Saving Lives Through Newborn Screening: Making the Most of a Simple Blood Test

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Director - NBS Program
Washington State Public Health Laboratories
What is Newborn Screening?

An integrated system that includes:

• Universal screening - all infants
• Follow-up to assure appropriate clinical response
• Referral for diagnosis of affected infants
• Appropriate treatment and clinical care
• Evaluation of system effectiveness (long-term follow-up)
Why is Newborn Screening Important?

- It prevents death and disability to affected infants by providing early treatment.
- It benefits the public through savings in health care and disability support costs.

Two 6 year old girls with congenital hypothyroidism.
Annual Newborn Screening Numbers

Screen ~90,000 newborns
Receive ~180,000 specimens
Track ~5,000 infants with abnormal results
Prevent ~200 babies from death or disability
Annual Newborn Screening Numbers

Screen ~90,000 newborns

Prevent ~200 babies from death or disability
NBS Sequence of Events

- **Follow-up**
- **NBS lab testing**
- **Transit time**
- **Hospital – blood collection**

**Recommended window for NBS specimen collection**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The chart illustrates the sequence of events from birth to follow-up, highlighting the recommended window for NBS specimen collection.
Washington Criteria for NBS

- Early identification benefits the newborn
- Treatment is available
- Nature of the condition justifies population-based screening
- A good screening test exists
- The benefits justify the costs of screening
Level of Urgency

HIGH!!

- CAH
- Galactosemia
- MSUD
- CIT/ASA
- VLCAD/LCHAD/TFP
- MCAD
- IVA
- MMA/PROP
- CCHD

Diagnosis and treatment should be initiated ASAP!
Level of Urgency

MODERATE

- Biotinidase deficiency
- Congenital Hypothyroidism
- Homocystinuria
- PKU
- GA-I
- BKT

Treatment recommended by 1 to 3 weeks of age
Level of Urgency

Low – can wait over a weekend to contact baby’s provider

- Cystic Fibrosis
- Hemoglobin disorders
- TYR-I
- HMG/MCD
- CUD
- Hearing Loss
- SCID

Treatment recommended by 3 to 4 weeks of age
Distribution of lab values
Laboratory Testing

Distribution of lab values

False (+)          False (-)

Normal            Cases
Laboratory Testing

Ways to mitigate false (+) results:

• Stratify results
  • birthweight
  • age at collection
  • secondary analytes or ratios

• Routine 2\textsuperscript{nd} screens

End result – reduce the impact on families
How is screening done?

Specimen Testing

- A variety of testing platforms are used to screen for the 28 disorders
- Any abnormal result is repeated in duplicate for confirmation

Tandem Mass Spectrometer
19 disorders
1 punch

Fluoroimmunoassay Instrument
3 disorders
3 punches

Hemoglobin Gel Electrophoresis
3 disorders
1 punch

Spectrophotometer
2 disorders
2 punches

PCR Instrument
1 disorder
1 punch

Washington State Department of Health
Case Study #1
Sickle Cell Disease
Sickle Cell Disease: Historical Overview

• 1910 “sickle shaped” red blood cells described

• 1949 Linus Pauling: Abnormal hemoglobin protein described as cause of disease

• 1956 Molecular basis described
Sickle Cell Disease: Historical Overview

- 1975 New York begins screening all newborns using the dried blood spot
- 1986 Efficacy of penicillin prophylaxis shown
- 1987 NIH Consensus Development Conference recommends screening all newborns
Sickle Cell Disease: Simple Ideas

- Screen all infants
- Enroll those affected into comprehensive care that includes penicillin prophylaxis
- Save lives, improve outcomes
Sickle Cell Disease: Complex Realities

- Multiple genotypes and phenotypes: homozygous SS vs compound heterozygotes (Sβ thalassemia, SC, SD, SE)
- Clinical heterogeneity
- Screening test casts a wide net:
  - Other structural abnormalities (Hb C, D, E)
  - Thalassemias
  - Hemoglobin traits
  - > 700 other hemoglobin variants
Sickle Cell Disease: Complex Realities

- Very large numbers of patients - clinical care capacity for affected individuals
- Genetic counseling resources for those identified with hemoglobin traits
- Treatments (transfusions, hydroxyurea, bone marrow transplants) – who pays?
- Language and Cultural Barriers – need interpreters and case managers
Case Study #2
New Screening Method for Congenital Hypothyroidism
Congenital Hypothyroidism (CH)

