Don’t let newborn screening have an Achilles heel.
What to do with abnormal and borderline newborn screening results and the resources to help you do it

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January 10, 2013
The first documented blood spot sampling

Achilles Dying. Ernst Herter. Achilleion, Greece
Categories of Screening of newborns

Formal newborn screening program:
• Hearing screening
• Pulse oximetry screening
• Blood metabolite screening

Additional screening measures:
• Prenatal screening
• Perinatal monitoring
• Newborn physical exam
• Family history
Most common newborn screening diagnoses in the US (2006)

1. Hearing loss
2. Congenital hypothyroidism
3. Cystic fibrosis
4. Sickle cell disease
5. MCAD deficiency
6. Galactosemia
7. PKU
Hearing screening

• The Washington State Early Hearing-loss Detection, Diagnosis and Intervention (EHDDI) program recommends screening all infants for hearing loss <1 month of age.
• Perform screening as close to discharge as possible, preferably >12 hours after birth.
• If neonate does not pass first screening, perform second screening prior to hospital discharge.
• Infants in the NICU >5 days require an automated ABR.
• Further in-patient assessment by audiology if indicated.
Cyanotic heart disease screening

• Pulse oximetry screening pioneered at TG
• 10,600 neonates screened 2008-2012 with 41 cases of significant congenital heart disease identified

<table>
<thead>
<tr>
<th>Screening Result</th>
<th>Criteria</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Screen</td>
<td>Saturation ≥ 95% in right hand and either foot and ≤ 3% difference between extremities</td>
<td>Screening Complete</td>
</tr>
<tr>
<td>Equivocal Screen</td>
<td>Saturation 90-94% in right hand or either foot or &gt; 3% difference between extremities</td>
<td>Repeat screening in one hour Three equivocal screens -&gt; Positive result</td>
</tr>
<tr>
<td>Positive Screen</td>
<td>Saturation &lt; 90% in right hand or either foot</td>
<td>Echocardiogram Follow up with Pediatric Cardiologist</td>
</tr>
</tbody>
</table>
Blood metabolite screening

• Endocrine disorders
• Hemoglobinopathies
• Cystic fibrosis
• Inborn errors of metabolism
  – Amino acid disorders
  – Organic acid disorders
  – Fatty acids disorders
  – Others (acid-free)
Washington blood spot screening, 2011

- 9.1 million tests on 167,000 specimens
- 85,000 neonates tested
- 172 confirmed diagnoses (i.e. approx 1/500)
- Cost per newborn screened is $69
- Bloodspots banked for at least 21 years
- Bloodspots can be used for DNA testing if necessary
Random audience screening:

• True or False?:
  A. Parent(s) may opt out of NBS
  B. By law, NBS cards must be submitted before hospital discharge but no later than day 5 of life
  C. A minimum of two NBS cards must be submitted to NBS to be considered complete
  D. A third NBS card may necessary even if the first two are normal
A. Parents may opt out of NBS.
   *True.* A parent may opt-out by signing the back of the card. But only for religious reasons and only after a provider has informed the parent(s) of the risk.

B. By law, NBS cards must be submitted before hospital discharge but no later than day 5 of life.
   *True.* Even for premature neonates.

C. A minimum of two NBS cards must be submitted to NBS to be considered complete.
   *True.* One card after birth, the next at approx. 7-14 days.

D. A third NBS card may necessary even if the first two are normal.
   *True.* Infants <1500 g at birth and sick infants in hospital for >3 weeks should have three specimens collected.
Newborn screening is screening
It is not diagnostic testing
False positive and borderline results

Screening Results
what we would like ...

Normal

100% specificity

Affected

100% sensitivity

Screening Results
what we usually get ...

Normal

Affected

Specificity vs. Sensitivity
How often is positive (or borderline) really positive in NBS?

• Positive predictive value (PPV): the proportion of positive results that are true positives (TP) rather than false positives (FP):
  \[ PPV = \frac{TP}{TP + FP} \]

• Average PPV for most metabolic disorders screened is 25%: for every TP there are three FP

• Newborn screening is just that—*screening*

• Further diagnostic testing is *always* (and often urgently) indicated for these
Endocrine disorders

• Congenital hypothyroidism
  – Thyroid stimulating hormone (TSH) level measured by fluoroimmunoassay

• Congenital adrenal hypoplasia (CAH)
  – 17-hydroxyprogesterone (17-OHP) measured by fluoroimmunoassay
    – 17-OHP is elevated in classic CAH
    – 17-OHP level may not correlate with clinical severity
Cystic Fibrosis

- Immunoreactive trypsinogen (IRT) measured by fluoroimmunoassay
- Elevated in affected neonates
- Elevation in two consecutive NBS blood spot specimens is considered a presumptive positive
- Confirmatory testing is necessary
Amino acid disorders

