LABORATORY STANDARDS AND PROCEDURES WORKGROUP

May 10, 2016

Kellie Kelm, PhD, chair
Susan Tanksley, PhD, co-chair
# WORKGROUP ROSTER

<table>
<thead>
<tr>
<th>Name</th>
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<tbody>
<tr>
<td>Stanton Berberich</td>
<td>Harry Hannon</td>
<td>Dieter Matern</td>
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<tr>
<td>Roberto Zori</td>
<td>Bill Slimak</td>
<td>Jane Getchell</td>
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<tr>
<td>Fred Lorey</td>
<td>Mei Baker</td>
<td>Michael Watson</td>
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<tr>
<td>Carla Cuthbert</td>
<td>Rebecca Goodwin</td>
<td>George Dizikes</td>
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<tr>
<td>Clem McDonald</td>
<td>Michele Caggana</td>
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</tbody>
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Ad Hoc Experts: Koon Lai, Joann Bodurtha, Jelili Ojodu, Ed McCabe

Chair: Kellie Kelm
Co-chair: Susan Tanksley
HRSA staff: Debi Sarkar, Kathryn McLaughlin, Alaina Harris
Laboratory Standards & Procedures Workgroup

Charge

Define and implement a mechanism for the periodic review and assessment of

1. The conditions included in the uniform panel
2. Laboratory procedures utilized for effective and efficient testing of the conditions included in the uniform panel.
3. Infrastructure and services needed for effective and efficient screening of the conditions included in the uniform panel.
New Project 1

1. Laboratory procedures: Explore the role of next generation sequencing in newborn screening
   • Screening is currently based on phenotypic data. How do we accumulate the data to identify correlation between phenotypic & genotypic data?
   • Are there conditions for which sequencing is the only screening method?
   • What do you gain/lose from NGS?
   • Which data do you report? VUS? Carrier status?
   • What new infrastructure needs to be built for NGS?
New Project 2

2. Infrastructure and services:
   • Review data related to testing (Timeliness 1.0)
   • What are the implications of earlier specimen collection (<24 hrs)?
   • What are the unforeseen consequences and costs of timeliness?
Agenda

1. Welcome & roll call
2. Review charge & new projects
3. Role of whole genome sequencing in newborn screening
   a. Overview of APHL Molecular Subcommittee – Michele Caggana – 15 min
   b. Next Gen Sequencing in a state NBS Program – Mei Baker – 15 min
4. Timeliness –
   a. Early specimen collection – Lisa Feutchbaum - 20 min
   b. Unintended consequences and costs of timeliness – Marci Sontag - 20 min
Overview of APHL Molecular Subcommittee – Michele Caggana

1. History
2. Molecular Quality Improvement Program
3. NBS Molecular Workshops
4. Molecular Assessment Program
5. NBS Molecular Resources Website
6. Paradigm for NBS Molecular Pilots
7. NextGen sequencing meeting for the NBS community - APHL/CDC, Q1 2017
Next Generation Sequencing in NBS: Are we there yet?

Mei Baker, MD, FACMG

Co-Director, Newborn Screening Laboratory at WSLH
Professor, Department of Pediatrics
University of Wisconsin School of Medicine and Public health

SACHDNC Laboratory Procedures and Standards Subcommittee
May 9, 2016
Progression of CF NBS Tests

IRT → IRT/DNA → IRT/DNA* → IRT/DNA/DNA**
(F508del) (CFTR-23) (CFTR > 200)

1979 → 1991 → 2003 → 2012-16

*With IRT/DNA, 10 heterozygote carriers are detected for every CF infant diagnosed.

**IRT/NGS algorithm applying CFTR2 knowledge and next generation sequencing capability, which may be a “game-changer”.
## CFTR2 Mutation List History

<table>
<thead>
<tr>
<th></th>
<th>V1 4/10/2012</th>
<th>V2 7/22/2013</th>
<th>V3 2/27/2015</th>
<th>V4 8/13/2015</th>
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<tbody>
<tr>
<td>Number of Patients</td>
<td>35,312</td>
<td>39,696</td>
<td>39,696</td>
<td>88,664</td>
</tr>
<tr>
<td>CF-causing</td>
<td>123</td>
<td>175</td>
<td>179</td>
<td>242</td>
</tr>
<tr>
<td>Varying Clinical</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>Consequence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non CF-causing</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Unknown Significance</td>
<td>15</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>158</td>
<td>203</td>
<td>207</td>
<td>276</td>
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Prospective Study

- **SPECIFIC AIM 1.** We will further modify the established Illumina NGS method to expand CFTR mutation panel up to 250 CF-causing mutations.

