A Brief History of Newborn Screening

The first 50 years.

Ken Pass
I didn’t do this alone...

Thanks to:
Amy Hoffman
Alex Kemper
Kathy Harris
Piero Rinaldo
…and others
Asbjorn Follin

Liv

Dag

Borgny and Harry Egeland

Pediatrics 2000;105;89-103
“It all began with Johnny.”

Dr. Robert Guthrie
1916-1995
There were critics…

“At this time, the American Academy of Pediatrics favors neither the extension of current compulsory legislation nor passage of new legislation for the compulsory testing of newborn infants for the presence of congenital metabolic disease.”

April 1967
And still today...

Newborn screening: A spot of trouble
By raising hell about newborn blood-spot screening, Twila Brase could jeopardize public-health programmes and derail research. The problem is, she has a point.

Nature 2011
Then...and....Now

1963
- 3-5 days
- PKU
- one lab test
- no DNA
- 20 positive cases

2013
- 24 hours
- PKU + 50 more
- 8 tests
- DNA
- 900+ cases
Newborn screening today

- every state provides a screening program
- 15,000 newborns tested daily
- 58 newborns referred daily
- 3 infants identified during this talk
New York State Newborn Screening 1965-2012
12,775,000 Newborns Screened
17,521 Newborns Diagnosed

Tests Added
- PKU
- GALT
- MSUD
- SCD
- HCY
- CH
- HIV-1
- BIOT
- CAH
- CF
- MCAD
- MS/MS Krabbe
- SCID

# Babies Screened
# Babies Diagnosed
Specimen type

- Diaper or urine
- Guthrie specimen
- Cord blood
- Archived specimens
Guthrie BIA test for PKU
Basic Questions in Newborn Screening

**Who** should be tested?
**When** should the test be done?
**How** should the analysis be done?
**What** should be done with the results?
<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>CONC. RANGE</th>
<th>AMT IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-OHP</td>
<td>15-750 pmol/mL</td>
<td>23-1125 fmol</td>
</tr>
<tr>
<td>T4</td>
<td>26-400 pmol/mL</td>
<td>39-600 fmol</td>
</tr>
<tr>
<td>Carnitines</td>
<td>0.5-10 nmol/mL</td>
<td>2-30 pmol</td>
</tr>
<tr>
<td>Amino acids</td>
<td>60-900 nmol/mL</td>
<td>0.2-2.7 nmol</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.3-3 umol/mL</td>
<td>1-8 nmol</td>
</tr>
<tr>
<td>TSH</td>
<td>1-36 ng/mL</td>
<td>2-50 pg</td>
</tr>
<tr>
<td>IRT</td>
<td>10-400 ng/mL</td>
<td>30-1200 pg</td>
</tr>
<tr>
<td>Hemoglobins</td>
<td>0.9-180 mg/mL</td>
<td>2.7-540 ug</td>
</tr>
<tr>
<td>DNA</td>
<td>6 pg/cell</td>
<td>190 ng</td>
</tr>
<tr>
<td></td>
<td>10,000 cell/uL</td>
<td></td>
</tr>
</tbody>
</table>
Evolution of NBS

1957  Diaper test in California
1958  Phenistix in Europe
1963  Guthrie & Susi publish: BIA for PKU and develop the use of dried blood specimens
1963  Massachusetts universal screening
1975  Electrophoresis for SSD – first multiplex test
1994  MS/MS – second multiplex test
2000  DNA analysis as second tier
2006  LSDs – first to challenge functioning of system
2010  SCID – DNA as first tier
2020  ???
NBS – A Slave of Technology

- Wet Diaper
- BIA
- Electrophoresis
- RIA
- EIA
- Isoelectric focusing
- msms
- PCR
Some Disorders Detectable by MS-MS

❖ Amino acidemias
  ● Phenylketonuria
  ● Maple syrup urine disease
  ● Homocystinuria
  ● Citrullinemia
  ● Hepatorenal tyrosinemia

❖ Organic acidemias
  ● Propionic acidemia
  ● Methylmalonic acidemia
  ● Isovaleric acidemia
  ● 3-Methylcrotonylglycinemia
  ● Glutaric acidemia type 1
  ● Hydroxymethylglutaric acidemia

❖ Fatty acid oxidation disorders
  ● SCAD deficiency
  ● MCAD deficiency
  ● VLCAD deficiency
  ● LCHAD and trifunctional protein deficiency
  ● Glutaric acidemia type II
  ● CPT-II deficiency

…and more
NBS – A Slave of Technology

- Wet Diaper
- BIA
- Electrophoresis
- RIA
- EIA
- Isoelectric focusing
- msms
- microarrays
Preliminary results indicate that the Guthrie spot will provide suitable DNA, both in quantity and quality, for better than acceptable performance on Affymetrix DNA chips.

