What have I been doing since 2002?
Milestones of a personal journey
and memorable encounters along
the metabolic path

Piero Rinaldo, MD, PhD
T. Denny Sanford Professor of Pediatrics
Professor of Laboratory Medicine
Mayo Clinic, Rochester (MN)

Why 2002?
What Was I Doing in 2002?

Piero Rinaldo, MD, PhD
T. Denny Sanford Professor of Pediatrics
Professor of Laboratory Medicine
Mayo Clinic, Rochester (MN)

National Coalition for PKU & Allied Disorders Conference
Orlando, FL
October 3rd, 2002

Looking Back

Looking Back (2002)

“News You can Use”
(U.S. News & World Report - January 17, 2000)

Awareness in the general public and attention given to this issue are mounting and soon there will be a sweeping demand for changes in screening programs.

CHALLENGE:
We, as geneticists, are better to be prepared to deal with the “sorting” of disorders (screened vs not screened).

“News You can Use”
(U.S. News & World Report - January 17, 2000)

We, as geneticists, are better to be prepared to deal with the “sorting” of disorders (screened vs not screened).
Retrospective Analysis of Original NBS Sample

Nora’s NBS sample

Normal control


New Cases at Mayo (2002)
Facts to Know about NBS

- More than 4 million babies are born every year in the United States (~130 million worldwide)
- In the vast majority of cases (term pregnancy, no perinatal issues), newborn screening is the first encounter with a laboratory test after birth
- Awareness of parents is often limited, and most are unprepared to cope with it, especially in case of an apparently abnormal result

False Positives: The Dark Side of Newborn Screening

- Recall and repeat analysis (2nd, 3rd, 4th...)
- Disruption of care (premature, sick newborns)
- ER visit(s), admission(s), follow up visits
- Confirmatory testing ($$$)
- Referral to multiple specialists, 2nd opinions
- Disruption of working parents schedule
- Impact on extended family life (stress)

Facts to Know About NBS

- More than 4 million babies are born every year in the United States (~130 million worldwide)
- Vast majority receives a normal result
- Other outcomes (IEM by MS/MS only) include
  - True positives (TP) ~0.05% ~2,500/yr
  - False positives (FP) ~0.5% ~25,000/yr
  - Positive predictive value (PPV) ~10% (1 in 10)
  - False negatives (FN) ~1: ???? ???/?yr

FN: The Darkest Side of NBS

The price of being wrong

Dec. 9, 2016

FN: The Darkest Side of NBS
Lab’s standards missed baby’s serious disorder
Uniformity lacking in states’ screenings


My Goals in the Years after 2002
• Improve newborn screening performance
• Achieve uniformity of testing panel by MS/MS to maximize detection of affected newborns
• Set and sustain lowest achievable rates of false positive results of primary test (first tier)
• Development and implementation of second tier tests to further reduce false positives
• Define criteria for success

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Performance Targets
• False positive rate (FPR) <0.3%
• Positive predictive value (PPV) >20%

NBS by MS/MS: Examples of Performance Metrics
◆ US States (N=37)
(MN not shown)
2004

- 50/50 split of NBS in the US
- NBS left to consumer initiative
- HRSA grants (genesis of R4S)
- The MN model

**Newborn Screening Left to Consumer Initiative**

Department of Health & Human Services, Maternal and Child Health Bureau

Letter sent in July 2004 to every NBS programs in the USA

Additionally, I also encourage you to facilitate the development of educational material that inform parents about the option to have their babies screened for additional conditions that could be routinely tested in the newborn period but at this time are not covered or required by your State program.
To be eligible for funding, a regional proposal must include at least 4 participating states.