- **SPECIFIC AIM 2.** We will demonstrate that the IRT/NGS CF screening protocol can significantly reduce false positive results caused by identification of CF heterozygote carrier infants in a real-world NBS environment.
Early specimen collection in California – Lisa Feutchbaum

1. We used California population-level data to determine whether early specimens (collected from 12 to 23 hours) would also be considered satisfactory based on screening performance.

2. CA analyzed for false-negative and false-positive rates in four disease categories:
   - metabolic disorders detectable by tandem mass spectrometry (MS/MS);
   - congenital adrenal hyperplasia (CAH);
   - congenital hypothyroidism (CH);
   - initial immune reactive trypsinogen (IRT) for cystic fibrosis (CF).

3. We compared the rates between the early-collection group (12 to 23 hours) and the standard-collection group (24 to 48 hours).
4. Conclusion:

- No significant difference of false-negative rate was detected between the two collection-timing groups.
- Early specimens had a significantly higher false-positive rate for CH (0.10 vs. 0.01%) and IRT (1.85 vs. 1.54%) but a lower false-positive rate for MSMS metabolic disorders (0.11 vs. 0.18%) and CAH (0.10 vs. 0.14%).

UNINTENDED CONSEQUENCES OF TIMELINESS

OBJECTIVES

Provide overview of NBS timeliness concerns
Discuss methods for collecting/analyzing data
Review state data provided (NY, MN, WI, IA) and discuss implications
Discuss challenges and next steps
Concerns

• Pre-Analytic
  • Less time to consult parents in hospital prior to screen
  • Collecting specimens from NICU/VLBW newborns

• Analytic
  • Repeat testing due to more out-of-range/borderline results
  • Asking for additional specimens due to more out-of-range/borderline results

• Post-Analytic
  • Increase in missed cases (false negatives)
  • Increase in presumptive positives (false positives)
Methods

• Report proportion of presumptive positives for IRT and TSH by age/time of collection
  • Correlation of analyte vs. age at time of collection
  • Proportion of presumptive positives by age of collection
• Look at borderline cases separately
• Remove VLBW babies (<1000g) from analysis as they tend to have atypical values
<table>
<thead>
<tr>
<th>Age at Time of Collection (hrs.)</th>
<th>Total IRT results reported</th>
<th>Total Out-of-Range (PP+ Borderline)</th>
<th>PP n (%)</th>
<th>Borderline n (%)</th>
<th>True +</th>
<th>PPV</th>
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<tbody>
<tr>
<td>0:00 – 11:59</td>
<td>381</td>
<td>15</td>
<td>4 (1.05%)</td>
<td>11 (2.89%)</td>
<td>1</td>
<td>6.7%</td>
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<tr>
<td>12:00 – 23:59</td>
<td>175</td>
<td>1</td>
<td>1 (0.57%)</td>
<td>0 (0%)</td>
<td>0</td>
<td>0.0%</td>
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<tr>
<td>24:00 – 29:59</td>
<td>47105</td>
<td>174</td>
<td>174 (0.37%)</td>
<td>0 (0%)</td>
<td>17</td>
<td>9.8%</td>
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<td>30:00 – 47:59</td>
<td>19439</td>
<td>76</td>
<td>75 (0.39%)</td>
<td>1 (0.01%)</td>
<td>8</td>
<td>10.5%</td>
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<tr>
<td>≥ 48:00</td>
<td>5173</td>
<td>15</td>
<td>14 (0.27%)</td>
<td>1 (0.02%)</td>
<td>3</td>
<td>20.0%</td>
</tr>
<tr>
<td>0:00 – 11:59</td>
<td>330</td>
<td>9</td>
<td>3 (0.91%)</td>
<td>6 (1.82%)</td>
<td>0</td>
<td>0.0%</td>
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<tr>
<td>12:00 – 23:59</td>
<td>175</td>
<td>3</td>
<td>2 (1.14%)</td>
<td>1 (0.57%)</td>
<td>0</td>
<td>0.0%</td>
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<tr>
<td>24:00 – 29:59</td>
<td>40803</td>
<td>137</td>
<td>137 (0.34%)</td>
<td>0 (0%)</td>
<td>17</td>
<td>12.4%</td>
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<td>30:00 – 47:59</td>
<td>18460</td>
<td>36</td>
<td>36 (0.2%)</td>
<td>0 (0%)</td>
<td>2</td>
<td>5.6%</td>
</tr>
<tr>
<td>≥ 48:00</td>
<td>4700</td>
<td>14</td>
<td>13 (0.28%)</td>
<td>1 (0.02%)</td>
<td>1</td>
<td>7.1%</td>
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Next Steps

• Need more data (i.e. how many babies are being called out as abnormal by age of collection)
• Identify some analytes that may be called out as normal when drawn early
• Need to monitor for missed cases
• Pre-analytic concerns and measures