Collaboration: Robin Pietropaolo, Wadsworth Center; Michelle Caggana, Wadsworth Center; Kenneth Pass, Wadsworth Center; John Palma, Affymetrix; Janet Warrington, Affymetrix
NBS Chip®

P/N: 520019
Lot #: 1
Exp. Date: 040125
<table>
<thead>
<tr>
<th>Currently screened conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PKU</strong></td>
</tr>
<tr>
<td><strong>BIOT</strong></td>
</tr>
<tr>
<td><strong>HCY</strong></td>
</tr>
<tr>
<td><strong>MSUD</strong></td>
</tr>
<tr>
<td><strong>GAL</strong></td>
</tr>
<tr>
<td><strong>CH</strong> ✓</td>
</tr>
<tr>
<td><strong>CAH</strong></td>
</tr>
<tr>
<td><strong>CF</strong></td>
</tr>
<tr>
<td><strong>SSD</strong></td>
</tr>
<tr>
<td><strong>MCADD</strong></td>
</tr>
<tr>
<td><strong>HIV</strong> ✓</td>
</tr>
<tr>
<td><strong>TYR</strong></td>
</tr>
<tr>
<td><strong>G-6-PD</strong></td>
</tr>
<tr>
<td><strong>TOXO</strong> ✓</td>
</tr>
</tbody>
</table>
### And in the future NBS panel?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Disorder or Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>Duchenne MD</td>
</tr>
<tr>
<td>Cancer</td>
<td>Becker MD</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>SCID</td>
</tr>
<tr>
<td>Asthma</td>
<td>Turner Syndrome</td>
</tr>
<tr>
<td>Astrocytomas</td>
<td>Wiskott-Aldrich Syndrome</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Fabry Syndrome</td>
</tr>
<tr>
<td>Hirschsprung Syndrome</td>
<td>Krabbe Syndrome</td>
</tr>
<tr>
<td>Hurler-Scheie Syndrome</td>
<td>Pompe Syndrome</td>
</tr>
<tr>
<td>OTC deficiency</td>
<td>Hurler-Scheie Syndrome</td>
</tr>
<tr>
<td>Citrullinemia</td>
<td>GALK</td>
</tr>
<tr>
<td>Argininemia</td>
<td>GALE</td>
</tr>
<tr>
<td>E3 deficiency</td>
<td>CDG</td>
</tr>
</tbody>
</table>

*All identifiable by mutation analysis*
Issues to address in DNA primary screening

• Genotype/phenotype correlations
• Private mutations
• Controls (CDC program? Private?)
• QA and QC (CDC program? Private?)
• Cost
• Others?
Primary DNA screening
One spot
One test
One result

Guthrie test for PKU
Each day:

1000 specimens
8 screening analyses
8 confirmatory analyses on 10%
yields 9000 spots used
From "One Punch - One Disease"

To **Whole Spot Extraction**

- Collect MORE blood
  - or....
  - SOLVENT extraction
    - AC
    - AA
    - Others
  - Buffer (H$_2$O) extraction
    - Proteins
    - Steroids
    - Others
  - DNA extraction
    - ID
    - CF
    - Others
  - Repeat analyses,
    - 2$^{nd}$ tier tests, storage
Should We Collect More than Blood?

"Universal" collection card

Blood

Urine

How many spots?
Multiplex Technology

- msms supreme example
- Hgb electrophoresis was first
- Targeted profile
Time, Tide, and Tech
Wait for No One

Time and Tide
Wait for No One

X
Cellulose Acetate Membranes

CA membranes can be cast directly onto silicon chips using a standard fabrication process.