Goals of Regional Collaborative

- Implement universal screening and confirmatory testing of newborns for inborn errors of amino acid, organic acid, and fatty acid metabolism
- Reduce inequities in access to genetic services
- Utilize a regional approach to improve public health infrastructure for supporting optimal diagnosis, follow up and management of children with heritable disorders and birth defects

Objectives of Project 1

- Achieve uniformity of testing panel by MS/MS to maximize detection of affected newborns within the region
- Improve overall analytical performance
- Set and sustain lowest achievable rates of false positive results

Standardization of Outcomes and Guidelines for State Newborn Screening Programs

Contract No. 240-01-0038

Contractor
American College of Medical Genetics
Michael S. Watson, PhD, FACMG, Project Director

SPONSOR
Maternal and Child Health Bureau
Genetic Services Branch
Health Resources and Service Administration
Michele A. Lloyd-Puryear, MD, PhD, Chief
**Criteria and Scoring System**

- Incidence of conditions
- Identifiable at birth
- Burden of disease
- Availability of test
- Test characteristics
- Availability of treatment
- Cost of treatment
- Efficacy of treatment
- Benefits to individual
- Benefits to F&S
- Mortality prevention
- Diagnostic confirmation
- Acute management
- Simplicity of therapy

**Uniform Panel Survey**

**Work Group**

- Donald Bailey
- Celia Kay
- Alex Kemper
- Michele Lloyd-Puryear
- Marie Mann
- Kenneth Pass
- Jennifer Puck

(Chair) Piero Rinaldo

Brad Therrell

Michael Watson

**HRSA/ACMG Screening Panel (2004)**

- 28/42 (66%)

20 Primary targets ("Uniform panel")

22 Secondary targets

**The Minnesota Model**

A public-private partnership between

The Minnesota Department of Health (MDH)

The University of Minnesota

Mayo Clinic (Mayo Medical Laboratories)
Start Date June 15, 2004

MN Performance by MS/MS (False Positive Rate 2004-2013)
Testing performed by Mayo Clinic

<table>
<thead>
<tr>
<th>Period</th>
<th>Births</th>
<th>Abnormal cases</th>
<th>True positives</th>
<th>False positives</th>
<th>Detection rate</th>
<th>FPR</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>71,207</td>
<td>55† (N=37)</td>
<td>38 USA</td>
<td>17</td>
<td>1:1,874</td>
<td>0.024%</td>
<td>69%</td>
</tr>
</tbody>
</table>

†NO repeat requests
†NO TPN

Delta 42%

2005

In Memory of Kay Tanaka, M.D. (1929-2005)

1st R4S Meeting (01/24-25/05)
2006

- Implementation progress

- The armageddon prediction

- The RUSP

Newborn Screening 2006

Conditions screened for in North America

...our story would have been different had our child been born in a state like Minnesota that has such a comprehensive newborn screening program
that an individual test for a disorder had a specificity of 99.99%, we estimated that ~2375 infants would have received false-positive results through screening with tandem mass spectrometry in 2005. If specificity was assumed to be 99.9%, then the number increased to ~31,800.

Predicted scenarios FPR

Best case  81  0.11%
Intermediate  808  1.14%
Worst case  1606  2.27%

ACTUAL  52  0.07%

The Recommended Uniform Screening Panel (RUSP) of 2006

- R4S continues to grow
- HRSA nomination process
- The beginning of the storm
**Project Objectives (2007)**

- Development and implementation in screening practice of clinically validated cut-off values and post-analytical tools
- Training course at Mayo in small groups
- Development of customized software to manage data collection, analysis, and reporting of NBS data
- Collection, compilation and monitoring of performance metrics, with definition of targets of acceptable performance
- Monthly conference calls and bi-annual face-to-face meetings
- Continuing clinical validation of 2nd tier tests
- Round robin sample exchange

**Operating Principles**

- Web-based access, password protected, large (100-200) number of total users with the expectation of multiple simultaneous log-ins
- Peripheral data submission (participants enter own data, not done centrally)
- Automation of administrative functions (e-mail reminders, monthly posting of general updates)
- On demand, user-driven production of project tools (score cards, plots)
- Easy generation of customize reports (comparison tools of own data vs. cumulative data)
- Flexibility to add new conditions and markers (with potential applicability beyond MS/MS panel), and to query the database to generate novel reports

**Nomination Form for New Conditions to be Added to Uniform Panel was posted on HRSA website on May 18, 2007**
The Beginning of the Storm

FIVE DROPS OF BLOOD:
INVASION OF PRIVACY?

Minnesota doctors say the state's newborn screening program saves lives and money; some privacy advocates say it amounts to involuntary genetic testing.