- Condition of thyroid hormone deficiency that is present at birth

- Left untreated, severe forms lead to permanent growth failure and intellectual disability

- Treatment in the newborn period leads to virtually normal growth and development
Timing of the Screen

• First newborn screen is required by 48 hours of age (>97% compliance)

• Second screen recommended for all infants between 7 & 14 days (>90% compliance)

• Third screen recommended for NICU infants at ~30 days of age
Background

• Prior to July 2004: primary thyroxine (T4) screen ⇒ lowest 10% pulled for secondary thyroid stimulating hormone (TSH) screen (normal cutoff: TSH < 20 µIU/mL)

• ~15% of babies with hypothyroidism were only identified on the second screen because of normal T4 levels on first screen

• Data from retrospective TSH analyses for babies missed on the first screen suggested that we could reduce the false negative rate by 50%
Day 1 – normal cutoff: TSH < 20.00

The following specimen(s) have borderline or other abnormal (non-presumptive) TSH results:

<table>
<thead>
<tr>
<th>Lab #</th>
<th>TSH</th>
<th>Classification</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>20041880004</td>
<td>25.52</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880005</td>
<td>22.29</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880006</td>
<td>22.47</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880043</td>
<td>37.46</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880044</td>
<td>47.89</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880056</td>
<td>37.46</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880072</td>
<td>82.73</td>
<td>CHB1C</td>
<td></td>
</tr>
</tbody>
</table>

Comments: 20,950 is ~ 2% of total specimens screened. Happy primary TSH!!!

Day 2

The following specimen(s) have borderline or other abnormal (non-presumptive) TSH results:

<table>
<thead>
<tr>
<th>Lab #</th>
<th>TSH</th>
<th>Classification</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>20041880005</td>
<td>40.91</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880006</td>
<td>46.96</td>
<td>CHB1B</td>
<td></td>
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<tr>
<td>20041880118</td>
<td>28.32</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880147</td>
<td>49.23</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880167</td>
<td>36.61</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880172</td>
<td>45.66</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880173</td>
<td>24.60</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880176</td>
<td>62.93</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880194</td>
<td>36.02</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880211</td>
<td>40.78</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880215</td>
<td>25.74</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880372</td>
<td>26.56</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880373</td>
<td>27.69</td>
<td>CHB3B</td>
<td></td>
</tr>
<tr>
<td>20041880383</td>
<td>24.31</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880390</td>
<td>20.95</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880400</td>
<td>20.64</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880412</td>
<td>61.95</td>
<td>CHB1B</td>
<td></td>
</tr>
<tr>
<td>20041880462</td>
<td>21.93</td>
<td>CHB3A</td>
<td></td>
</tr>
<tr>
<td>20041880464</td>
<td>42.63</td>
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<td></td>
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<tr>
<td>20041880477</td>
<td>20.95</td>
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<td></td>
</tr>
<tr>
<td>20041880505</td>
<td>23.22</td>
<td>CHB3A</td>
<td></td>
</tr>
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</table>

More comments: 21,707 ~ 3% of total specimens tested.
# TSH Cutoff Stratification

<table>
<thead>
<tr>
<th>TSH µIU/mL serum</th>
<th>1 to 12 hrs</th>
<th>13 to 24 hrs</th>
<th>25 to 36 hrs</th>
<th>37 to 48 hrs</th>
<th>49 to 504 hrs</th>
<th>&gt; 504 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 14.99</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
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<tr>
<td>15.00 – 19.99</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Borderline&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>20.00 – 24.99</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>25.00 – 29.99</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Borderline&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>30.00 – 44.99</td>
<td>Normal</td>
<td>Normal</td>
<td>Borderline&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>45.00 – 54.99</td>
<td>Normal</td>
<td>Borderline&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>55.00 – 59.99</td>
<td>Borderline&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>60.00 – 99.99</td>
<td>Borderline&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Borderline&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Presumptive&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Presumptive&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Presumptive&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Presumptive&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>≥ 100.00</td>
<td>Presumptive&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Presumptive&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Presumptive&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Presumptive&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Presumptive&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Presumptive&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Superscript letters (a,b,c,d) denote general guidelines for the different follow-up responses that will be initiated by Follow-up staff.