The membranes have good adhesion, good structural integrity, and are biocompatible.

The membranes are made with variable rejection characteristics to restrict the passage of molecules as small as 300Da and up to 700Da (with current capabilities).

Membranes’ rejection characteristics, charge, thickness, and/or flux rate can be altered using different casting and treatment conditions.

CA membranes, combined with electrophoresis can be used to purify DNA from PCR inhibitors such as heme.
Microfluidic Screening Assays
Device with Three Assays in Parallel

Assay 1: PKU
Assay 2: Galactosemia
Assay 3: CAH

lines: microfluidic channels
sample: drop of blood
filter: sample cleanup
standard: calibration or standard addition
The “Price” of Extra Tests

- More false positives - more parental anxiety
- Delayed Dx for false negatives
- Heterozygotes - what to do with them?
4 Landmark Reports

1975 NAS - Proven public benefit, feasibility, consent, tests and follow-up, advisory committees

1994 IOM- Benefit to child, Dx, Rx and F/U

1997 Task Force on Genetic Testing - Direct benefit to child, mandatory screening

2006 Newborn Screening: Toward a Uniform Screening Panel and System.

N Green
Premise: “Newborn screening should be conducted only when science and technology can serve both the individual and the public good.”

NBS Task Force Report 2000
ELSI-type Points to Consider

- Do the diseases in the expanded panel meet the criteria for a screened disease?
- Diagnosing patients who would never present with a problem
- Diagnosing patients that may already be severely compromised or dead when the results of testing are available
- Identifying disease carrier status in children
- Screening for diseases where is no clear treatment protocol
Traditional Screening Criteria

- Important health problem
- Natural history understood
- Detectable early stage
- Treatment at an early stage is of better benefit
- A suitable test is available
- Test should be acceptable
- Intervals for repeating the test should be determined
- Adequate services available to cover screening-induced need
- Risks should be less than the benefits
- The costs should be balanced against the benefits

Wilson, Jungner, World Health Organization, 1968

David and Patrick will expand on these.
Charge of SACHDNC

• To make systematic evidence-based and peer-reviewed recommendations that include the heritable disorders that have the potential to significantly impact public health for which all newborns should be screened

• To develop a model decision-matrix for newborn screening expansion, including an evaluation of the potential public health impact of such expansion and periodically update the recommended uniform screening panel
SACHDNC Recommendations
Adopted by the Secretary, HHS

Conditions recommended
• Severe Combined Immunodeficiency
• Critical Congenital Heart Disease (CCHD)
• Pompe disease

Conditions considered but not recommended
• Hemoglobin H disease
• Krabbe disease
• Chronic Bilirubin Encephalopathy
The Newborn Screening Translational Research Network (NBSTRN)

- Newborn Screening Saves Lives Act of 2007
- Hunter Kelly Newborn Screening Research Program
- 5-year contract from NICHD to ACMG
- Develop a research infrastructure to support investigators with projects related to newborn screening
Virtual Repository of Dried Blood Spots (VRDBS)

- Secure, centralized & web-based
- Pilot phase 6/12 to 9/12 – production date: 9/26/12
- Inventory of DBS samples – over 2.6 million
- Investigators can request letters of support, submit questions to participating states, browse & request specimens, track shipments & provide feedback
- States can respond to questions, review & manage requests, approve requests & control distribution

![VRDBS Image]
Unintended Consequences

- Carrier detection
  - Sickle cell trait
  - Cystic fibrosis
  - PKU?

- Late onset
  - Krabbe

- Affected mother
  - Maternal PKU
And the DBS?

Better known as the Guthrie Specimen....
## Analytes identified in DBS

**Harry Hannon**

### Appendix 1. List of analytes that have been analysed in dried blood spot (DBS) samples (not including analytes listed in appendix 2).