Minneapolis Star Tribune, November 11, 2007, A1

“Some researchers might be trying to convince the state to test day-old infants for genes linked to a 'tendency towards violence'”


2008

• US(A) vs. them

ACMG Expert Group recommendations

Mandatory
- screening for primary target conditions
- reporting of all secondary target conditions
- reporting of any abnormal results that may be associated with clinically significant conditions, including the definitive identification of carrier status

What Europe Thinks of Us

If it moves, shoot it!

If it's too Hard (to do it right), Skip it
San Antonio November 2, 2008

Why OPT-IN is a Recipe for Disaster

2009

Nine Families Sue State Of Minnesota - Alleges Violation Of State Genetic Privacy Law In Newborn Screening

Main Category: Litigation / Medical Malpractice
Article Date: 12 Mar 2009 - 4:00 PDT

2010

• The complete R4S website

• The post-analytical tools

The R4S Website

http://www.region4genetics.org/

Collection
The R4S Website

http://www.region4genetics.org/

Post-Analytical Tools

OTC  CUD  CPT1  CPT2

VLCAD  LCHAD  GA1  GA2

VLCAD Tool

May 2009

Sep 2010

4th R4S User Workshop

Orlando May 2, 2010

The R4S Papers (No.1)

2011

• R4S paper No. 1
The R4S Papers (No. 2)

Enhanced interpretation of newborn screening results without analyte cutoff values

The end of HRSA grant

The “struggles” of R4S

Minnesota statute 144.125

Prevention of false negatives

2012

R4S paper No. 2

The evolution of the R4S Project

Region 4 Stork (R4S) started as a regional laboratory quality improvement project of expanded newborn screening by tandem mass spectrometry (7 state programs)

R4S was selected as one of three projects of a Regional Genetics collaborative funded by the Health Resources and Services Administration (2004-2012)

In May 2012 the R4S database became part of the Newborn Screening Translational Research Network, which is funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development

Prevention of False Negatives (using R4S Tools)

- 23 conditions
- 67 false negative cases
- 59 informative tools (88%)

Evolution of the R4S Project

2012

- R4S paper No. 2
- Prevention of false negatives
- The end of HRSA grant
- The “struggles” of R4S
- Minnesota statute 144.125

Parallel, not Sequential Algorithms

A parallel (rather than sequential), objective, and automated evaluation of complex metabolic profiles to achieve better sensitivity and specificity

Prevention of False Negatives (using R4S Tools)

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- 67 false negative cases
- 59 informative tools (88%)

Evolution of the R4S Project

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• Parental right to request deletion of records
• Destruction of 1.1 million archived specimens
• Destruction of new specimens after 71 days
• Prohibition to use residual specimens for new test development (even with oversight of 2 IRBs)
• Destruction of ALL results after 2 years

2013
• And destruction it was.....

21 Million Mayo Results (2004-2013) Had to Be Destroyed

It is hard to imagine how such things could come to such a result to satisfy imaginary problems of a dramatically few people, and a few lawyers who need something to do and will do anything for a buck.

R. Rodney Howell, M.D., FAAP, FACMG
Emeritus Chair of Pediatrics, University of Miami
President, International Society for Newborn Screening (ISNS)
(cited with permission)

2014
• R4S paper No. 3
• A memorable encounter
• The switch

The R4S Papers (No. 3)

The Legacy of Egil Jellum (Oslo, Norway)
One of the trends in modern medicine is the increasing understanding that many, if not all, diseases may be linked in some way with deviations from, or alterations in, one or more of the several thousand chemical reactions that normally take place inside the cells and body.

It does not seem unreasonable to assume that if one were able to identify and determine the concentration of all the compounds inside the human body, including both high- and low-molecular-weight substances, one would probably find that almost every known disease would result in characteristic changes in the biochemical composition of the body fluids.


Oslo, May 7, 2014

FPR by MS/MS (Mayo births only) (2010-15)

- Another memorable encounter (again from Norway)
- CLIR replaces R4S

Collaborative Laboratory Integrated Reports (CLIR)
- CLIR is a multivariate pattern recognition software and interactive web tool that was initially developed to support Region 4 Stork (R4S), a federally-funded (2008-2012) collaborative project for laboratory quality improvement of newborn screening by tandem mass spectrometry.