- Argininosuccinic acidemia
- Citrullinemia
- Homocystinuria
- Maple syrup urine disease (MSUD)
- Phenylketonuria (PKU)
- Tyrosinemia type I
Organic acid disorders

- 3-hydroxy-3-methylglutaric aciduria
- $\beta$-ketothiolase deficiency
- Glutaric acidemia type I
- Isovaleric acidemia
- Methylmalonic acidemias (CblA,B and MUT)
- Multiple carboxylase deficiency
- Propionic acidemia
Fatty acid disorders

- Carnitine uptake deficiency
- Long-chain L-3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency
- Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency
- Trifunctional protein deficiency
- Very-long chain Acyl-CoA dehydrogenase (VLCAD) deficiency
Others (acid-free)

- Biotinidase deficiency
- Galactosemia
- Congenital adrenal hyperplasia
- Congenital hypothyroidism
- Cystic fibrosis
- Hemoglobinopathies
So your patient has an abnormal NBS…

- The State Newborn Screening Lab will contact the neonate’s provider of record
- Additional testing recommendations will be provided
- Clinical surveillance and treatment may be suggested
- Genetics clinic contacts are provided if needed
NBS Lab protocol for PKU

<table>
<thead>
<tr>
<th>Blood Phe (μM)</th>
<th>Age &lt;24 hrs</th>
<th>Age &lt;24 hrs</th>
<th>Age&gt;24 hrs</th>
<th>Age&gt;24 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phe/Tyr &lt;2</td>
<td>Phe/Tyr &gt;2</td>
<td>Phe/Tyr &lt;2</td>
<td>Phe/Tyr &gt;2</td>
</tr>
<tr>
<td>&lt;152</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>152-179</td>
<td>Normal</td>
<td>Borderline</td>
<td>Normal</td>
<td>Borderline</td>
</tr>
<tr>
<td>180-239</td>
<td>Borderline</td>
<td>Presumptive</td>
<td>Borderline</td>
<td>Borderline</td>
</tr>
<tr>
<td>&gt;240</td>
<td>Presumptive</td>
<td>Presumptive</td>
<td>Presumptive</td>
<td>Presumptive</td>
</tr>
</tbody>
</table>

- **Borderline result**: Health care provider is contacted by phone to recommend a repeat newborn screening specimen

- **Presumptive positive**: Health care provider contacted by phone to recommend a repeat newborn screening specimen and/or plasma phe and tyr levels

- Once diagnosis is confirmed (within several days), PKU metabolic formula and urgent referral to PKU Clinic is provided
# NBS Lab protocol for methylmalonic and propionic acidemias

<table>
<thead>
<tr>
<th>C3-species (µM)</th>
<th>Age ≤ 6 days</th>
<th>Age &lt; 6 days</th>
<th>Age ≤ 6 days</th>
<th>Age &gt; 6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N 2° markers</td>
<td>↑2° markers</td>
<td>N 2° markers</td>
<td>↑2° markers</td>
</tr>
<tr>
<td>&lt;4.1</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>4.1-4.89</td>
<td>Normal</td>
<td>Normal</td>
<td>Borderline</td>
<td>Presumptive</td>
</tr>
<tr>
<td>4.9-6.09</td>
<td>Normal</td>
<td>Borderline</td>
<td>Borderline</td>
<td>Presumptive</td>
</tr>
<tr>
<td>6.1-8.39</td>
<td>Borderline</td>
<td>Presumptive</td>
<td>Borderline</td>
<td>Presumptive</td>
</tr>
<tr>
<td>8.4-11.99</td>
<td>Borderline</td>
<td>Presumptive</td>
<td>Borderline</td>
<td>Presumptive</td>
</tr>
<tr>
<td>≥12.0</td>
<td>Presumptive</td>
<td>Presumptive</td>
<td>Presumptive</td>
<td>Presumptive</td>
</tr>
</tbody>
</table>

- Normal secondary markers: C3/C2 < 0.2, C3/C16 < 2.2
- **Borderline result**: If first screen, wait for 2nd screen. If second screen, health care provider contacted by telephone and diagnostic testing recommended
- **Presumptive positive**: Health care provider contacted by telephone and *urgent* diagnostic testing recommended (urine organic acids, acylcarnitines)
NBS Lab protocol for MCAD deficiency

<table>
<thead>
<tr>
<th>C8-species (µM)</th>
<th>Normal 2° markers</th>
<th>Elevated 2° markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.5</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>0.5-0.99</td>
<td>Borderline</td>
<td>Borderline</td>
</tr>
<tr>
<td>&gt;1.0</td>
<td>Borderline</td>
<td>Presumptive</td>
</tr>
</tbody>
</table>