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>(Czernavski, Fitzgerald et al. 2001; Dug, Dug et al. 2000, Dug and Dug 2001)</td>
</tr>
<tr>
<td>Alpha-fetoprotein</td>
<td>(Migration, Baltierra et al. 1997, Perkins, Perkins et al. 2003)</td>
</tr>
<tr>
<td>Amodiacine/Deoxycorticosteroids</td>
<td>(Giten, Muccheli et al. 2003)</td>
</tr>
<tr>
<td>Bioneurite</td>
<td>(Yamaguchi, Fujita et al. 1981; Peters, Anandor et al. 1987, Brode, Braungart et al. 2001)</td>
</tr>
<tr>
<td>Broccoli carbohydrates</td>
<td>(Klocke, Igland et al. 1995)</td>
</tr>
<tr>
<td>Catechin (Wilkinson’s disease)</td>
<td>(Chen, Abakumov et al. 1999)</td>
</tr>
<tr>
<td>Chloroquine Chlorophyllins</td>
<td>(Onwude, Chukwu et al. 1996; Niami, Rais et al. 2003)</td>
</tr>
<tr>
<td>Dichlorobisphenylchlorobisphenyl</td>
<td>(Chrise, Demopoulos 1997)</td>
</tr>
<tr>
<td>Dihydroxyacetone/Reductase</td>
<td>(Gew, silicone 1958)</td>
</tr>
<tr>
<td>Diphenyl/Thiocyanate</td>
<td>(Aron 1958, Hong, Ko et al. 1969)</td>
</tr>
<tr>
<td>Erythrocyte protoporphyrin</td>
<td>(Coffman, Murphy et al. 1977)</td>
</tr>
<tr>
<td>Ferric acid/Acrylicitin</td>
<td>(Schiff/Schmiede, Papp et al. 1993; Bannett, Bannett et al. 1962)</td>
</tr>
<tr>
<td>Glycoproteins</td>
<td>(Kerkel, Slavenia et al. 1996; Lemon 2000; Kimera, Yone et al. 2002)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>(Takii, Hanafusa et al. 1996)</td>
</tr>
<tr>
<td>Galactose (Galactose-1-phosphate)</td>
<td>(Chun, Han et al. 1996; Hone, Yoon et al. 2001)</td>
</tr>
<tr>
<td>Galactose A</td>
<td>(Klock, Elal et al. 1998; Fujimoto, Okano et al. 2000)</td>
</tr>
<tr>
<td>Glutathione</td>
<td>(Chun, Han et al. 1997)</td>
</tr>
<tr>
<td>Hemoglobin variant</td>
<td>(Egidi, Perry et al. 2003)</td>
</tr>
<tr>
<td>Homocystine</td>
<td>(Accini, Campello et al. 2003)</td>
</tr>
<tr>
<td>Human chorionic/Grandisoester hormone</td>
<td>(Mori, Anderson et al. 1996; Yallaha, Kreutz et al. 2000)</td>
</tr>
<tr>
<td>Human Immunodeficiency virus</td>
<td>(Yours and Conroy 1992)</td>
</tr>
<tr>
<td>3-Methoxyxantracetone</td>
<td>(Burke, World et al. 1981)</td>
</tr>
<tr>
<td>Immunoactive Trypsin</td>
<td>(Kerby, Applegard et al. 1981; Carlin, Pederneti et al. 1990; Xu, Peterson et al. 1992)</td>
</tr>
<tr>
<td>Lecithin</td>
<td>(Bomra, Worth et al. 1981)</td>
</tr>
<tr>
<td>Lactate</td>
<td>(Chammas, Bianco et al. 2001)</td>
</tr>
<tr>
<td>Maldonante</td>
<td>(Nhao, Efraim et al. 1997)</td>
</tr>
<tr>
<td>Melatonin</td>
<td>(Vladimr, Giubert et al. 1990)</td>
</tr>
<tr>
<td>Metformin</td>
<td>(Chammas, Bianco et al. 2001)</td>
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<tr>
<td>Mucin</td>
<td>(Shamelin, Sila et al. 1995; Verillo, Rofield et al. 1996; Halfad, Kavering et al. 2001)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>(Bergfroy, Al Khabash et al. 1990)</td>
</tr>
<tr>
<td>Nucleobase</td>
<td>(Rodeigues-Perez, Dain-Junatsu et al. 1999)</td>
</tr>
<tr>
<td>PEGacylated/Polynucleotide</td>