Status of R4S as of May 31st, 2018

| Countries | 69 |
| Locations (programs) | 269 |
| Registered active users | 1,295 |
| Positive cases (94 conditions) | 21,038 |
| Current count website user login | 156,316 |
| Total count user website page views | 1,507,762 |
| Newborns tested in R4S (2012-2018) | 28,289,051 |
| Calculated post-analytical tool scores | 393,747,192 |

R4S will be turned off on September 1, 2018
CLIR is a multivariate pattern recognition software and interactive web tool that was initially developed to support Region 4 Stork (R4S), a federally-funded (2008-2012) collaborative project for laboratory quality improvement of newborn screening by tandem mass spectrometry. Since 2012, CLIR is supported by institutional funding and has been approved as an official product of Mayo Clinic.

What Does CLIR Do, Exactly?

- Replaces conventional reference ranges
  - With continuous, (covariate)-adjusted %iles
- Replaces analyte cutoff values
  - With a condition-specific degree of overlap
- Enhances the clinical utility of individual markers
  - With all possible permutation of ratios
- Replaces sequential algorithms (“AND”)
  - With tool-based parallel algorithms (“OR”)

2016

- Back in the game: Kentucky

In March 2015, KY legislature passed bill KRS 214.155 amending their NBS program to add Krabbe disease.

Rationale for Mayo Response

- Screening for ANY LSD in isolation is problematic, regardless of the method used, because there will be too many false positives (artifacts causing multiple low activities, carriers and pseudo-deficiency cases)
- The solution is to look at a simultaneous profile of 6 lysosomal enzymes and 4 lysophosphatidylcholines and calculate any possible permutation of ratios
- We can report only Krabbe and effectively “silence” other potentially relevant findings, but we cannot report Krabbe with good performance without measuring the other markers
- Eventually decided to also screen for Pompe disease and MPS I in addition to Krabbe disease

Kentucky

In March 2015, KY legislature passed bill KRS 214.155 amending their NBS program to add Krabbe disease.

As a potential (short term?) alternative to in-house testing, KY contacted Mayo to inquire whether we would be able to offer testing for galactocerebrosidase activity (only)

Day 1 (February 17th, 2016)

so…. what happened?
## 2017

- **Kentucky year 1**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Krabbe</th>
<th>Pompe</th>
<th>MPS I</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>55,151</td>
<td>55,151</td>
<td>55,151</td>
<td>55,151</td>
</tr>
<tr>
<td>Abnormal (CLIR)</td>
<td>11</td>
<td>15</td>
<td>57</td>
<td>83</td>
</tr>
<tr>
<td>Second tier test</td>
<td>PSY/30kb del</td>
<td>(Cre/Crn)/GAA</td>
<td>HS DS</td>
<td></td>
</tr>
<tr>
<td>Reported positive</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Confirmed TP</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Confirmed FP</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Detection rate</td>
<td>1:55,151</td>
<td>1:27,581</td>
<td>1:55,151</td>
<td>1:13,788</td>
</tr>
<tr>
<td>FPR</td>
<td>0%</td>
<td>0%</td>
<td>0.002%</td>
<td>0.002%</td>
</tr>
<tr>
<td>PPV</td>
<td>100%</td>
<td>100%</td>
<td>50%</td>
<td>80%</td>
</tr>
</tbody>
</table>

## 2018

- **Kentucky year 2**
- **Precision newborn screening**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Krabbe</th>
<th>Pompe</th>
<th>MPS I</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>56,109</td>
<td>56,109</td>
<td>56,109</td>
<td>56,109</td>
</tr>
<tr>
<td>Abnormal (CLIR)</td>
<td>12</td>
<td>12</td>
<td>14</td>
<td>38</td>
</tr>
<tr>
<td>Second tier test</td>
<td>PSY/30kb del</td>
<td>(Cre/Crn)/GAA</td>
<td>HS DS</td>
<td></td>
</tr>
<tr>
<td>Reported positive</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Confirmed TP</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Confirmed FP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Detection rate</td>
<td>n/a</td>
<td>1:18,703</td>
<td>n/a</td>
<td>1:18,703</td>
</tr>
<tr>
<td>FPR</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>PPV</td>
<td>n/a</td>
<td>100%</td>
<td>n/a</td>
<td>100%</td>
</tr>
</tbody>
</table>