• Normal secondary markers: C8/C2 < 0.02, C8/C10 < 0.92, C10:1 < 0.18

• Borderline result: Health care provider contacted by phone and diagnostic testing recommended (acylcarnitine profile and MCAD gene DNA testing)

• Presumptive positive: Health care provider contacted by phone and urgent diagnostic testing recommended (acylcarnitine profile and MCAD gene DNA testing) and urgent referral to Genetics clinic
NBS clinical consultants

- The State Newborn Screening Lab has contracted clinical consultants to assist in the interpretation and management of positive and borderline NBS results.
- Consultants include: clinical geneticists, genetic counselors, clinical nutritionists, and clinical chemists
- Consultants are based in Tacoma (Mary Bridge, Tacoma General) and Seattle (Children’s, UWMC)
Abnormal NBS follow-up

• Testing after an abnormal result may include:
  – Repeat NBS blood specimen
  – Electrolytes, glucose, ammonia
  – Urine organic acids
  – Plasma amino acids
  – Plasma acylcarnitine profile
  – Hormone testing (thyroid, adrenal)
  – Targeted enzyme testing
  – Targeted DNA testing
South Sound (and SW Washington) Assistance for NBS abnormalities

- Immediate medical genetics assistance is available 24/7/365 through MultiCare
- As consultants to the State NBS Lab, MultiCare/Mary Bridge Medical Genetics service participates in weekly conference calls reviewing all “incomplete” NBS cases
- Mary Bridge Genetics clinic will see all urgent NBS-positive neonates in clinic in <2-3 days
ACT Sheet for PKU

- http://www.acmg.net/StaticContent/ACT/Phenylalanine.pdf
ACT sheet for methylmalonic aciduria and propionic aciduria

- [http://www.acmg.net/StaticContent/ACT/C3.pdf](http://www.acmg.net/StaticContent/ACT/C3.pdf)
ACT Sheet for MCAD

- [http://www.acmg.net/StaticContent/ACT/C8_C6_C10.pdf](http://www.acmg.net/StaticContent/ACT/C8_C6_C10.pdf)
Protein metabolic disorders: organic acidemias, urea cycle defects

- **Acute findings:**
  - Fussiness, lethargy, altered mental status
  - Reflux, nausea, vomiting

- **Subacute findings:**
  - Failure to thrive
  - Developmental delay
  - +/- seizures and spasticity
  - Risk for acute decompensation during catabolic stress (e.g. infection)

- **Acute (and subacute) lab findings:**
  - Hypoglycemia
  - Metabolic acidosis with elevated anion gap
  - Hyperammonemia
Initial interventions for acute presentations of protein metabolic disorders

• Limit protein intake (po, IV) for 2-3 days
• Start IV D10-1/2NS (+/- K) at 1.5X maintenance rate
• STAT labs:
  – chemistry panel, CBC, ammonia
• If CO2<15, consider IV bicarbonate or acetate
• If ammonia>200, consider IV ammonia-scavengers (Ammonul®)—bolus then constant infusion
Initial investigations for NBS+ for a protein metabolic disorder

• Confirm clinical status with family same day
• If acute symptoms, send to ED
• If no acute symptoms, obtain diagnostic specimens:
  – Urine organic acids
  – Plasma amino acids
  – Acylcarnitine profile
• If subacute symptoms, obtain in addition:
  – Chemistry panel, CBC, ammonia
• Call your genetics service for friendly assistance
Coming soon to NBS testing!

- State-mandated pulse oximetry
- Blood spot screening for severe combined immunodeficiency (SCID)
- Lysosomal storage disease (LSD) screening (a specified subset of LSDs)
Take-home messages

1. NBS reduces mortality and morbidity in a cost-effective manner
2. NBS resources for providers in Washington are readily available
3. Medical genetics direct assistance is always available
4. NBS is screening, not diagnostic testing
5. Diagnostic testing is essential to realize the benefits of NBS
NBS References

- NBS Providers’ manual:

- NBS “Health professionals page”:
  http://www.doh.wa.gov/YouandYourFamily/InfantsChildrenandTeens/NewbornScreening/NBSProfessionals.aspx

- ACMG ACT sheets:
  http://www.acmg.net/AM/Template.cfm?Section=NBS_ACT_Sheets_and_Algorithms_Table&Template=/CM/HTMLDisplay.cfm&ContentID=5072

- Recommended for parents:
  http://savebabies.org/

- Hearing screening:
  http://www.doh.wa.gov/Portals/1/Documents/Pubs/344-023_EHDDINBScnrProto.pdf
Newborn screening
Saving babies one foot at a time

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Non physicians
• Cut and paste the following link into your browser, to access the post test. Successfully pass the post test and you will receive a participation certificate.
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• *AMA prohibits non physicians from being awarded CME credit.