<td>(Boudt, Van der Hoeven et al. 1995; Kolewicz, Taylor et al. 1995; Bergfroy, Fudina et al. 1998)</td>
</tr>
<tr>
<td>Phenolic antioxidants</td>
<td>(Chammas, Bianco et al. 2001)</td>
</tr>
<tr>
<td>Phosphatidylcholine/nucleotides</td>
<td>(Ostuni, Middle et al. 2005)</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>(Crowe, McCarthy et al. 1996)</td>
</tr>
<tr>
<td>Riboflavin-7-carboxylic acid</td>
<td>(Duarte, Gyanes et al. 2002)</td>
</tr>
<tr>
<td>Ribonucleic acid</td>
<td>(Parker and Cohlin 1999)</td>
</tr>
<tr>
<td>RNA</td>
<td>(Nelson, Siemion et al. 2003)</td>
</tr>
<tr>
<td>Ribonucleic acid</td>
<td>(Fawcett and Ratko 1999)</td>
</tr>
<tr>
<td>Ribonucleic acid</td>
<td>(Rama, Rama et al. 1999)</td>
</tr>
<tr>
<td>Ribonucleic acid</td>
<td>(Bergfroy, Al Khabash et al. 1990; Fujimoto, Tera et al. 1995)</td>
</tr>
<tr>
<td>Ribonucleic acid</td>
<td>(Teri, Law et al. 1996; Wavan, Olivares et al. 2001)</td>
</tr>
<tr>
<td>Ribonucleic acid</td>
<td>(Gutierrez, May et al. 1997)</td>
</tr>
<tr>
<td>Ribonucleic acid</td>
<td>(Dossman, Morrisette et al. 1980)</td>
</tr>
<tr>
<td>Transaminase/Transaminase</td>
<td>(Pawson and Enstrom 1999; Sorensen, Spanier et al. 2002)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>(Bachhuber, Lee et al. 1992)</td>
</tr>
<tr>
<td>Triglyceride/Triglycerides</td>
<td>(Vlaso, Vlaso et al. 2007)</td>
</tr>
<tr>
<td>Transaminase/Transaminase</td>
<td>(Glicker, Smith et al. 1990)</td>
</tr>
<tr>
<td>Trypsin/Chymotrypsin</td>
<td>(Nal, Gruensova et al. 1987)</td>
</tr>
<tr>
<td>Urea</td>
<td>(Kallen, Husain et al. 1996; Hum, Fishos et al. 1987; Chapman, Chapman et al. 1990)</td>
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<tr>
<td>Urea</td>
<td>(Bergfroy, Al Khabash et al. 1990)</td>
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<tr>
<td>Urine</td>
<td>(Kusuma, Hara et al. 1997)</td>
</tr>
<tr>
<td>Urinary protein/Thrombin</td>
<td>(Larsen and Brodin 1975)</td>
</tr>
<tr>
<td>Urinary protein/Thrombin</td>
<td>(Issid 1971; Newton andStarr 1971; Rudy, Rodwell et al. 1997; Pons, Alonso et al. 2001)</td>
</tr>
<tr>
<td>Urinary protein/Thrombin</td>
<td>(Filipp, Catenzio et al. 1990)</td>
</tr>
<tr>
<td>Urinary protein/Thrombin</td>
<td>(Cortetti, Guterre et al. 1998; Kissi and NaCeli 1995)</td>
</tr>
<tr>
<td>Urinary protein/Thrombin</td>
<td>(Leshner, Gruensova 1990)</td>
</tr>
<tr>
<td>Urinary protein/Thrombin</td>
<td>(Akelew, Meads et al. 1985)</td>
</tr>
<tr>
<td>Urinary protein/Thrombin</td>
<td>(Albend, Guerz et al. 2004)</td>
</tr>
<tr>
<td>Urinary protein/Thrombin</td>
<td>(Dann, Taug et al. 1995)</td>
</tr>
<tr>
<td>Urinary protein/Thrombin</td>
<td>(Jeffrey, Millsius et al. 1975; Wurt, Danso et al. 1969; Bioser 2004)</td>
</tr>
</tbody>
</table>
However, some things won’t change...
...despite some strong objections.
Thank you.

..and by the way...
“Mary had a little lamb!”
No mas.
Clarrisa Ball
9lbs 8oz
Feb 16, 2006
Multiplex Technology

- msms supreme example
- Hgb electrophoresis was first
- Targeted profile