## R4S & CLIR Performance

(Interpretive Tools w/o cutoff values)

<table>
<thead>
<tr>
<th>System</th>
<th>R4S</th>
<th>CLIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBS test</td>
<td>MS/MS</td>
<td>KMP</td>
</tr>
<tr>
<td>Conditions</td>
<td>RUSP</td>
<td>MPS</td>
</tr>
<tr>
<td>From (date)</td>
<td>01/01/13</td>
<td>02/17/16</td>
</tr>
<tr>
<td>To (date)</td>
<td>12/31/13</td>
<td>02/28/18</td>
</tr>
<tr>
<td>State</td>
<td>MN</td>
<td>KY</td>
</tr>
<tr>
<td>Newborns tested</td>
<td>71,207</td>
<td>111,260</td>
</tr>
<tr>
<td>True positive cases</td>
<td>38</td>
<td>7</td>
</tr>
<tr>
<td>False positive cases</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>False positive rate (FPR)</td>
<td>0.024%</td>
<td>0.001%</td>
</tr>
<tr>
<td>Pos. predictive value (PPV)</td>
<td>67%</td>
<td>88%</td>
</tr>
</tbody>
</table>

## The Era of Precision Screening

*Genet Med, August 2018 issue*
Near 0% FPR for Pompe, MPS-I, and X-ALD is achievable without additional patient contact and molecular testing.

2019-2020ish?

• Enough NBS, my job is done
• End of my career will refocus on the patients…….
• CLIR tools for patients!

Beyond 2018… Back to the Patients!

New CLIR Functionalities

• NBSTRN has requested some form of PUBLIC ACCESS, not dependent on data contribution
  – Limited to selected tools and reports
  – Time limited (30 days)
  – Not renewable
• We will also add a login process to PATIENT ACCESS to a customized tool for long term monitoring of individual patients
• Rather than being a “diagnostic” tool, the longitudinal plot can provide an integrated, age-adjusted trend toward illness (score going up) or wellness (score going down)

Patient/Family Folders
3 Year Follow-up by Plasma ACRN of Propionic Acidemia Case (partial list)

<table>
<thead>
<tr>
<th>Case Id</th>
<th>Age (yr)</th>
<th>Gender</th>
<th>C0</th>
<th>C2</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>65.00-6</td>
<td>Male</td>
<td>10.87</td>
<td>7.91</td>
<td>64.84</td>
</tr>
<tr>
<td>7</td>
<td>6.27</td>
<td>Male</td>
<td>17.35</td>
<td>8.96</td>
<td>92.09</td>
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<tr>
<td>8</td>
<td>6.74</td>
<td>Male</td>
<td>14.12</td>
<td>19.27</td>
<td>82.03</td>
</tr>
<tr>
<td>9</td>
<td>7.00</td>
<td>Male</td>
<td>9.82</td>
<td>10.51</td>
<td>93.16</td>
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<tr>
<td>10</td>
<td>7.45</td>
<td>Male</td>
<td>9.90</td>
<td>7.48</td>
<td>75.08</td>
</tr>
<tr>
<td>11</td>
<td>7.74</td>
<td>Male</td>
<td>7.62</td>
<td>7.50</td>
<td>49.88</td>
</tr>
<tr>
<td>12</td>
<td>7.90</td>
<td>Male</td>
<td>13.67</td>
<td>15.91</td>
<td>57.80</td>
</tr>
<tr>
<td>13</td>
<td>8.29</td>
<td>Male</td>
<td>13.65</td>
<td>17.34</td>
<td>51.00</td>
</tr>
<tr>
<td>14</td>
<td>8.50</td>
<td>Male</td>
<td>9.37</td>
<td>10.90</td>
<td>70.33</td>
</tr>
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