fasting, \pm carnitine supplement



Child A - MCAD Before Screening

- Healthy until 18 months of age
- Became ill and slept for 20 hours without eating or drinking
- Unarousable and taken to the hospital
- Severe hypoglycemic episode with brain injury
- Intractable seizures requiring 3 medications
- Spastic quadriplegia
- Seizure disorder
- Mental retardation
- G-tube fed

Child B - MCAD After Screening

- Detected by newborn screening at 10 days of age
- Follow-up testing confirmed MCAD deficiency
- Never became ill
- Followed regularly in *Genetics Clinic*
- Dietary management with avoidance of fasting and carnitine supplement
- Normal growth and development

Cystic Fibrosis (2007)

- Immunoreactive trypsinogen first tier method
- Elevated IRT is seen in pancreatic insufficiency
- DNA-based second tier testing was added in Missouri in 2012
- If IRT is abnormal, DNA testing is done for the 40 most common mutations
- If one or two mutations found, baby is referred to a CF Center for management
- 4 Cystic Fibrosis Treatment Centers in Missouri

Selected Errors in Sample Collection

- Cards not filled out properly and completely
- Contamination of blood spots
- Inadequate blood on spots
- Cards not air dried
- Not delivered to laboratory promptly
- Obtained too early (<24 hours)
- Baby received a transfusion
- Baby is ill or premature---can cause false positives or negatives

Newborn Hearing Screening

- Started in Missouri in 2002
- Detection of hearing loss and treatment prior to 6 months of age significantly improves outcomes for development
- OAE or ABR method
- Almost all states have mandated screening
- 95% of all newborns in the US are tested

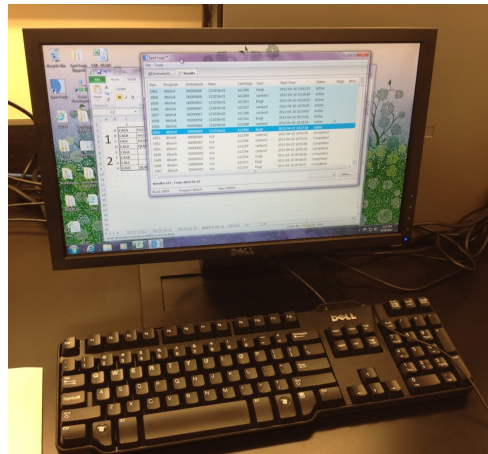


Newborn Screening for Critical Congenital Heart Disease

- Now in place in most states in the US
- Screening performed by pulse oximetry in the birth hospital nursery
- Low blood oxygenation may indicate cyanotic heart defect
- Baby is referred for an echocardiogram and evaluation by a cardiologist

Newborn Screening for Lysosomal Storage Diseases

- Screening for LSDs by several methods is feasible
- Krabbe disease testing performed in NY since 2006
- Missouri started LSD screening in August 2012 for Krabbe and for Gaucher, Fabry, Hurler and Pompe diseases in January 2013
- Illinois is starting a pilot in the Chicago area for 5 disorders using TMS method
- Enzyme replacement therapy available for some diseases:
 - Gaucher
 - Fabry
 - Hurler
 - Pompe
- Other treatments include bone marrow or cord blood stem cell transplant, such as in Krabbe disease



Future Additions to MO NBS

- Severe combined immune deficiency (SCID) will be added in 2014
- Testing being done in other states already using TRECs (T- cell receptor excision circles) to detect lymphopenia

Newborn Screening Challenges

- False positives
- False negatives
- Current treatment not completely effective for some disorders identified
- Adequate parental education about these disorders
- Detection of compounds of unknown significance
- Funding

Controversies in Newborn Screening

- Should screening be done for disorders with no definitive treatment?
- Should screening be done if treatment options are only under development or experimental?
- Examples:
 - Niemann-Pick syndrome
 - Duchenne muscular dystrophy
 - Fragile X syndrome

Genomic Screening for Newborns

- DNA sequencing technology is making this possible
- Research studies are being done to determine the feasibility of this on a large population scale
- Many challenges are anticipated
 - Clinical
 - Psychosocial
 - Ethical

Newborn Screening in the State of Missouri



Missouri Department of Health
and Senior Services
Division of Community and Public Health
PO Box 570
Jefferson City, MO 65102
Phone: 1-800-877-6246
Fax: 1-573-751-6185
<http://www.dhss.mo.gov>

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1-800-TEL-LINK
(1-800-835-5465)



**Newborn
Screening**



**Protect your
Newborn**

#14
Revised 9/08

As of July 2011 NBS Samples are Saved for 5 Years

INITIAL NEWBORN SCREENING
MO 580.000.1 (05)

2014.02
[G3] 01/20/2011 / 1101326

Detach This Copy and Give to Mother of Newborn!