- a - if first test, wait for routine second specimen; if second test, call health care provider immediately to request a follow-up specimen
- b - wait for routine second specimen
- c - call health care provider immediately to request follow-up specimen
- d - call health care provider immediately to recommend immediate diagnostic work-up
Number of babies diagnosed with CH per 100,000 births: one year prior to and one year after switch to primary TSH

* False (-) tally excludes NICU babies and mild forms of CH
** Mild forms (TSH < 30) tally excludes NICU babies
Case Study #2 – lessons learned

• Why isn’t this working (better)?
  – utility of additional investigation/analyses

• What is the impact of these changes?
  – unintended consequences of changing protocols

• How can we make this better?
  – utility of stratification to reduce the false positive rate

• How did things turn out?
  – importance of evaluation
## TSH Thresholds – What is *Urgently* Presumptive?

<table>
<thead>
<tr>
<th>TSH Level (≥)</th>
<th>True (+)</th>
<th>False (+)</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>411</td>
<td>111</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>300</td>
<td>150</td>
<td>4*</td>
<td>97%</td>
</tr>
<tr>
<td>200</td>
<td>188</td>
<td>9</td>
<td>95%</td>
</tr>
<tr>
<td>150</td>
<td>208</td>
<td>29</td>
<td>88%</td>
</tr>
<tr>
<td>125</td>
<td>229</td>
<td>60</td>
<td>79%</td>
</tr>
<tr>
<td>100</td>
<td>258</td>
<td>172</td>
<td>60%</td>
</tr>
<tr>
<td>75</td>
<td>296</td>
<td>587</td>
<td>34%</td>
</tr>
<tr>
<td>55</td>
<td>348</td>
<td>1985</td>
<td>15%</td>
</tr>
<tr>
<td>25</td>
<td>535</td>
<td>3736</td>
<td>13%</td>
</tr>
<tr>
<td>15</td>
<td>666</td>
<td>4308</td>
<td>13%</td>
</tr>
<tr>
<td><strong>ALL</strong></td>
<td>780</td>
<td>8007</td>
<td>9%</td>
</tr>
</tbody>
</table>

*Mom was on thyroid medication in all 4 cases*
Case Study #3
Congenital Adrenal Hyperplasia Kits
Case study - CAH

Congenital Adrenal Hyperplasia

- Disorder of hormone synthesis in the adrenal glands
- Can be life threatening in the first weeks of life
- Kits: 3-4 months
## Case study - CAH

### EP9 Method Comparison

**X Method:** 261333  
**Y Method:** 262130

#### Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>Deming</th>
<th>Regular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>1.505 (1.443 to 1.568)</td>
<td>1.352 (1.290 to 1.413)</td>
</tr>
<tr>
<td>Intercept</td>
<td>-3.044 (-3.040 to -5.049)</td>
<td>-1.310 (-5.222 to 2.602)</td>
</tr>
<tr>
<td>Std Err Est</td>
<td>29.587</td>
<td>28.953</td>
</tr>
</tbody>
</table>

95% Confidence intervals are shown in parentheses.

#### Medical Decision Point Analysis

Calculated by Partitioned Biases (R<0.975)

<table>
<thead>
<tr>
<th>X Method MDP</th>
<th>Y Method Pred. MDP</th>
<th>95% Conf. Limits Low</th>
<th>95% Conf. Limits High</th>
</tr>
</thead>
<tbody>
<tr>
<td>59.99</td>
<td>93.555</td>
<td>86.914</td>
<td>100.195</td>
</tr>
<tr>
<td>90.00</td>
<td>123.565</td>
<td>116.924</td>
<td>130.205</td>
</tr>
</tbody>
</table>
Case study - CAH

PRINCIPLES OF THE ASSAY

The AutoDELFIA Neonatal 17α-OH-progesterone (17-OHP) assay is a solid phase, time-resolved fluorimunoassay based on the competitive reaction between europium-labeled 17-OHP and sample 17-OHP for a limited amount of binding sites on 17-OHP specific polyclonal antibodies (derived from rabbit). Danazol facilitates the release of 17-OHP from the binding proteins (12). A second antibody, directed against rabbit IgG, is coated to the solid phase, giving convenient separation of the antibody-bound and free antigen.
Case study - CAH
Case Study #4
DNA Testing for Galactosemia
Enzyme Activity Assays

Enzyme + substrate → Product

Enzyme activity assays involve the addition of a substrate to an enzyme, leading to the formation of a product.
Galactosemia – Lab Test

• GALT enzyme activity
  – Profound deficiency < 2 units/gHb
  – Partial deficiency – 2 to 2.5 units/gHb
  – Normal > 2.5 units/gHb