Dear Parents of Newborn Baby:

To help give your baby a good start, Missouri law requires your baby's blood to be tested for more than 50 disorders. A few small drops of blood taken from the baby's heel are put on special paper and then sent to the State Public Health Laboratory for testing.

Many disorders are not noticed at birth. Yet they can put the baby at risk for disability or death if not found early. If results from this screening test are not normal, your doctor will tell you about more testing that needs to be done. Please make sure the hospital staff gives you a Newborn Screening pamphlet.

Once the newborn screening test is done, the Missouri State Public Health Laboratory will store the remaining newborn screening sample for five (5) years. The storage is secure. Missouri state law allows for the stored sample to be used for research. The research may help improve methods for spotting illnesses. The research may also find better ways to test, treat and cure major childhood diseases. Your baby is not identified to the researcher in any way. After five years, the rest of the newborn screening sample will be destroyed.

The law allows the parent or legal guardian the option of not having their baby's extra newborn screening sample stored or studied. You may ask the State Laboratory to:

- Give the extra newborn screening sample back to you
- Destroy the newborn screening sample after the newborn tests are done
- Store the extra newborn screening sample for 5 years but do not release it for study

If you choose NOT to allow your baby's extra newborn screening sample to be studied, select one of the above three options. Then write to the Laboratory Director at:

Missouri State Public Health Laboratory
Newborn Screening Laboratory
P.O. Box 570
Jefferson City, MO 65102-0570

Give this information:
Baby's name
Baby's date of birth
Mother's first and last name
Place where baby was born
The option you selected from above
State if you are the parent and legal guardian
Your current address
Your signature and current date (See other side)

If you have questions or need assistance, please contact the Newborn Screening Laboratory at 573-751-2662.

LABORATORY COPY

See collection instructions on back of form.

Fold back cover at fold line to expose sample area.

When dry, cover blood spots with overlay.

Send to lab within 24 hours.

BIOHAZARD

- Give the extra sample back to the family
- Destroy the sample after testing is completed
- Store the sample for 5 years, but do not release for research

Information Provided to Parents About Sample Storage and Research

Benefits of storing newborn screening samples:

There are many reasons why newborn screening samples are kept, many of which benefit your family and other Missouri families.

- In some cases, samples are requested by the family or the baby's health care team.
- The baby's sample is available to you for other health-related testing within five years of storage.
- The baby's sample is available to help identify a missing or deceased child within five years of storage.
- If your child has an illness and is enrolled in a research study, parents may request that their baby's newborn screening sample be returned to them in order that they may send it to the researcher within five years of storage.

For research purposes, all identifying information is removed from the samples (baby's name, parent's name, parent's address, hospital of birth, etc.). The researcher does not know who the baby is. These samples may be used to:

- Provide quality assurance in the screening.
- Do public health studies and research to help develop newborn screening tests and better understand diseases for the benefit of the general public.
- Search for new markers for chronic diseases such as childhood leukemia, sickle cell disease, autism and diabetes.

Only those research projects that undergo careful scientific and ethical review will be given approval to use newborn screening samples.

H O S P I T A L U S E		1. Baby's Name (Last, First)		14. Baby's Sex		
		2. Date of Birth	Military Time	3. Date of Collection	Military Time	15. <input type="checkbox"/> White
		4. Baby's Medical Record Number	5. Mother's Medical Record Number	16. <input type="checkbox"/> Black		
		6. Mother's Name (Last, First)	17. <input type="checkbox"/> Asian			
		7. Mother's Street Address (P.O. Box)	18. <input type="checkbox"/> Unknown			
		8. City	9. State	10. Zip Code	19. Feeding	
		11. Mother's Telephone Number	Alternate Telephone Number	<input type="checkbox"/> 1 - BRE		
		12. Mother's Maiden Name		<input type="checkbox"/> 2 - MB		
		13. Baby's Primary Physician (Last, First) or Clinic / Telephone Number		<input type="checkbox"/> 3 - No		
				<input type="checkbox"/> 4 - TP		

Web Resources

- www.genes-r-us.uthscsa.edu
 - National Newborn Screening and Genetics Resource Center
- www.savebabies.org
 - Save Babies Through Screening Foundation, Inc.
- www.dhss.mo.gov/lab/newborn/
 - Missouri Newborn Screening Program
- www.acmg.net
 - American College of Medical Genetics
 - ACT sheets for recommended follow-up of abnormal screen results
- <http://dhss.mo.gov/living/families/genetics/newbornhearing/>
 - Missouri Newborn Hearing Screen Program
- www.genetests.org
 - List of international genetics clinics, laboratories, education materials