• Specimen handling is very important for accurate results – potential for false positives if specimen is exposed to heat or direct sunlight
lactose → glucose + galactose

INTESTINAL LUMEN

cell membrane

aldose reductase

galactose → galactitol

CYTOPLASM

ATP → ADP

hexokinase

gal-1-P uridyl transferase

gal-1-P

galactokinase

UDP-glu → UDP-gal

UDP gal-4-epimerase

glu-1-P

phosphoglucomutase

glu-1,6-bis-P

phosphoglucomutase

phosphoglucomutase

glycogen

GLYCOLYSIS

glu-6-P

dehydrogenase

6-phosphogluconate

PENTOSE SUGARS

CITRIC ACID CYCLE

A lactase deficiency (AR)
lactose intolerance (AR)

B galactokinase deficiency (AR)

C galactosemia (AR)

D glucose-6-phosphate dehydrogenase (G6PD) deficiency (XR)
Classic Galactosemia

Symptoms within first two weeks
• Jaundice
• Vomiting and diarrhea after ingesting milk

Can be life-threatening if untreated
• Sepsis
• Liver failure
• Kidney failure
• Brain damage
Mild Galactosemia

- Some infants with mild galactosemia will show signs of poor feeding and digestion and may benefit from treatment
- Some infants will not require treatment
# Genotype/Phenotype Correlation

<table>
<thead>
<tr>
<th>Genotype</th>
<th>GALT activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>100%</td>
</tr>
<tr>
<td>ND</td>
<td>75%</td>
</tr>
<tr>
<td>NG</td>
<td>50%</td>
</tr>
<tr>
<td>DD</td>
<td>50%</td>
</tr>
<tr>
<td>DG</td>
<td>5-20%</td>
</tr>
<tr>
<td>GG</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>

N = normal  
G = severe galactosemia allele (Q188R, S135L or K285N)  
D = Duarte allele (N314D: present in 5% of the U.S. population)
NBS resources

Initial GALT results

Time of tests
• GALT assay = 4.5 hours
• DNA assay = 2.5 hours

Alleles available
• Q188R, S135L and K285N (severe)
• N314D (Duarte)
Considerations

• Are we sure this is real?
  – check other enzyme activity results
  – repeat in duplicate to confirm original results

• How can we minimize the amount of “crying wolf”?
  – DNA testing for alleles that cause severe disease
  – DNA testing for Duarte allele (mild disease and prevalent in population)
GALT DNA Case

- LBW African-American baby boy
- 1st NBS drawn at 24 hours GALT= 2.79 (cut-off of 3.76)
- In NICU w/ hyperbilirubinemia but sepsis had already been ruled out
- Baby had multiple congenital deformities
- DOH recommended shifting to soy formula until we ran in-house DNA
GALT DNA Case

- DNA results = Q188R/N314D
- DOH recommended stopping soy formula
- Pending diagnostic labs showed profound enzyme deficiency
- DNA sequencing revealed 3 mutations: Q188R/N314D/R25P.
- False reassurance from limited DNA panel
Tyler Mize  5/1/98 to 5/10/98
NBS Program Goals

• Test every baby
• Assure hospital compliance
  – Quality specimens
  – Complete and accurate information
  – Quick transit time
• Perform accurate testing
  – Balance sensitivity with specificity
• Assure that babies with abnormal test results get appropriate and timely additional testing
• Assure that diagnosed babies get needed treatments without delay
• Provide clear and understandable information to the public
• Quality improvement
Final Thoughts

• Be courteous

• Ask questions!
  – what do/don’t we know?
  – what would be important to find out?
  – how are we doing and what could we do better?

• Data are our friends – get acquainted, try to understand them and question them if they seem wrong

• Don’t fly on autopilot!

• Where can we get the biggest bang for our buck?
Washington State Newborn Screening

www.doh.wa.gov/nbs

(206) 418-5410

or

1-866-660-9050

Come visit our lab!
What Not to Do

• Initial hemoglobin results were indicative of transfusion (AA)
• Results from second NBS were inconsistent with first results (FA)
• Analyzed all four blood spots – obviously blood from two individuals: one baby and one adult
What Not to Do

• Initial hemoglobin results were indicative of transfusion (AA)

• Results from second NBS were inconsistent with first results (FA)

• Analyzed all four blood spots – obviously blood from two individuals: one baby and one adult

• Baby did not bleed well and phlebotomist supplemented with someone else’s blood to fill circles

• Letter from our Program Director to Hospital Management about this dangerous practice